Manzoor *et al.*
*Egyptian Journal of Biological Pest Control* (2023) 33:40
https://doi.org/10.1186/s41938-023-00688-x

Egyptian Journal of Biological Pest Control

RESEARCH

Open Access

# Comparative evaluation of temperate, subtropical, and tropical isolates of nucleopolyhedrovirus against tomato fruit borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae)

Saika Manzoor[1], Mudasir Gani[1*], Taskeena Hassan[2], Insha Shafi[1], Fehim Jeelani Wani[3], Shabnum Mumtaz[1], Gozde Busra Eroglu[4], Munazah Yaqoob[1] and Mohd Ayoob Mantoo[1]

## Abstract

**Background** The tomato fruit borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), is a serious insect pest owing to its polyphagous nature, migrating long distances, greater capacity to develop resistance against insecticides and persistence in cropping areas. The comparative evaluation of temperate, sub-tropical and tropical *H. armigera* NPV (HearNPV) isolates was carried out to determine the potential of local and exotic HearNPV isolates for the management of *H. armigera* in Kashmir, India, having a temperate climate.

**Results** Mortality of 2nd instar *H. armigera* larvae with three different HearNPV isolates at different concentrations ranged between 13.40 and 73.25%, with significant differences between the concentrations in each isolate under laboratory conditions. The median lethal concentration ($LC_{50}$) values of HearNPV-IND-K, HearNPV-IND-J, and HearNPV-IND-B isolates against 2nd instar *H. armigera* larvae were $4.62 \times 10^3$, $5.99 \times 10^4$, and $7.24 \times 10^4$ OBs/larva at 10th day post inoculation, with significant differences among the isolates. In time response bioassays, the cumulative mortality (%) caused by median lethal concentration ($LC_{50}$) of HearNPV-IND-K, HearNPV-IND-J and HearNPV-IND-B isolates over a period of 10 days was 50.33, 49.00 and 49.00%, respectively. Their median survival time ($ST_{50}$) values against 2nd instar *H. armigera* larvae were 8.10, 8.94 and 9.50 days, respectively, with significant differences among the isolates. The results revealed that the $LC_{50}$ and $ST_{50}$ values of HearNPV-IND K isolate were significantly lower than HearNPV-IND-J and HearNPV-IND-B isolates. The cumulative mortality of *H. armigera* larvae with HearNPV-IND-K, HearNPV-IND-J and HearNPV-IND-B isolates at different concentrations ranged between 22.00–68.32, 20.54–55.17, 11.66–44.33%, respectively, on tomato crop under field conditions. The highest mortality rate of 68.32% was observed at the local isolate (HearNPV-IND-K).

**Conclusions** It's concluded that the local isolate had the potential for the development of species-specific and environmentally safe biocontrol agent for organic farming and its incorporation into Integrated Pest Management program may reduce the use of chemical insecticides in Kashmir.

**Keywords** *Helicoverpa armigera*, Baculovirus, Nucleopolyhedrovirus, $LC_{50}$, $ST_{50}$, Tomato

*Correspondence:
Mudasir Gani
mudasir32@gmail.com
Full list of author information is available at the end of the article

## Background

The tomato fruit borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), is one of the most destructive insect pest which attacks a great variety of food, fiber, oil seed, fodder, and horticultural crops of more than 300 plant species (Arora et al. 2005). This pest is polyphagous and highly mobile in nature and continues to spread to new environments around the world (Koch et al. 2003). The polyphagy, high mobility, high fecundity, and facultative diapause help *H. armigera* to attain the status of a major pest (Fite et al. 2020). In India, *H. armigera* feeds on 181 cultivated and uncultivated species belonging to 45 families (Wubneh 2016). Infestations that reach the Economic Threshold Level (ETL) are normally managed by synthetic insecticides; however, *H. armigera* has developed resistance to numerous insecticides (Musser et al. 2015).

Biocontrol agents are being mass-produced and applied for ecofriendly management of this pest owing to a reduction in pesticides in the environment as well as the development of resistance to chemical insecticides (Martin and Oliveira 2005). The microbial control agents viz. entomopathogenic viruses (NPV and GV), entomopathogenic bacteria (*Bacillus thuringiensis* Berliner), entomopathogenic fungi (*Beauveria bassiana* (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metchnikoff) Sorokin), and entomopathogenic nematodes (*Steinernemma* and *Heterorhabiditis*) are widely used for eco-friendly management of *H. armigera* (Eroglu 2022). The entomopathogenic viruses (EPV) are naturally involved in the regulation of insect pest populations, including many agricultural, horticultural, and forest pests, and individual virus isolates have a very narrow host range, making them harmless to non-target organisms, humans, and the environment (Groner 1986). The *H. armigera* Nucleopolyhedrovirus (HearNPV) is extremely effective against *H. armigera* larvae and a component of integrated pest management program in many crops (Kalia et al. 2001). The widespread use of HearNPV in India and abroad is well documented and the demand for commercial formulation of these viruses is increasing. The HearNPV isolates have been reported from temperate (Sofi 2020), sub-tropical (Gupta et al. 2010) and tropical (Jeyarani et al. 2010) regions of India, however, their comparative evaluation under laboratory and field conditions against the *H. armigera* larval population has not been conducted till now and the same was investigated in this study.

## Methods

### Helicoverpa armigera

Laboratory culture of *H. armigera* was established from field collected larvae from experimental farm of FoA, Wadura. The 1st instar larvae were reared on Rumex (*Rumex obtusifolius*) in large Petri plates that were lined with a tissue paper at the bottom. Larvae were regularly monitored for their growth. The leaves were supplemented regularly to ensure sufficient food and proper care was taken for sanitation. The culture was maintained at 28 ± 2 °C, 60% R.H., and 16L: 8D photoperiod. From 2nd instar onwards, the larvae were reared on the semi-synthetic diet (Chitneni 2008) and transferred into individual glass vials or multi-well plates to avoid cannibalism. The pupae for culture maintenance were transferred into plastic jars (7 cm × 5 cm × 12 cm) for adults' emergence. After 7 to 8 days, adults were separated based on sex and released into oviposition cages (45 cm length: 30 cm diameter) in 1:2 ratio of males to females. Adults were provided by 10% honey solution as food using cotton swabs that was kept hanging inside the jar. Oviposition cages were lined internally with muslin cloth for egg laying. The larvae on hatching were maintained as described earlier.

### *Helicoverpa armigera* NPV isolates

The temperate, subtropical and tropical HearNPV isolates available with the Division of Entomology, Faculty of Agriculture, SKUAST-Kashmir were used in the present investigation. The temperate HearNPV was previously isolated from *H. armigera* larval population in Kashmir (Sofi 2020). The subtropical and tropical HearNPV isolates were provided by Division of Entomology, Faculty of Agriculture, SKUAST-Jammu and Indian Council of Agricultural Research—National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Bangalore, respectively. The HearNPV isolates were mass multiplied on laboratory reared late 3rd instar healthy *H. armigera* larvae that were inoculated with the crude virus of temperate, subtropical and tropical isolates, individually. All precautions were taken to prevent any cross-contamination. Semi-synthetic diet was prepared and cut into pieces of 1 $cm^2$ and 0.5 cm thick. Virus inoculation was done by the surface contamination method as given by Evans and Shapiro (1997). The larvae were allowed to feed on the virus contaminated diet and subsequently on the healthy diet. The NPV infected moribund larvae were collected and the isolation and purification of HearNPV was conducted as per the methods described by Gani et al. (2021).

### Concentration response and time response bioassays

The temperate, subtropical and tropical HearNPV isolates were bio-assayed on laboratory reared freshly molted second-instar host larvae that were starved for 12 h and then inoculated with six concentrations of HearNPV viz., $1.0 \times 10^2$, $1.0 \times 10^3$, $1.0 \times 10^4$, $1.0 \times 10^5$, $1.0 \times 10^6$ and $1.0 \times 10^7$ OBs/larva, using semi-synthetic

diet as described earlier. For time-response the larvae were inoculated with the median lethal concentration ($LC_{50}$) in order to produce similar mortality rates and facilitate comparisons among the isolates. Serial dilutions with distilled water were performed to achieve the desired concentrations. Ten microliter (µl) of each viral concentration from each viral isolate was spread on each diet plug using a blunt end of glass rod. Control larvae were fed a diet plug sprayed with distilled water and healthy larval extract. The diet plugs were air-dried and then placed individually in the wells of the multi-well plates. One larva was then placed in each well. Larvae having eaten the entire diet plug within 24 h, were transferred to fresh uncontaminated diet and reared at 28 ± 2 °C, 60% RH, and 16L: 8D photoperiod. Larvae that did not consume the entire plug were discarded. Bioassay of each viral concentration and a corresponding experimental control group was replicated three times in case of concentration–response and 7 times in case of time-response with 20 larvae per replicate. The virus induced mortality was recorded daily starting from third day to the tenth day after inoculation. Mortality caused by virus was diagnosed from typical virus disease characteristics and confirmed by microscopic examination through Giemsa staining.

### Field trial

The tomato plants (variety: S2) were grown in the experimental farm of Division of Entomology, Faculty of Agriculture, Wadura, Jammu & Kashmir. The pest incidence was monitored visually and the treatments were started when the pest incidence was noticed. Only a single application of the virus was made to the plants at vegetative period. The crude preparation of the HearNPV from infected larval cadavers was applied at dusk with the motorized knapsack sprayer using the treatments viz. $2 \times 10^4$, $2 \times 10^5$, $2 \times 10^6$, $2 \times 10^7$, $2 \times 10^8$ and $2 \times 10^9$ OBs/plot. The control plots were sprayed with distilled water and healthy larval extract. The treatments were replicated three times in a way that each plant represents a single independent replicate in Randomized Complete-Block Design (RCBD). The plot size was 1 × 2 m and the spacing between the plants was 45 × 30 cm. Observations on the number of live larvae was recorded at one day before spray and then three, seven and ten days after spray. The per cent larval mortality on 3rd, 7th and 10th day and cumulative mortality (%) in treated and control plants were calculated.

### Statistical analysis

All analyses were performed utilizing SPSS software. The data on mortality was analysed through one-way ANOVA and further subjected to post hoc tests for comparison of means. Probit analysis was performed for the calculation of median lethal concentration ($LC_{50} \pm 95\%$ C.L.). Median Survival times ($ST_{50}$) and corresponding confidence interval was calculated using log rank test under Kaplan–Meier analysis. The data on mortality of larvae (%) in the treatment and control plants in the field was analysed by one-way ANOVA and further subjected to post hoc tests.

## Results

### Bioassays

The Kashmir, Jammu and Bangalore isolates of HearNPV were named as HearNPV-IND-K, HearNPV-IND-J and HearNPV-IND-B, respectively. The virulence of three HearNPV isolates against 2nd instar *H. armigera* larvae were determined under laboratory conditions. The mortality in the larvae was confirmed by signs and symptoms of the NPV disease and also by microscopic examination.

The mortality of 2nd instar *H. armigera* larvae with the three different HearNPV isolates at concentrations of $1 \times 10^2$, $1 \times 10^3$, $1 \times 10^4$, $1 \times 10^5$, $1 \times 10^6$ and $1 \times 10^7$ OBs/larva ranged from 13.45–33.40, 25.00–46.70, 33.20–51.60, 43.00–58.50, 51.60–63.35, 56.60–73.25%, respectively, with significant differences between the concentrations in each isolate (HearNPV-IND-K: $F = 108.308$, $df = 7, 16$; $p < 0.05$; HearNPV-IND-J, $F = 37.434$, $df = 7, 16$; $p < 0.05$; HearNPV-IND-B: $F = 28.590$, $df = 7, 16$; $p < 0.05$). It was found that the mortality of NPV infected larvae increased with increase in the concentration with a significant positive correlation (r values; HearNPV-IND-K = 0.591; HearNPV-IND-J = 0.572; HearNPV-IND-B = 0.558) between concentration and mortality. The highest mortality was observed with the HearNPV-IND-K isolate (73.25%), followed by HearNPV-IND-J isolate (61.70%) and HearNPV-IND-B (56.60%) isolate at a concentration of $1 \times 10^7$ OBs/larva. A mortality rate of 0–1.66% was observed in the larvae in control group.

Probit analysis of the mortality data of *H. armigera* larvae with the HearNPV-IND-K, HearNPV-IND-J and HearNPV-IND-B isolates showed median lethal concentration ($LC_{50}$) values of $4.62 \times 10^3$, $5.99 \times 10^4$ and $7.24 \times 10^4$ OBs/larva, respectively, at 10th day post inoculation with significant differences among them as evidenced by non-overlapping confidence limits (Table 1). The ascending order of $LC_{50}$ values were HearNPV-IND-K < HearNPV-IND-J < HearNPV-IND-B isolate. The HearNPV-IND-K isolate had the lowest $LC_{50}$ value of $4.62 \times 10^3$ OBs/larva revealing that it is highly virulent to *H. armigera* larvae than HearNPV-IND-J and HearNPV-IND-B isolates. The contribution of different HearNPV

**Table 1** Concentration response bioassays of three different HearNPV isolates against *Helicoverpa armigera* larvae over a period of 10 days

| Isolates | LC50 (OBs/larva) | Confidence limits 95% | Heterogeneity ($\chi^2$) | Coefficient of determination ($r^2$) | *p* value |
|---|---|---|---|---|---|
| HearNPV-IND-K | $4.62 \times 10^3$ | $2.73 \times 10^3$–$8.84 \times 10^3$ | 45.651 | 0.96 | < 0.001 |
| HearNPV-IND-J | $5.99 \times 10^4$ | $3.15 \times 10^4$–$1.94 \times 10^5$ | 60.144 | 0.95 | < 0.001 |
| HearNPV-IND-B | $7.24 \times 10^4$ | $4.04 \times 10^4$–$2.48 \times 10^5$ | 51.998 | 0.95 | < 0.001 |

isolates towards the mortality of *H. armigera* larvae varied up to the magnitude of 95–96%.

The time response bioassays were conducted using the $LC_{50}$ value of the three HearNPV isolates. The first virus induced mortality was observed on day 3 and continued up to day 10 post-inoculation. After 10th day, no further virus induced mortality was observed. The daily mortality observed on day 1 to day 10 varied from 0.00 to 12.83% with significant differences (HearNPV-IND-K, $F = 9.407$; $df = 9, 12$; $p = 0.00$; HearNPV-IND-J, $F = 5.724$; $df = 9, 12$; $p = 0.01$; HearNPV-IND-B, $F = 1.62$; $df = 9, 12$; $p = 0.369$) between the days in each isolate. The cumulative mortality (%) caused by HearNPV-IND-K, HearNPV-IND-J and HearNPV-IND-B isolates over a period of 10 days was 50.66, 49.12 and 49.02%, respectively (Fig. 1).

The statistical analysis related to the time-response bioassays revealed that the $ST_{50}$ values followed the same pattern as that of $LC_{50}$ in comparing the virus isolates. The $ST_{50}$ values of HearNPV-IND-K, HearNPV-IND-J and HearNPV-IND-B isolates against 2nd instar *H. armigera* larvae were 8.32, 8.94 and 9.10 days, respectively, at their respective $LC_{50}$, with significant differences among them as evidenced by the non-overlapping confidence limits. The HearNPV-IND-K isolate had the highest speed of kill, followed by HearNPV-IND-J and HearNPV-IND-B isolates (Table 2).

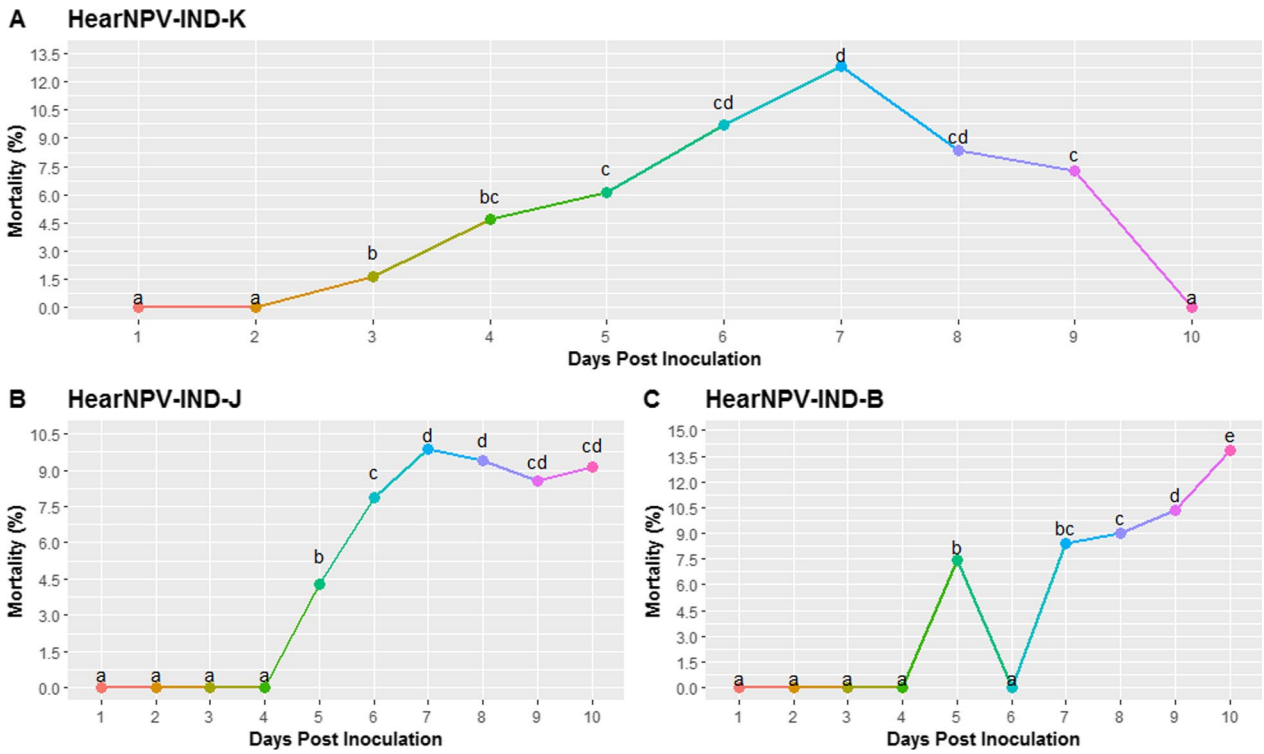


**Fig. 1** Comparative daily mortality (%) of three different HearNPV isolates against 2nd instar *Helicoverpa armigera* larvae in time response bioassays. The first virus induced mortality was observed on day 3 with the HearNPV-IND-K isolate and day 5 with the other two isolates. The virus induced mortality peaked on 7th day with the HearNPV-IND-K and HearNPV-IND-J isolates and 10th day with the HearNPV-IND-B isolate. After 10th day, no further virus induced mortality was observed with all isolates

**Table 2** Time response bioassays of three different HearNPV isolates against *Helicoverpa armigera* larvae at their respective median lethal concentrations

| Isolates | $ST_{50}$ (days) | Confidence limits 95% | Heterogeneity/ degrees of freedom ($\chi^2/df$) | *p* value |
|---|---|---|---|---|
| HearNPV-IND-K | 8.32 | 8.06–8.98 | 131.88/1 | < 0.001 |
| HearNPV-IND-J | 8.94 | 8.57–9.30 | 93.98/1 | < 0.001 |
| HearNPV-IND-B | 9.10 | 8.72–9.47 | 96.38/1 | < 0.001 |

### Field trial

To determine whether the performance of HearNPV isolates under laboratory conditions was entirely reflective of their performance under field conditions, the experiments were conducted on tomato crop at Faculty of Agriculture, Wadura. The cumulative mortality (%) of *H. armigera* larvae with HearNPV-IND-K, HearNPV-IND-J and HearNPV-IND-B isolates at concentrations of $2 \times 10^4$, $2 \times 10^5$, $2 \times 10^6$, $2 \times 10^7$, $2 \times 10^8$ and $2 \times 10^9$ OBs/plot ranged from 22.00–77.82, 20.64–55.11, 11.68–44.42%, respectively, with significant differences {HearNPV-K ($F = 1.034$, $df = 23, 16$; $p < 0.01$) HearNPV-J ($F = 2.638$, $df = 23,16$; $p < 0.01$) HearNPV-B ($F = 2.211$, $df = 23, 16$; $p < 0.01$)} among the concentrations in each isolate. The highest mortality of 77.82% was observed with the HearNPV-IND-K isolate at a concentration of $2 \times 10^9$ OBs/plot. The larval mortality observed in the control plots varied from 3 to 5.43% (Fig. 2). It's concluded that the local HearNPV isolate was more virulent and had the potential for development of effective microbial control agent against *H. armigera* in Kashmir, India.

## Discussion

Baculoviruses play a major role in the suppression of a variety of insect pests in agro-ecosystems. They act as natural regulators of pest population dynamics of economically important insect pests primarily in the order Lepidoptera such as *H. armigera*, *Spodoptera litura* (Fabricius), *Autographa californica* (Speyer) and *Cydia pomonella* (Linnaeus) (Williams et al. 2017). They have been developed as a promising biocontrol agent of insect pests in agriculture due to their specificity, high virulence, persistence in the environment and eco-friendly nature (Malinga and Laing 2022). The HearNPV has been found widely in nature and the local isolates was mass produced and applied to different crops for management of *H. armigera* in India. The commercial formulations of HearNPV viz. Helicovex®

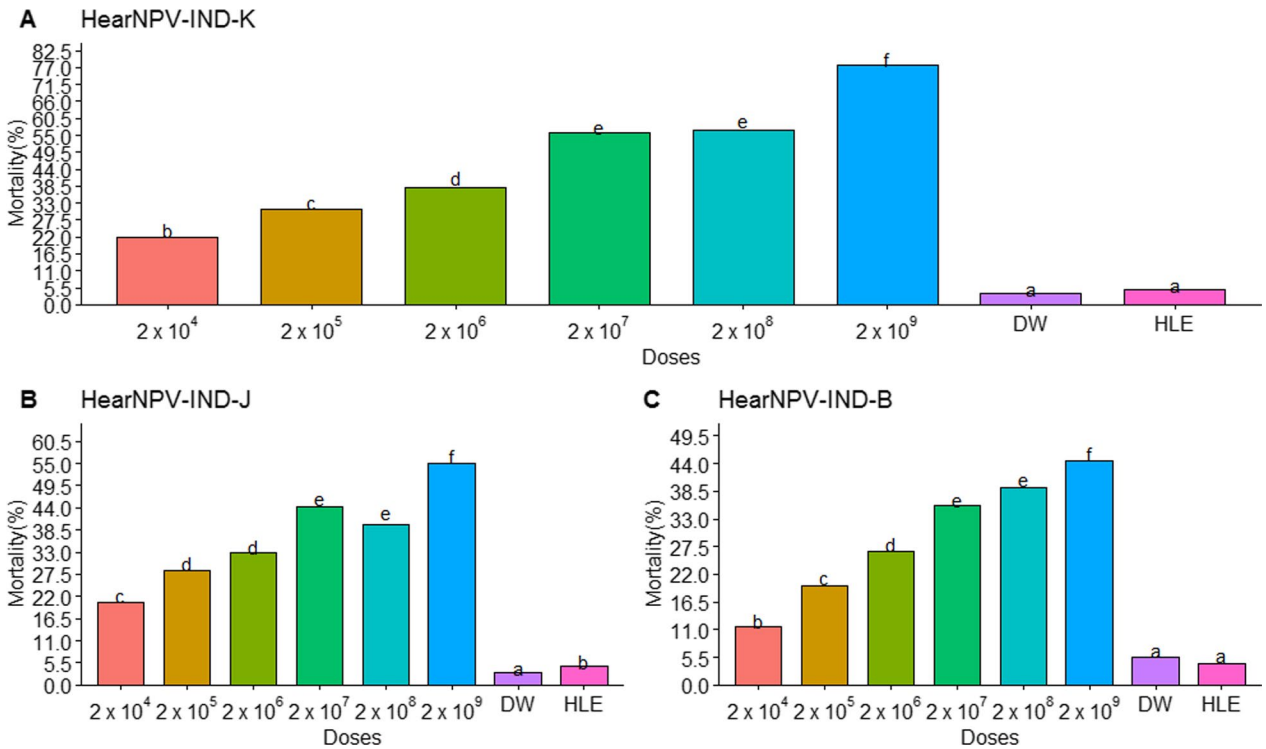


**Fig. 2** Comparative mortality (%) of three different HearNPV isolates against *Helicoverpa armigera* larvae on tomato plants under field conditions (DW = Distilled water; HLE = Healthy larval extract). The highest mortality of 77.82% was observed with the HearNPV-IND-K isolate at a concentration of $2 \times 10^9$ OBs/plot

(Corteva Agriscience, USA; Andermatt Biocontrol, Brazil), Heligen® (Agbitech LLC., USA), Gemstar® (Certis USA) and Diplomata (Koppert, Brazil) are being used for eco-friendly management of *H. armigera* in different parts of the world.

The HearNPV isolates used in this study were found virulent to the 2nd instar *H. armigera* larvae. The HearNPV-IND-K isolate was found highly effective, followed by HearNPV-IND-J and HearNPV-IND-B isolates against *H. armigera* larvae. The HearNPV was reported to cause 90–100% mortality against neonate and 2nd instar *H. armigera* larvae (Ginting et al. 2018). The HearNPV was more effective against early instar larvae of *H. zea* and caused 99% mortality in 1st–3rd larval instars in 4–6 days and only 35% mortality in 4th and 5th larval instars (Black et al. 2022). The variation in biological activity of NPV isolates reported in this study and also by other authors is attributed to the genotype of the virus and the strain of the host (Kitchin and Bouwer 2018). Further, insects have evolved methods to inhibit or block virus replication and the early instars are more susceptible than late instars due to the increased presence of anti-microbial peptides, gut proteases, midgut-based mechanism and developmental resistance (Sauer et al. 2021). The $LC_{50}$ values of the HearNPV-IND-K, HearNPV-IND-J and HearNPV-IND-B isolates against the 2nd instar *H. armigera* larvae were $4.62 \times 10^3$, $5.99 \times 10^4$ and $7.26 \times 10^4$ OBs/larva, respectively. Our findings corroborate with many other studies on differences in the $LC_{50}$ values of NPVs against the host larvae (Ali et al. 2018). It was found that the HearNPV-IND-K isolate had the highest speed of kill, followed by HearNPV-IND-J and HearNPV-IND-B isolates. Obtained results revealed that the virulence of the HearNPV varied with respect to the isolate and the HearNPV-IND-K was found highly effective against the 2nd instar *H. armigera* larvae. It is a well-established fact that the baculoviruses isolated from the same species at different locations frequently vary in their biological activity *i.e.,* pathogenicity and virulence (Erlandson 2009). These differences in biological activity are attributed to a number of factors. The significant differences in virus infectivity can be associated with virus structural changes or genetic composition, particularly the presence or absence of key genes and their functions (Fleischmann 1996). The variation in the pathogenicity of baculoviruses isolated from different hosts from different parts of the world has also been confirmed through functional genomics studies (Eberle et al. 2009). Therefore, the HearNPV-IND-K isolate may be genetically heterogeneous from the other two isolates. Another reason for the highest efficacy of HearNPV-IND-K isolate against the *H. armigera* was that this isolate was isolated from local host population and hence is well established and adapted in agro-ecological conditions of Kashmir.

The cumulative mortality (%) of *H. armigera* larvae with HearNPV-IND-K, HearNPV-IND-J and HearNPV-IND-B isolates at different concentrations ranged from 22.00–77.82, 20.64–55.11, 11.68–44.42%, respectively, with significant differences between the concentrations in each isolate under field conditions. The highest mortality of 77.82% was observed with the HearNPV-IND-K isolate at a concentration of $2 \times 10^9$ OBs/plot. The field application of HearNPV at $1 \times 10^{12}$ OBs $ha^{-1}$ resulted in effective management of *H. armigera* in India and Thailand on a variety of crops (Arrizubieta et al. 2016). Previous attempts to use HearNPV for management of *H. armigera* have usually met with success and it was found that the local isolates were more infective against host populations presumably as a result of continuous host–pathogen coevolution (Escribano et al. 1999).

## Conclusions

The results indicated that the local isolate of HearNPV was likely better suited for the development as a biological insecticide for eco-friendly management of *H. armigera* in Kashmir. Future studies should be focussed towards the development of HearNPV-IND-K based formulations against *H. armigera* and their commercialization for conventional and organic farming in Kashmir.

**Acknowledgements**
The authors are grateful to Professor & Head, Division of Entomology, SKUAST-Kashmir for providing the facilities for successful implementation of this research work. Our thanks are due to Prof. Subhash Chander, Director, ICAR-National Research Centre for Integrated Pest Management, New Delhi, India for suggestions and guidance during the course of investigation.

**Author contributions**
SM, MG, TH and IS have planned the outline of this research, designed the methodology and prepared the manuscript. FJW, SM and GBE assisted in the drafting of the manuscript and statistical analyses. SM, MY, MG and MAM conducted the laboratory and field experiments. All the authors read and approved the final manuscript.

**Funding**
The work was supported by the Division of Entomology, Faculty of Agriculture, SKUAST-Kashmir. No external funding was received.

**Availability of data and materials**
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

**Author details**
[1]Division of Entomology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu & Kashmir, India. [2]Department of Zoology, Aligarh Muslim University, Uttar Pradesh, Aligarh, India. [3]Division of Agricultural StatisticsFaculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu & Kashmir, India. [4]Department of Molecular Biology and Genetics, Faculty of Science, Erzurum Technical University, Erzurum, Turkey.

Received: 23 January 2023 Accepted: 6 April 2023
Published online: 15 April 2023

**References**

Ali G, Abma-Henkens MH, Van der Werf W, Hemerik L, Vlak JM (2018) Genotype assembly, biological activity and adaptation of spatially separated isolates of *Spodoptera litura* nucleopolyhedrovirus. J Invertebr Pathol 153:20–29
Arora R, Sharma HC, Dreissche EV, Sharma KK (2005) Biological activity of lectins from grain legumes and garlic against the legume pod borer. Helicoverpa Armigera J SAT Agric Res 1(1):1–3
Arrizubieta M, Simon O, Williams T, Caballero P (2016) Determinant factors in the production of a co-occluded binary mixture of *Helicoverpa armigera* alphabaculovirus (HearNPV) genotypes with desirable insecticidal characteristics. PLoS ONE 11(10):e0164486
Black JL, Lorenz GM, Cato AJ, Bateman NR, Seiter NJ (2022) Efficacy of *Helicoverpa Armigera* nucleopolyhedrovirus on soybean for control of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in Arkansas Agriculture. Insects 13(1):91
Chitneni SK (2008) Development and evaluation of diagnostic tools for nucleopolyhedroviruses (NPVs) infecting major lepidopteran pests of legume crops in the Semi-Arid Tropics (Doctoral dissertation, Acharya Nagarjuna University)
Eberle KE, Sayed S, Rezapanah M, Shojai-Estabragh S, Jehle JA (2009) Diversity and evolution of the *Cydia pomonella* granulovirus. J Gen Virol 90(3):662–671
Erlandson MA (2009) Genetic variation in field populations of baculoviruses: mechanisms for generating variation and its potential role in baculovirus epizootiology. Virol Sin 24(5):458–469
Eroglu GB (2022) Pest insects and their biological control. In: Abdurakhmonov IY (ed) Cotton [Internet]. London: IntechOpen; [cited 2023 Jan 17]. Available from: https://www.intechopen.com/chapters/80016. https://doi.org/10.5772/intechopen.101980
Escribano A, Williams T, Goulson D, Cave RD, Chapman JW, Caballero P (1999) Selection of a nucleopolyhedrovirus for control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae): structural, genetic, and biological comparison of four isolates from the Americas. J Econ Entomol 92(5):1079–1085
Evans HF, Shapiro M (1997) Viruses. In: Lacy LA (ed) Manual of techniques in insect pathology. Academic Press, San Diego, pp 17–54
Fite T, Tefera T, Negeri M, Damte T, Sori W (2020) Evaluation of *Beauveria bassiana*, *Metarhizium anisopliae*, and *Bacillus thuringiensis* for the management of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) under laboratory and field conditions. Biocontrol Sci Technol 30(3):278–295
Fleischmann Jr WR (1996) Viral genetics. Medical microbiology. 4th edn
Gani M, Senger S, Lokanath S, Saini P, Bali K, Gupta R, Sivaprasad V, Jehle JA, Wennmann JT (2021) Patterns in genotype composition of Indian isolates of the *Bombyx mori* nucleopolyhedrovirus and *Bombyx mori* bidensovirus. Viruses 13(5):901
Ginting S, Santoso T, Munara YK, Anwar R, Sudirman LI (2018) Pathogenicity of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) and *Lecanicillium* sp. against *Helicoverpa armigera*. J Biopes 11:128–135
Groner A (1986) Specificity and safety of baculoviruses. Biol Baculoviruses 1:177–202
Gupta RK, Raina JC, Arora RK, Bali K (2010) Selection and field effectiveness of nucleopolyhedrovirus isolates against *Helicoverpa armigera* (Hubner). Int J Virol 6(3):164–178
Jeyarani S, Sathiah N, KaruppucHamy P (2010) Field efficacy of *Helicoverpa armigera* nucleopolyhedrovirus isolates against *H. armigera* (Hubner) (Lepidoptera: Noctuidae) on cotton and chickpea in Tamil Nadu. Plant Prot Sci 46(3):116–122
Kalia V, Chaudhari S, Gujar GT (2001) Optimization of production of nucleopolyhedrovirus of *Helicoverpa armigera* throughout larval stages. Phytoparasitica 29(1):23–28
Kitchin D, Bouwer G (2018) Significant differences in the intra-host genetic diversity of *Helicoverpa armigera* nucleopolyhedrovirus dnapol after serial in vivo passages in the same insect population. Arch Virol 163:713–718
Koch RL, Hutchison WD, Venette RC, Heimpel GE (2003) Susceptibility of immature monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae: Danainae), to predation by *Harmonia axyridis* (Coleoptera: Coccinellidae). Biol Control 28(2):265–270
Malinga LN, Laing MD (2022) Efficacy of biopesticides in the management of the cotton bollworm, *Helicoverpa armigera* (Noctuidae), under field conditions. InSects 13(8):673
Martins T, Oliveira L (2005) Occurrence and characterization of a nucleopolyhedrovirus from *Spodoptera littoralis* (Lepidoptera: Noctuidae) isolated in the Azores. J Invertebr Pathol 89:185–192
Musser FR, Greene JK, Herbert DA, Jones M, Kerns D, Lorenz GM, Stewart SD (2015) Update on bollworm pyrethroid resistance monitoring. In: Beltwide cotton conferences, pp 5–7
Sauer AJ, Fritsch E, Undorf-Spahn K, Iwata K, Kleespies RG, Nakai M, Jehle JA (2021) Cross-resistance of the codling moth against different isolates of Cydia pomonella granulovirus is caused by two different but genetically linked resistance mechanisms. Viruses 13(10):1952
Sofi BA (2020) Evaluation of nucleopolyhedrovirus (NPV) for management of the tomato fruit borer, *Helicoverpa armigera* (Hubner) M.Sc. Thesis submitted to Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir, pp18–38
Williams T, Bergoin M, van Oers MM (2017) Diversity of large DNA viruses of invertebrates. J Invertebr Pathol 147:4–22
Wubneh WY (2016) Biological control of chickpea pod borer, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae): a global concern. World Sci News 2(45):92–110

## Publisher's Note