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Research Article

Evaluation of native nucleopolyhedrovirus isolates for eco-friendly management of tomato fruit borer, *Helicoverpa armigera* (Hubner)

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ABSTRACT: The tomato fruit borer, *Helicoverpa armigera* (Hubner), is a serious insect pest of many crops in India and several other countries. The native *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) isolates were characterised and evaluated for eco-friendly management of the *H. armigera* in the temperate region of Jammu and Kashmir, India. The biological characterisation of three NPV isolates, one each from North Kashmir, Central Kashmir, and South Kashmir were conducted under laboratory conditions, and the efficacy of the most potent isolate for the management of *H. armigera* was evaluated under field conditions. The natural incidence of HearNPV at different locations was 1.66 to 5.50 per cent. The HearNPV-IND-K was closely related to the HearNPV Turkey and China isolates as per the phylogenetic analysis based on the *polh*, *lef-8* and *lef-9* genes. A mortality of 28.33 - 73.33 per cent was observed with HearNPV isolates at different concentrations against 2nd instar *H. armigera* larvae under laboratory conditions. The LC_{50} and ST_{50} values of three HearNPV isolates from Kashmir against *H. armigera* larvae varied from 9.79×10^3 to 9.46×10^4 OBs/larvae and 8.10 to 9.16 days, respectively. The LC_{50} and ST_{50} values of HearNPV-IND-NK isolate was significantly lower as compared to other two isolates and it caused 30.66 - 69.33 per cent mortality in *H. armigera* larvae on tomato plants under field conditions. The HearNPV has a considerable potential for eco-friendly management of *H armigera* and can be used as a promising viable alternative to chemical insecticides.

KEYWORDS: Baculovirus, *Helicoverpa armigera*, LC_{50}, nucleopolyhedrovirus, ST_{50}, tomato

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INTRODUCTION

The tomato fruit borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), is a polyphagous, cosmopolitan, and extremely mobile insect pest that continues to spread to new habitats worldwide (Koch *et al.*, 2003). It is the most destructive insect pest that targets more than 300 plant species from a large variety of fruit, oilseed, fibre, fodder and horticultural crops (Arora *et al.*, 2005). The pest is widely distributed in Asia, Africa, Australia and Mediterranean Europe (Sharma *et al.*, 2005) and causes annually estimated global economic losses of over 3 billion US dollars (Riaz *et al.*, 2021). It was reported from more than 20 crops and 180 wild plant species in India (Sharma *et al.*, 2005). It is the major pest of tomato and feeds on every stage of the crop from seedling to maturity in most agro-ecological areas of India, from the Andaman Nicobar Islands to Jammu and Kashmir (Singh *et al.*, 2002; Shankar *et al.*, 2014). *H. armigera* has been estimated to cause both quantitative and qualitative damage to the tomato crop by 40-50 per cent (Pareek & Bhargava, 2003; Ambule *et al.*, 2015).

Infestations of *H. armigera* exceeding the economic threshold are usually regulated by synthetic insecticides; however, the pest's ability to adopt new environments in a short period has intensified the inappropriate use of insecticides resulting in the development of resistance to different groups of insecticides (Armes *et al.*, 1992; Musser *et al.*, 2015; Eroglu, 2022). For safety concerns over the

use of chemical insecticides and to provide target-specific insecticidal action, a greater number of natural enemies must be explored and utilised in biological control programs. The use of entomopathogens as biopesticides has been encouraged and promoted by national and international pest management authorities (Ahmad, 2003; Martins *et al.*, 2005). Baculoviruses (Baculoviridae) are DNA viruses that have been reported from three orders of class Insecta, viz. Lepidoptera, Hymenoptera and Diptera. A good number of baculoviruses are used as biopesticides for eco-friendly management of the insect pests of agricultural, horticultural and forest crops (Lacey *et al.*, 2002; Abhilash *et al.*, 2016; Pavan *et al*., 2024; Gundreddy *et al*., 2024).

The *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) is quite effective against *H. armigera* larvae and is an important component of the Integrated Pest Management (IPM) program in many crops (Kalia *et al.*, 2001). The widespread use of HearNPV in India is well known, and demand for commercial formulation of this virus is growing, but only subtropical or tropical regions have been explored hitherto. Local strains of biocontrol agents are often favoured for resilience, adaptability, and effectiveness under a given agro-ecosystem. The concentration response and time response bioassays need to be thoroughly evaluated when comparing the response variability of populations to NPV infection and developing them as biopesticides (Kaur *et al*., 2021Grant & Bouwer, 2009). The exploration of local isolates of HearNPV from the temperate zone of Jammu and Kashmir, India, has ample scope for widespread multiplication and commercial use as novel biopesticides. In accordance with the above, the native isolates of HearNPV were collected and evaluated with the rationale to utilise them for eco-friendly management of *H. armigera* in the temperate region of Jammu and Kashmir, India.

MATERIALS AND METHODS

Survey

The exploratory surveys for the presence of native HearNPV isolates from *H. armigera* larvae were conducted during 2020 April–July in the North, Central and South Kashmir. The infected larvae were collected individually in micro-centrifuge tubes (2 ml) from different crops, viz., tomato, chickpea, field pea, beans, etc. and brought to the laboratory for isolation of Occlusion Bodies (OBs). The NPV-infected larvae and the healthy larvae on a particular crop and at a particular place and time were counted. The percentage infection was calculated by using the formula:

$$\frac{\text{No. of infected larvae} \times 100}{\text{Total no. of larvae } (\text{healthy} + \text{infected})}$$

Establishment of laboratory culture of *H. armigera*

The culture of *H. armigera* in the laboratory was established and maintained from field-collected larvae from different locations in Kashmir. The larvae were kept individually in sterile plastic vials capped with ventilated lids on the semi-synthetic diet as described by Chitneni (2008). The diet was provided regularly to ensure enough food and care was taken for sanitation. The pupae for culture maintenance were transferred into plastic jars (7cm × 5cm × 12cm) for adult emergence. The emerging adults were released into oviposition cages (45cm length: 30cm diameter) and lined internally with muslin cloth for egg laying. The larvae on hatching were reared together until the second instar stage and later reared individually in glass vials.

Virus identification and phylogenetic analysis

The HearNPV was isolated and purified as per the methods described by Gani *et al* (2017). The crude OBs were centrifuged at a speed of 100 × g for 10 s to remove any contaminant. The supernatant was removed carefully; the pellet was washed with 0.1 per cent Sodium Dodecyl Sulfate (SDS) and centrifuged as described above. The supernatants were combined and centrifuged at a speed of 15000 × g for 5 min using a Remi R8C rotor to pellet the virus. The virus pellet was resuspended in 1 ml of distilled water and stored at −20°C for further studies. The HearNPV was identified through light microscopy and Polymerase Chain Reaction (PCR) amplification of *late expression factor 8* (*lef-8*), *late expression factor 9* (*lef-9*) and *polyhedrin* (*polh*) genes as per the methods described by Gani *et al* (2021). The gene sequences were aligned in the BioEdit (7.1.3.0) program, and the phylogenetic tree was constructed by the Maximum Likelihood method in the MEGA 11 program.

The HearNPV was mass multiplied on laboratory-reared 4th instar *H. armigera* healthy larvae by inoculating *per os* (through the mouth) with the respective strain of virus. For each virus strain, NPV was mass multiplied on 250 larvae in 10 batches, with twenty-five fourth instar larvae in each batch. The moribund larvae with typical NPV symptoms were collected and subjected to virus isolation, as the said larvae are more suitable for handling and mass production of virus than dead ones.

Biological characterisation

The concentration response bioassays of HearNPV isolates were conducted against laboratory-reared freshly moulted 2nd instar *H. armigera* larvae that were starved for 12 h and then inoculated with six concentrations of HearNPV, viz., 1.0×10^2, 1.0×10^3, 1.0×10^4, 1.0×10^5, 1.0×10^6 and 1.0×10^7 OBs/larva. For time-response, the larvae were inoculated with a concentration of 1.0×10^4 OBs/larva to cause similar mortality rates in order to make comparisons among the

isolates. The larvae were inoculated with 10 µl of the viral concentration of each HearNPV isolate individually by the diet surface contamination method. Control larvae were fed on a diet plug with distilled water only. Larvae, having eaten the entire piece of diet within 24 h, were transferred to a fresh uncontaminated diet and reared at 25 ± 2ºC, 60 per cent R.H. and 16L: 8D photoperiod in the multi-well insect bioassay trays. The larvae that did not consume the entire diet within 24 h were discarded. The experiment was replicated three times in the case of concentration-response and seven times in the case of time-response, with 20 larvae per replicate. The mortality caused by NPV was recorded daily from the 2nd to the 10th day after inoculation.

Field trial

The efficacy of unformulated HearNPV was evaluated on tomato plants (variety: S1) under polyhouse conditions (Plate 1). Each tomato plant was artificially infested with twenty-five 2nd instar *H. armigera* larvae one day before spraying to allow the larvae to establish on the plants. The larvae were released on the top half of the tomato plants containing tender leaves. Only a single application of the virus was made to the plants. The control plots were sprayed with distilled water. The crude preparation of the HearNPV @ 2 x 10^4, 2 x 10^5, 2 x 10^6, 2 x 10^7, 2 x 10^8 and 2 x 10^9 OBs/plot from infected larval cadavers was applied at dusk with the motorised knapsack sprayer with hollow-cone nozzle. The experiment included three replications per treatment with five plants per replication in a Randomised Complete Block Design (RCBD). The plot size was 1 x 2 m² and the spacing between the plants was 45 x 30 cm² (variety: S1). The host larvae were counted immediately before spray and at regular intervals for 10 days. The mortality (%) of the larvae in treated and control plants was calculated.



Plate 1: Evaluating the efficacy of *Helicoverpa armigera* nucleopolyhedrovirus on tomato plants under polyhouse conditions

Data analysis

SPSS version 16 was used for statistical analysis of data. The mortality data were analysed through one-way Analysis of Variance (ANOVA), and means were subjected to post hoc tests for comparison. Median lethal concentration (LC_{50}) and corresponding confidence intervals were calculated through Probit analysis. The log-rank test under Kaplan-Meier analysis was used to calculate the median survival times (ST_{50}) and corresponding confidence intervals.

RESULTS

The typical symptoms of NPV infection, such as discolouration, slow movement and rupture of the cuticle, were observed in a few *H. armigera* larvae that were collected from different locations of Kashmir during 2020. A microscopic examination of the haemolymph and tissue smears of the infected larvae showed the presence of polyhedral under a phase contrast microscope (@1000 magnification). The virus was also identified through PCR amplification of *late expression factor 8* (*lef-8*), *late expression factor 9* (*lef-9*) and *polyhedrin* (*polh*) genes. The North, Central and South Kashmir isolates of *H. armigera* were named as HearNPV-IND-NK, HearNPV-IND-CK and HearNPV-IND-SK, respectively, as per the most commonly used methods (Bonsall, 2004; Fauquet & Fargette, 2005; Erlandson, 2009). The HearNPV-IND-K was closely related to the HearNPV Turkey and China isolates as per the phylogenetic analysis based on the *polh*, *lef-8* and *lef-9* genes (Figure 1).

The natural infection of NPV in *H. armigera* larvae varied from 3.33–5.5 per cent, 1.66–4.16 per cent and 2.38–4.65 per cent in North, Central and South Kashmir, respectively. North Kashmir had the highest percentage of NPV infection (4.33 per cent), followed by Central Kashmir (3.19 per cent) and South Kashmir (2.49 per cent). Overall, the NPV infection in *H. armigera* larvae varied from 1.66–5.50 per cent in the Kashmir valley.

The mortality of *H. armigera* larvae with three different NPV isolates at a dosage of 1 × 10^2, 1 × 10^3, 1 × 10^4, 1 × 10^5,1

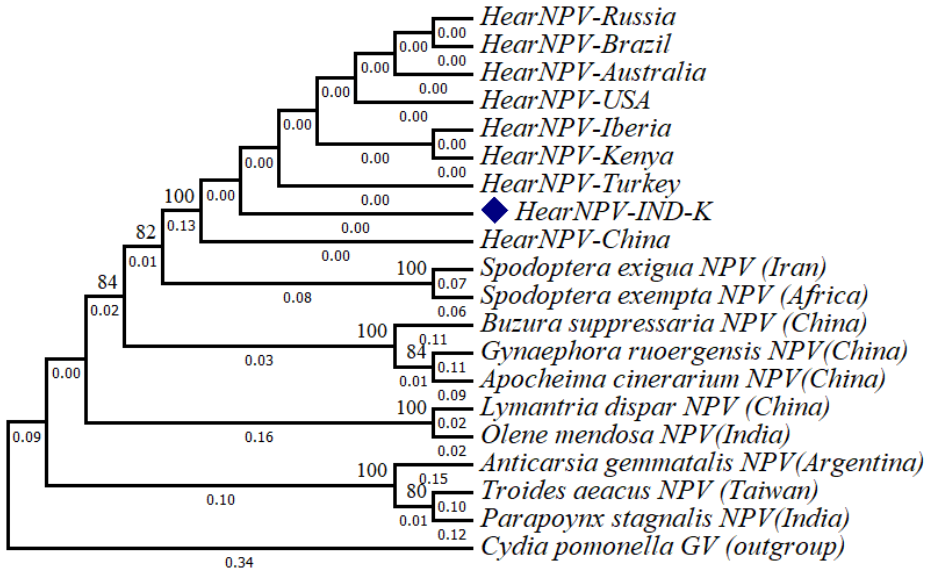


Figure 1. Phylogenetic analyses of already reported Helicoverpa NPVs with HearNPV-IND-K using concatenated nucleotide sequences of the partial *lef-8*, *lef-9* and *polh* genes. *Cydia pomonella* GV was used as an outgroup. Bootstrap values are indicated at the nodes of the tree. A phylogenetic tree was drawn using the Maximum Likelihood method in the MEGA 11 program.

Table 1. Concentration response bioassays of three nucleopolyhedrovirus isolates against 2nd instar *Helicoverpa armigera* larvae

Isolate	LC_{50} (OBs/larva)	95 % confidence limits	Slope	Intercept	χ^2	r^2	p value
HearNPV-IND-NK	9.79×10^3	3.57×10^2 - 5.32×10^5	0.18	-0.74	0.10	0.8782	0.81
HearNPV-IND-CK	9.46×10^4	5.70×10^3 - 7.62×10^6	0.20	-1.00	0.11	0.9435	0.96
HearNPV-IND-SK	4.26×10^4	2.98×10^3 - 9.95×10^6	0.17	-0.79	0.03	0.8954	0.92

$\times 10^6$ and 1×10^7 OBs/larva ranged from 28.33–73.33 per cent, with significant differences (HearNPV-IND-NK: F = 26.058, df = 6, 14; $p<0.05$; HearNPV-IND-CK: F = 16.50, df = 6, 14; $p<0.05$; HearNPV-IND-SK: F = 19.58, df = 6, 14; $p<0.05$) between the concentrations in each isolate. The mortality of larvae increased with an increase in the NPV concentration, with a significant positive correlation (r values; HearNPV-IND-NK = 0.558; HearNPV-IND-CK = 0.589; HearNPV-IND-SK = 0.537) between concentration and mortality. The maximum mortality was observed with the HearNPV-IND- NK isolate (73.33 per cent), followed by HearNPV-IND-SK isolate (66.66 per cent) and HearNPV-IND-CK (56.66 per cent) isolate at a concentration of 1×10^7 OBs/larva.

The median lethal concentration (LC_{50}) values of HearNPV-IND-NK, HearNPV-IND CK, and HearNPV-IND-SK isolates against 2nd instar larvae were 9.79×10^3, 9.46×10^4 and 4.26×10^4 OBs/larvae, respectively, with significant differences between them owing to the non-overlapping confidence limits (Table 1). The HearNPV-IND-NK isolate had the lowest LC_{50} value of 9.79×10^3 OBs/larva, suggesting that it was highly virulent to *H. armigera* larvae as compared to HearNPV-IND-CK and HearNPV-IND-SK isolates.

The time response bioassays of *H. armigera* larvae with three different NPV isolates at a dosage of 1×10^4 OBs/larva showed that the first NPV induced mortality started from day 2 and continued up to day 10 post-inoculation. After 10th day, no further NPV-induced mortality was recorded. The daily mortality observed up to 10 days varied from 0.00–10.71 per cent with significant differences (HearNPV-IND-NK, F = 4.75; df = 1, 12; $p<0.01$; HearNPV-IND-CK, F = 6.58; df = 1, 12; $p<0.02$; HearNPV-IND-SK, F = 9.20; df = 1, 12; $p<0.01$) between the days in each isolate. The cumulative mortality per cent caused by HearNPV-IND-NK, HearNPV-IND-CK and HearNPV-IND-SK isolates during 10 10-day periods was 52.78 ± 15.32, 41.38 ± 12.42 and 46.38 ± 10.54, respectively (Figure 2).

The analysis of the mortality data of time-response bioassays revealed that the ST_{50} values follow the same trend as that of LC_{50} in comparing the HearNPV isolates. The ST_{50} values of HearNPV-IND-NK, HearNPV-IND-CK and HearNPV-IND-SK isolates against *H. armigera* larvae were 8.10, 9.16 and 8.89 days, respectively, with significant differences between them owing to the non-overlapping confidence limits. The speed of kill was highest with the HearNPV-IND-NK isolate, followed by HearNPV-IND-SK and HearNPV-IND-CK isolates (Table 2).

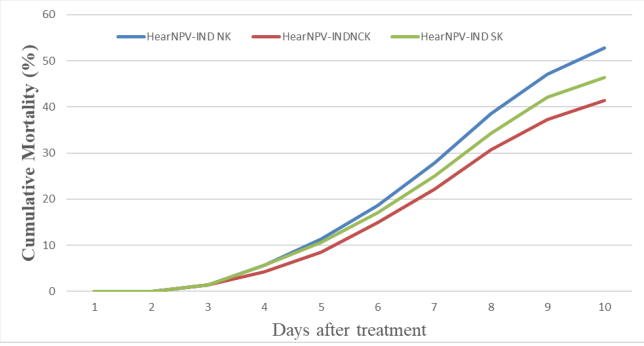


Figure 2. Comparative cumulative mortality (%) of three nucleopolyhedrovirus isolates against *Helicoverpa armigera* larvae with the concentration of 1.0×10^4 OBs/larva in time response bioassays. The highest mortality (52.78%) was caused by the HearNPV-IND-NK isolate over 10 days.

Table 2. Time response bioassays of three nucleopolyhedrovirus isolates against 2nd instar *Helicoverpa armigera* larvae with the concentration of 1.0×10^4 OBs/larva

Isolate	ST_{50} (days)	95 % confidence limits	χ^2/df	p value
HearNPV-IND- NK	8.10	7.59-9.19	115.48/1	<0.001
HearNPV-IND- CK	9.16	8.83-9.64	96.83/1	<0.001
HearNPV-IND- SK	8.89	8.58-9.37	89.84/1	<0.001

The results of experiments on biological characterisation showed that HearNPV-IND- NK isolate had the lowest LC_{50} (9.79×10^3 OBs/larva) and ST_{50} (8.10 days) in comparison to HearNPV-IND-CK and HearNPV-IND-SK isolates. Therefore, the HearNPV-IND-NK isolate was selected for the field experiments. The mortality of 2nd instar *H. armigera* larvae with HearNPV-IND-NK isolate at a dosage of 2×10^4, 2×10^5, 2×10^6, 2×10^7, 2×10^8 and 2×10^9 OBs/plot ranged from 30.66–69.33 per cent, with significant differences ($F = 58.13$, $df = 6, 14$; $p<0.01$) between them. The highest mortality of 69.33 per cent was observed at a concentration of 2×10^9 OBs/plot. The mortality of 2.66 per cent was observed in the control plot (Table 3).

DISCUSSION

The presence of NPV infected *H. armigera* larvae under field conditions is well documented, and the virus has been isolated from many countries (Figueiredo *et al.*, 1999). However, the present study established that the NPVs exist in the natural populations of *H. armigera* in the temperate region of Kashmir. Nevertheless, a low level of HearNPV infection varying from 2.49–4.33 per cent was observed. The *H. armigera* larval population from North Kashmir had the highest percentage of NPV infection, followed by South Kashmir and Central Kashmir. The differences in the occurrence of HearNPV infection at different locations or regions indicated that the baculovirus outbreaks are density dependent and occur during severe outbreaks of insect populations (Gupta *et al.*, 2016). In the present study, we observed a high larval population of the *H. armigera* on the tomato crop from the Kupwara region of North Kashmir that might have favoured the presence of a high level of NPV infection.

Table 3. Efficacy of unformulated nucleopolyhedrovirus against 2nd instar *Helicoverpa armigera* on tomato plants under field conditions

Isolate	Concentration (OBs/plot)	Mortality (No.) (Mean ± SE) *	Mortality (%) (Mean ± SE) *
HearNPV-IND-NK	2×10^4	7.66 ± 0.33^{b}	30.66 ± 1.33^{b}
	2×10^5	8.66 ± 0.33^{bc}	34.66 ± 1.33^{bc}
	2×10^6	10.66 ± 0.66^{cd}	42.66 ± 2.66^{cd}
	2×10^7	12.33 ± 0.33^{de}	49.33 ± 1.33^{de}
	2×10^8	14.3 ± 1.20^{e}	57.33 ± 4.80^{e}
	2×10^9	17.33 ± 0.88^{f}	69.33 ± 3.52^{f}
	Control	1.00 ± 0.57^{a}	2.66 ± 1.33^{a}

*Mean ± SE followed by different letters within the same column are significantly different at $p<0.05$; SE = Standard error of mean; Control = Distilled water
*No. of larvae/ replicate = 25

The mortality of *H. armigera* larvae with three Helocoverpa NPV isolates at different concentrations ranged from 28.33–73.33 per cent under laboratory conditions, the differences being significant between the concentrations in each isolate. The HearNPV-IND- NK isolate was found to be highly virulent, followed by HearNPV-IND-SK and HearNPV-IND-CK isolates. It was found that the larval mortality increased with an increase in the number of OBs. Our results are consistent with those of Nawaz *et al.* (2019), who also reported that an increase in the number of OBs led to an increase in the mortality of *H. armigera* larvae. The mortality of 18.3 to 100 per cent was observed in *H. armigera* larvae with different concentrations of HearNPV (Zhang *et al.*, 2015). The highest larval mortality was observed at a concentration of 10^8 OBs/ml and selected as the optimum concentration for mass production of HearNPV in 3rd and 4th instar *H. armigera* larvae (Gopali & Lingappa, 2001). The variation in biological activity of NPVs depends on the genotype of the virus and the strain of the host (Crook, 1981; Erlandson, 2009; Barrera *et al.*, 2011; Kitchin & Bouwer, 2018).

The HearNPV-IND-NK, HearNPV-IND-CK and HearNPV-IND-SK isolates used in this study were found

effective in causing mortality to the 2nd instar *H. armigera* larvae. The LC_{50} values of the HearNPV-IND-NK, HearNPV-IND-CK and HearNPV-IND-SK isolates against the *H. armigera* larvae were 9.79×10^3, 9.46×10^4 and 4.26×10^4 OBs/larva, respectively. Our findings are in line with many other studies on differences in the virulence or LC_{50} values of baculovirus isolates against the host larvae (Ali *et al.*, 2018). The LC_{50} values of 50 and 110 OBs were reported against the 1st and 2nd instar *H. armigera* larvae, respectively (Teakle *et al.*, 1985). Arrizubieta *et al.* (2014) reported non-significant differences in the LC_{50} values among seven isolates of HearNPV from the Iberian Peninsula against a *H. armigera* colony from Oxford (UK).

The analysis of the mortality data of the time-response bioassays showed that the ST_{50} values follow the same trend as that of LC_{50} in comparing three new HearNPV isolates. The ST_{50} values of HearNPV-IND-NK, HearNPV-IND-CK and HearNPV-IND-SK isolates against *H. armigera* larvae were 8.10, 9.16 and 8.89 days, respectively, with significant differences between them as per the non-overlapping confidence limits. The HearNPV-IND- NK isolate was found to have the highest killing speed, followed by HearNPV-IND-SK and HearNPV-IND-CK isolates. The LT_{50} values ranged from 2.8 to 6.8 days and 2.8 to 11.9 days for the South African and Kenyan isolates of HearNPV, respectively, against the first four larval instars of *H. armigera* (Ogembo *et al.*, 2005).

The results of our study revealed that the virulence of HearNPV is isolate dependent, and the HearNPV-IND-NK isolate is highly infective against *H. armigera* larvae. The baculoviruses isolated from the same insect species at different locations frequently vary in their pathogenicity and virulence due to a number of factors (Cory & Myers, 2003; Erlandson *et al*., 2007). The significant differences in baculovirus infectivity can be associated with structural or functional genomics (Crook, 1981; Fleischmann, 1996; Eberle *et al.*, 2009). Therefore, the HearNPV-IND-NK isolate may be structurally or functionally different from the other two NPV isolates, and the same needs to be elucidated.

To determine the field efficacy of highly infective HearNPV-IND-NK, experiments were conducted on a tomato crop that was artificially infested with 2nd instar *H. armigera* larvae under field conditions. The mortality of 2nd instar *H. armigera* larvae with different concentrations of HearNPV-IND-NK isolate ranged from 30.66–69.33 per cent, with significant differences between the concentrations. The highest mortality of 69.33 per cent was observed at a concentration of 2×10^9 OBs/plot. The previous studies have also reported significant reductions in *H. armigera* larval infestations after HearSNPV applications both in field-grown and protected tomato crops (Moore *et al.*, 2004; Ravi *et al.*, 2008; Gupta *et al.*, 2010). The use of NPV for the management of *H. armigera* is quite effective, and it has been reported that the local isolates are more infective than exotic isolates against host populations (Manzoor *et al*., 2023). The excellent results were obtained with the unformulated HearSNPV at different concentrations for management of *H. armigera* in field-grown and greenhouse tomatoes in South Africa (Moore *et al.*, 2004). The field application of HearNPV@ 1×10^{12} OBs ha^{-1} resulted in effective management of *H. armigera* on a variety of crops in India and Thailand (Arrizubieta *et al.*, 2016).

CONCLUSION

It's concluded that the native HearNPV has a considerable potential for eco-friendly management of *H armigera* and can be used as a promising biopesticide in Kashmir. There is no denial of the fact that already available HearNPV from tropical and sub-tropical regions of India can be effective against *H. armigera* in the temperate region of Kashmir, but the native isolates are always preferred for sustainability, adaptability and efficacy.

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