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Evaluation of Adjuvants on Enhancing the Bioefficacy of Hear NPV Against *Helicoverpa armigera* (Hubner) in Pigeonpea and Field Bean

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ABSTRACT: *Helicoverpa armigera* (Lepidoptera, Noctuidae) is polyphagous insect pest of pigeonpea and field bean crop causing yield losses of 10–90%. Chemical control has led to insecticide resistance and environmental concerns, making biological control with nucleopolyhedrovirus (NPV) a viable alternative. For effective infection, larvae must ingest sufficient virus before it is degraded by environmental factors. To improve NPV efficacy, adjuvants possessing phagostimulant properties were tested under field conditions. In both pigeonpea and field bean the treatments with adjuvants has shown significant differences in reducing the larval population which was on par with the chemical treatment and also enhancing the yield of the crops. The treatment T1 (HearNPV + Glycerol 0.1%) showed the highest larval reduction (1.17 and 1.43 larvae) and yield (13.25 and 12.95 q/ha), followed by Emamectin benzoate (1.32 and 1.23 larvae), (12.33 and 13.67q/ha). The untreated control had the highest larval count (2.40, 2.59 larvae) and lowest yield (6.67 and 7.33 q/ha) respectively in pigeonpea and field bean crop. Thus, adjuvants played a vital role in retaining the persistence of HearNPV and enhanced yield of crops.

Keywords: *Helicoverpa armigera*, Hear NPV, bioassay, field bean, pigeonpea, adjuvants.

INTRODUCTION

India is the global leader in pulse production, contributing 27–28% of the world's total output, and ranks first in consumption (27%) and import (14%) of pulses. Pulses account for approximately 20% of the food grain sector and contribute 7–10% to India's total food grain production. Despite this leadership, the country continues to face stagnation in productivity. Among the various constraints, biotic stress has been recognized as a major factor limiting pulse productivity (Kumar *et al.*, 2021). More than 250 insect pest species are known to attack legume crops in India, with the pod borer (*Helicoverpa armigera*) being one of the most destructive (Singh and Kumar 2003; Pandey *et al.*, 2024).

Among biotic factors, the gram pod borer (*H. armigera* Hübner; Noctuidae: Lepidoptera) is one of the most serious pests of pigeonpea. It is a notorious, polyphagous species with a host range exceeding 360 plant species (Manjunath *et al.*, 1987; Lalruatsangi *et al.*, 2019). In addition to pigeonpea, it also infests chickpea, mungbean, urdbean, lentil, field bean, and soybean (Sitanathan, 1983). The pest initiates damage in the early growth stages and becomes increasingly severe as the crop matures. It accounts for approximately 90–95% of total crop damage across India at various times of the year (Sachan, 1994). Losses caused by *H. armigera* are estimated at $350 million annually in pigeonpea (Vishakantaiah and Babu 1980) and approximately $2 billion across various crops in the semi-arid tropics (Sharma *et al.*, 2005). Larvae feed on leaves, flowers, and pods, causing up to 90% yield loss in the field (Ahmad *et al.*, 2015).

The larval stage lasts about 15–25 days and includes six instars. The 1st to 3rd instar larvae feed on young leaves, flowers, and buds, while later instars—being cannibalistic—are typically found singly, feeding on fruit or pods (Kakimoto *et al.*, 2003). As they develop, larvae transition from feeding on foliage to consuming developing seeds and pods (Reed and Pawar, 1982). Typically, larvae insert their head into the pod to feed on grains, with the rest of the body remaining outside (David and Ramamurthy 2012).

Chemical control remains the primary management strategy for most farmers. However, the indiscriminate use of synthetic pesticides has led to resistance development, pest resurgence, residue issues, and environmental degradation. Resistance to multiple classes of insecticides, including pyrethroids, carbamates, organophosphates, and even newer molecules like indoxacarb and fipronil, has been documented in *H. armigera* (Armes *et al.,* 1997; Kranthi *et al.*, 2002; Ahmad *et al.*, 2007; Ahmad *et al.*, 2008; Saleem *et al.*, 2008).

To maintain pest populations below the economic threshold, biological control strategies have been developed, particularly involving entomopathogenic viruses such as nucleopolyhedroviruses (NPVs) from the *Baculoviridae* family. These viruses exhibit high insecticidal activity and a narrow host range, making them ideal candidates for integrated pest management (Lapied *et al.*, 2009).

Baculoviruses, which are pathogenic to arthropods—mainly Lepidoptera, Hymenoptera, and Diptera—possess rod-shaped, enveloped nucleocapsids with a circular double-stranded DNA genome. A distinguishing feature is the production of occlusion bodies (OBs) (Blissard and Rohrmann 1990). Like other viruses, they are obligate pathogens and replicate only within the host larvae (Ignoffo, 1979). The viral dose is measured in OBs/ml, and the optimal concentration depends on both the virulence of the viral strain and the age of the host (Ignoffo and Couch 1981). Infection begins when larvae ingest OB-contaminated foliage. OBs dissolve under alkaline midgut conditions, releasing occlusion-derived virions (ODVs) that cross the peritrophic membrane and infect midgut epithelial cells (Erlandson *et al.*, 2019). Later, OBs are formed in the nuclei of infected cells. Infected larvae typically die within days, undergoing liquefaction on plant surfaces and releasing millions of OBs, perpetuating the transmission cycle (Williams, 2018).

The most widely used baculovirus for *H. armigera* management is the *Helicoverpa armigera* nucleopolyhedrosis virus (HaNPV) (Jones *et al.*, 1998). Baculoviruses have been effectively used in diverse cropping systems—field crops, vegetables, forests, and pastures—due to their specificity, safety, and environmental compatibility (Moscardi, 1999). One of the key advantages of NPVs is their host specificity, which ensures minimal impact on beneficial insects and pollinators, and overall environmental safety. OBs contribute to the environmental stability of the virus, allowing it to remain effective for extended periods.

However, challenges persist in using NPVs alone in IPM programs, including slow speed of kill, limited persistence, and the requirement for repeated applications (Mironidis *et al.*, 2013). To overcome these limitations, combining NPVs with microbial adjuvants or microbial insecticides has been suggested. Such synergistic approaches may enhance viral virulence and field efficacy.

Hence, the present study aims to enhance the efficacy of HaNPV against *H. armigera* through the use of adjuvants.

MATERIAL AND METHODS

A. Maintaining of the host insects

Field collected *H. armigera* larvae were brought to the laboratory and examined for parasitoid association and microbial contamination through standard entomological and pathological screening. Healthy larvae were individually reared on a semi-synthetic diet as per (Shorey and Hale 1965) and allowed to pupate. The culture was maintained in an incubator at 25 °C, 70% relative humidity, with a 14:10 h light–dark photoperiod. Adults emerged within 17–20 days, and the eggs laid by these moths were used to maintain the test insect colony for subsequent laboratory experiments.

B. Collection and extraction of baculovirus

H. armigera larvae showing typical HearNPV symptoms were collected from the field and transported to NBAIR, Hebbal (Bengaluru). Each larva was homogenized for 4 min in 5 ml sterile distilled water with a chilled pestle and mortar to release occlusion bodies (OBs). The homogenate was passed through glass wool, which was rinsed with an additional 0.5 ml sterile water. The filtrate was centrifuged at 15,000 × g for 5 min; the supernatant was discarded, and the pellet was washed with 2 ml sterile water and recentrifuged under the same conditions. The final pellet was resuspended in 1 ml sterile distilled water and stored at 4°C. OBs were enumerated with a Neubauer haemocytometer (depth 0.1 mm) under phase-contrast microscopy, using 100–1000-fold dilutions following Evans and Shapiro (1997). The stock suspension was adjusted to 1×10^9 OBs ml^{-1} and kept at 4 °C until required.

C. Counting, standardization and determination of NPV dosage

The concentration of any sample of NPV could be explained in terms of number of occlusion bodies per ml of solution (OBs/ml). The polyhedra could be easily counted by using an improved Neubauer haemocytometer. A sample of 5µl was poured into the chamber by using a micropipette and kept for 10 minutes to facilitate the settling of the polyhedral bodies at the bottom of the slide. The polyhedra were counted under phase contrast microscope at 400X. The polyhedral bodies present completely in the centre of the square were counted. The polyhedral touching the top and left side of the square were counted, while the polyhedra touching the bottom and right side were excluded. The number of PIBs per ml of the sample was determined by using the following formula,

$$\text{Number of inclusion bodies (PIB's/ml)} = \frac{D \times X}{N \times K}$$

Where, D = Dilution factor X = Total number of polyhedra counted N = Number of squares counted K = Volume above one small square in cm^3 ($2.5 \times 10^{-7} cm^3$) Area of each small square was 1/400 mm^2 = 0.0025 mm^2. Depth of the chamber was 0.1mm. Volume of liquid above a single small square was 0.0025 mm^2 × 0.1mm= 0.00025 mm^3. To convert it into cm^3 it was multiplied by 1/1000, to get a volume of $2.5 \times 10^{-7} cm^3$, above 1 small square. Hence, $K = 2.5 \times 10^{-7}$ cm^3.

D. Bioassay of HearNPV under laboratory conditions

The diet surface contamination method (Srinivasa *et al.*, 2008) was used to evaluate the efficacy of HearNPV against *Helicoverpa armigera* under laboratory conditions. A virus suspension with 1 × 10^5 OBs/ml was prepared, and eight concentrations (1 × 10^4 to 1 × 10^{10} OBs/ml) and control were tested. Ten microliters of virus suspension were applied to the diet surface using a sterile micropipette and spread evenly with a glass rod. Pre-starved second and early third instar larvae were placed individually into glass vials containing the treated artificial diet, with ten larvae per treatment and three replications. Control larvae received only distilled water. Vials were incubated at 25 ± 1°C, with larvae transferred to fresh diet as needed. Mortality was recorded daily from day 3 to day 10. NPV infection was confirmed by symptom observation and dark-field microscopy. Mortality data were analysed using Probit analysis in SPSS to determine LC_{50} at 95% confidence. Larval mortality in control was corrected using Abbott's correction formula (Abbott, 1925).

D. Field evaluation of H. armigera nucleopolyhedrovirus (HearNPV) against H.armigera

To evaluate the efficacy of HearNPVin the field, small-scale field trials were conducted in farmers field at Doddalahalli village of Kanakapura taluka at Ramanagar district. The experimental layout for pigeonpea and field bean, were laid out in randomized block design (RCBD) with seven treatments, each having four replications with the plot size of 3m × 3m and a gangway of one meter was allowed all around the plots. Crops were raised by following recommended agronomic practices. The recommended field dose of 1.0 × 10^{12} OBs/ha was applied during evening hours using a high-volume knapsack sprayer across different host plants. Various adjuvants were tested to enhance virus efficacy: Tinopal® (0.1%)**:** 1 g dissolved in 1000 ml water, Boric acid (0.1%)**:** 1 g in 1000 ml water, Robin blue® (0.1%)**:** 1 ml in 1000 ml water, Jaggery (1%)**:** 1 g in 100 ml water Teepol®**:** 2–3 drops per litre of water. These adjuvants were incorporated into the virus suspension prior to application.

E. Statistical Analysis

The data which are obtained from all the experiments were subjected to the statistical analysis to evaluate effects of treatments. Analysis was carried out by completely randomized design (CRD) using software WASP-2 tool (Duncan, 1995).

RESULT AND DISCUSSION

A. Survey of HearNPV infection on H. armigera in pigeonpea crop of Ramanagara district

The highest larval count of 2.6 ± 1.82 larva per plant was observed in Ramanagara taluka, followed by 2.2 ± 1.92 larva per plant in Magadi taluka. Channapatna and Kanakapura the larval count per plant were 2.0 ± 1.58. The mean NPV infection per plant was highest in Ramanagar showing 1.0 ± 0.71 NPV infection larva per plant was observed. which was followed by 0.8 ± 0.84 in Magadi taluka. 0.6 ± 0.55 mean NPV infection per larva per plant was observed in Kanakapura and Channapatna taluka. The highest larval infestation of 31.33 ± 18.80 per cent was observed in Ramanagar followed by Magadi 25 ± 23.29 per cent. The least larval infestation 21.67 ± 21.73 percent was found in Kanakapura and Channapatna taluka. Highest OBs of HearNPV was recorded in Ramanagara taluka (1.9 x 10^3 OBs/ larva, followed by Magadi with 1 × 10^3 OB per larva, followed by 2.3 × 10^2 OB per larva in Channapatna taluka, followed by 1.9 × 10^3 OB per larva in Ramanagara taluka and 1 × 10^1 OB per larva was observed in Kanakapura taluka (Table 1).

At present there is no evidence that covertly infected insects release OBs that could be transmitted horizontally. However, the baculovirus remains fully competent within the host and, at a certain moment, can be triggered to produce overt, lethal disease (Burden *et al.*, 2006). Similar studies were conducted by Madhusudan *et al.* (2011) from different geographical locations of India collected larva of the tomato fruit borer *Helicoverpa armigera* understanding these variations is essential for developing effective pest management strategies tailored to specific region. Rothman and Myers (1996) reported the natural epizootics caused by Nuclear Polyhedrosis Viruses (NPVs) are typically observed in regions where host populations reach high densities and the ability of occlusion-derived virions (ODVs) to bind to epithelial midgut cells plays a crucial role in the insect larva's oral infection susceptibility.

B. Survey on field bean for the infection of H. armigera and NPV infection in Ramanagara district

The highest 7.6 ± 2.30 larval population per plant was observed in Ramanagara taluk followed by Channapatna taluk showed the pest count of 6.2 ± 3.49 while the Magadi and Kanakapura taluk the larva per plant was almost similar recording the larval count of 5.4 ± 2.30 and 5.00 ± 3.16 respectively. Ramanagara and Channapatna the mean NPV infection per plant *i.e.*, natural occurrence of NPV in field condition against *H. armigera* were almost similar showing 1.6 ± 1.14 and 1.6 ± 0.89. While Kanakapura taluk recorded the NPV incidence on *H. armigera* was 1.4 ± 0.89. The NPV infection on the pest in Magadi was found to be 1.2 ± 0.84.

The highest percentage of NPV infection was observed in Channapatna taluk (25.00 ± 14.13) per cent infection. In Kanakapura fields the per cent NPV infection was 22.38 ± 12.97 per cent. It was 21.79 ± 11.84 per cent and 21.78 ± 13.64 per cent NPV infection on larva was observed in Ramanagara and Kanakapura taluk respectively. The highest occulssion bodies per ml of larva 1.9 × 10^3/ml was found in Kanakapura taluk followed by 1.8 × 10^3/ml in Channapatna. It was 1.6 × 10^3 OBs/ml in NPV infected larva of *H. armigera* in Ramanagara taluk and Kanakapura taluk respectively. Whereas, the Magadi taluk showed the lowest 1.2 × 10^1 OBs/ml from the NPV infected larva of *H. armigera* (Table 2). 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. 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).

Table 1: Survey of HearNPV infection on *H. armigera* in pigeonpea crop of Ramanagara district.

District	Taluk	GPS Co-Ordinates	Mean No. of healthy larva per plant	Mean No. of NPV infected larva per plant	Per Cent NPV infection	OBs yield per ml per larva
Ramanagara	Magadi	12.9577°N 77.2261°E	5	2	40.00	1.1×10^2
			2	1	50.00	2.1×10^1
			3	1	33.33	1.0×10^3
			0	0	0.00	0.00
			1	0	0.00	0.00
	Mean ± SD		**2.2 ± 1.92**	**0.8 ± 0.84**	**25.00 ± 23.29**	**0.00**
	Kanakapura	12.5462°N 77.4199°E	**3**	1	33.33	0.00
			4	1	25.00	1.6×10^1
			0	0	0.00	0.00
			2	1	50.00	1.0×10^1
			1	0	0.00	0.00
	Mean ± SD		**2.0 ± 1.58**	**0.6 ± 0.55**	**21.67 ± 21.73**	**0.00**
	Ramanagara	12.5462°N 77.4199°E	0	0	0.00	0.00
			3	1	33.33	1.1×10^2
			2	1	50.00	1.9×10^3
			5	2	40.00	0.00
			3	1	33.33	1.2×10^2
	Mean ± SD		**2.6 ± 1.82**	**1.0 ± 0.71**	**31.33 ± 18.50**	**0.00**
	Channapatna	12.4742° N 77.0424° E	2	1	50.00	3.1×10^1
			1	0	0.00	0.00
			4	1	25.00	0.00
			3	1	33.33	2.3×10^2
			0	0	0.00	0.00
	Mean ± SD		**2.0 ± 1.58**	**0.6 ± 0.55**	**21.67 ± 21.73**	**0.00**

Note: The mean values are the observations of five plants replications

C. Bio-efficacy of HearNPV NBAIR IX isolate against different larval instars of H. armigera

The 2nd instar larvae exhibited significantly higher susceptibility to HearNPV compared to 3rd instars across all concentrations and time intervals. At 3 DAT, mortality ranged from 23.33 per cent at 1×10^5 OBs/ml to 70.00 per cent at 1×10^{10} OBs/ml. At 9 DAT, mortality increased to a maximum of 96.67 per cent at 1×10^{10} OBs/ml, followed by 86.67 per cent 1×10^9 OBs/ml and 83.33 per cent at 1×10^8 OBs/ml of HearNPV. The progressive increase in mortality at 1×10^{10} OBs/ml (8.396) at 3 DAT to (9.854) at 9 DAT, indicated statistically significant improvement in mortality over time.

Even mid-level concentrations like 1×10^7 and 1×10^6 OBs/ml reached 83.33 per cent and 70.00 per cent mortality by 9 DAT, indicated that it is a potential isolate to control the pest at field level. Although mortality trends followed a similar dose- and time-dependent increase in 3rd instar larvae, the mortality levels were consistently lower than those observed in 2nd instars. Although mortality trends followed a similar dose- and time-dependent increase in 3rd instar larvae, the mortality levels were consistently lower than those observed in 2nd instars. At 3 DAT, mortality ranged from 13.33 per cent at 1×10^5 OBs per ml to 60.00 per cent 1×10^{10} OB per ml of HearNPV. At 9 DAT, mortality ranged from 56.67 per cent at 1×10^5 OBs per ml to 86.67 per cent at 1×10^{10} OBs per ml of HearNPV. This lag in virus ingestion due to lower feeding rate, shorter residual time for virus multiplication before pupation. Still, high doses like 1×10^9 and 1×10^{10} OBs per ml of HearNPV provided effective mortality, making them viable even for older larvae (Table 3). This isolate is NBAIR repository isolate it has retained the virulence and has shown greater virulence during the bioassay studies in lab. A higher peak was achieved in *H. armigera* after two days of infection (Plate 1 and 2).

Table 2: Survey of HearNPV infection on *H. armigera* in field bean crop of Ramanagara district.

District	Taluk	GPS Co-Ordinates	Mean No. of healthy larva per plant	Mean No. of NPV infected larva per plant	Per Cent NPV infection	OBs yield per ml per larva
Ramanagara	Magadi	12.9577°N 77.2261° E	6	2	33.33	2.3×10^{1}
			5	1	20.00	1.2×10^{1}
			3	1	33.33	0.00
			9	2	22.22	1.8×10^{2}
			4	0	0.00	0.00
	Mean ± SD		**5.4 ± 2.30**	**1.2 ± 0.84**	**21.78 ± 13.64**	**0.00**
	Kanakapura	12.5462°N 77.4199° E	0	0	0.00	0.00
			7	2	28.57	1.9×10^{3}
			4	1	25.00	0.00
			6	2	33.33	1.7×10^{2}
			8	2	25.00	1.2×10^{1}
	Mean ± SD		**5 ± 3.16**	**1.4 ± 0.89**	**22.38 ± 12.97**	**0.00**
	Ramanagara	12.3513°N 77.0828°E	8	1	12.50	1.7×10^{2}
			11	3	27.27	2.1×10^{2}
			8	1	12.50	1.6×10^{3}
			6	1	16.67	1.1×10^{2}
			5	2	40.00	1.8×10^{1}
	Mean ± SD		**7.6 ± 2.30**	**1.6 ± 0.89**	**21.79 ± 11.84**	**0.00**
	Channapatna	12.4742°N 77.0424°E	12	3	25.00	1.8×10^{3}
			3	1	33.33	0.00
			6	2	33.33	1.4×10^{1}
			4	0	0.00	0.00
			6	2	33.33	1.6×10^{2}
	Mean ± SD		**6.2 ± 3.49**	**1.6 ± 1.14**	**25.00 ±14.43**	**0.00**

Note: The mean values are the observations of five plants replications

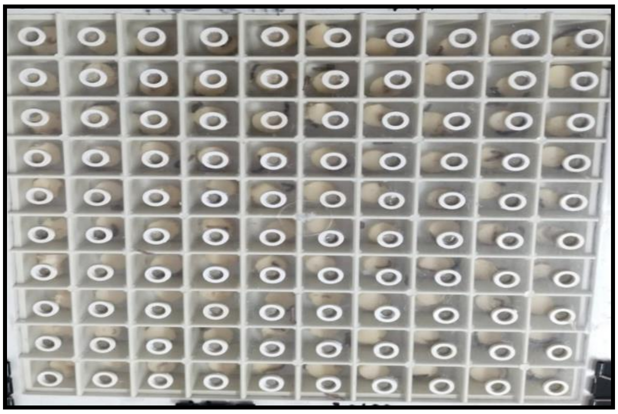

Plate 1: Bioassay of Hear NPV larvae against third instar larvae of *H. armigera*.

Plate 2: Typical hanging symptom of third instar diseased *H. armigera* with Hear NPV.

Table 3: Bio-efficacy of HearNPV NBAIR IX isolate against different larval instars of *H. armigera*.

Days after treatment	Larval Mortality (%) at various concentrations														control
	1×10^{8}		1×10^{5}		1×10^{6}		1×10^{7}		1×10^{8}		1×10^{9}		1×10^{10}		
	2nd Instar	3rd Instar	2nd Instar	3rd Instar	2nd Instar	3rd Instar	2nd Instar	3rd Instar	2nd Instar	3rd Instar	2nd Instar	3rd Instar	2nd Instar	3rd Instar	
3 DAT	23.33 (4.859)^{e}	13.33 (3.669)^{e}	43.33 (6.611)^{d}	23.33 (4.859)^{d}	50.00 (7.106)^{cd}	33.33 (5.803)^{c}	53.33 (7.33)^{c}	43.33 (6.611)^{b}	56.67 (7.554)^{bc}	50.00 (7.106)^{ab}	63.33 (7.984)^{ab}	53.33 (7.33)^{ab}	70.00 (8.396)^{a}	60.00 (7.778)^{a}	0.00 (0.707)^{f}
4 DAT	26.67 (5.191)^{e}	26.67 (5.191)^{e}	46.67 (6.859)^{d}	40.00 (6.364)^{d}	56.67 (7.554)^{c}	46.67 (6.859)cd	60.00 (7.778)^{bc}	53.33 (7.33)^{bc}	63.33 (7.984)^{abc}	60.00 (7.778)^{ab}	66.67 (8.19)^{ab}	63.33 (7.984)^{a}	73.33 (8.588)^{a}	66.67 (8.19)^{a}	0.00 (0.707)^{f}
5 DAT	30.00 (5.523)^{d}	46.67 (6.859)^{e}	50.00 (7.106)^{c}	50.00 (7.106)^{de}	53.33 (7.33)^{c}	53.33 (7.33)^{cd}	63.33 (7.984)^{b}	60.00 (7.778)^{bc}	66.67 (8.19)^{b}	66.67 (8.19)^{ab}	70.00 (8.396)^{ab}	70.00 (8.396)^{a}	76.67 (8.78)^{a}	73.33 (8.588)^{a}	0.00 (0.707)^{e}
6 DAT	36.67 (6.084)^{e}	50.00 (7.106)^{d}	53.33 (7.33)^{d}	53.33 (7.33)^{d}	56.67 (7.554)c^{d}	56.67 (7.554)^{cd}	60.00 (7.778)^{cd}	63.33 (7.984)^{bc}	63.33 (7.984)b^{c}	66.67 (8.396)^{ab}	73.33 (8.588)^{ab}	73.33 (8.588)^{a}	80.00 (8.961)^{a}	76.66 (8.78)^{a}	0.00 (0.707)^{f}
7 DAT	40.00 (6.364)^{d}	53.33 (7.33)^{e}	56.67 (7.554)^{c}	56.67 (7.554)^{de}	60.00 (7.778)^{c}	60.00 (7.778)^{cde}	63.33 (7.984)^{c}	63.33 (7.984)^{bcd}	76.67 (8.78)^{b}	70.00 (8.19)^{abc}	83.33 (9.153)^{ab}	73.33 (8.396)^{ab}	86.67 (9.333)^{a}	76.66 (8.588)^{a}	0.00 (0.707)^{e}
8 DAT	43.33 (6.611)^{e}	56.67 (7.554)^{b}	60.00 (7.778)^{d}	60.00 (7.966)^{ab}	66.67 (8.19)^{c}	66.67 (8.19)^{ab}	70.00 (8.396)^{c}	70.00 (8.396)^{a}	80.00 (8.972)^{b}	73.33 (8.588)^{a}	83.33 (9.153)^{a}	76.67 (8.755)^{a}	90.00 (9.513)^{a}	80.00 (8.78)^{a}	0.00 (0.707)^{f}
9 DAT	46.67 (6.859)^{e}	56.67 (7.33)^{c}	63.33 (7.984)^{d}	63.33 (7.76)^{c}	70.00 (8.396)^{cd}	73.33 (8.588)^{b}	73.33 (8.588)^{c}	76.67 (8.78)^{ab}	83.33 (9.153)^{b}	80.00 (8.972)a^{b}	86.67 (9.333)^{b}	83.33 (9.153)^{ab}	96.67 (9.854)^{a}	86.67 (9.333)^{a}	0.00 (0.707)^{f}

Note: Values are mean of three replications. Figures in parentheses are $\sqrt{x}+1$ transformed values. Mean values with different superscript within the same column represent a significant difference as determined by DMRT ($p \leq 0.05$)

Table 4: Larval mortality percentage of *Helicoverpa armigera* larva against the different concentration of HearNPV NBAIR IX.

HearNPV NBAIR IX Concentration	Larval mortality (%) (Mean ± SD) of *H. armigera*	
	2nd instar	3rd instar
1×10^{4}	35.24 ± 3.32^{e}	42.86 ± 6.19^{c}
1×10^{5}	53.33 ± 2.72^{d}	49.52 ± 5.22^{bc}
1×10^{6}	59.05 ± 2.69^{cd}	55.71 ± 4.97^{abc}
1×10^{7}	63.33 ± 2.52^{bcd}	61.43 ± 4.11^{abc}
1×10^{8}	70.00 ± 3.78^{abc}	66.67 ± 3.64^{ab}
1×10^{9}	75.24 ± 3.48^{ab}	70.00 ± 3.64^{a}
1×10^{10}	81.9 ± 3.63^{a}	73.33 ± 3.17^{a}

Note: Values are mean of three replications. Mean values with different superscript within the same column represent a significant difference as determined by Tukey's test ($p \leq 0.05$)

Table 4a: LC_{50} values HearNPV NBAIR IX against different instar of *Helicoverpa armigera* under laboratory conditions.

HearNPV NBAIR IX	LC_{50} (OB/ml)	Fiducial limits		Intercept	Slope	Chi-square	P value	df
		Lower	Upper					
2nd instar	1.19×10^{5}	5.89×10^{3}	7.29×10^{4}	-1.113	0.219	0.283	0.998^{b}	5
3rd instar	1.61×10^{5}	2.61×10^{6}	5.91×10^{6}	-1.63	0.314	2.665	0.751^{b}	5

D. LC_{50} values from the probit analysis for the HearNPV NBAIR IX

The larval mortality rate increased in the second instar and as the age increased the larval mortality decreased in third instar larva. Whereas the highest larval mortality of 81.9 and lowest larval mortality of 35.24 per cent was observed in the second instar larva. Whereas on third instar larva the lowest larval mortality recorded was 42.86 and highest larval mortality of 73.33 per cent larval mortality were observed. The LC_{50} values 1.19×10^5 OBs/ml and 1.61×10^5 OBs/ml were observed respectively in 2nd and 3rd instar larva. The fiducial limits were 5.89×10^3 to 7.29×10^4 and 2.61×10^6 to 5.91×10^6 OBs/ml were observed in second and third instar larva respectively (Table 4 and 4a).

The field populations of *H. armigera* demonstrated a low variation in susceptibility to HearNPV, with LC_{50} values ranging from 1.5×10^5 to 1.1×10^6 OBs/mL (7.3-fold variation). Similar variation in *H. armigera* susceptibility was observed to different HearNPV isolates, with LC_{50} values ranging from 1.6×10^4 to 3.5×10^4 OBs/mL (2.2-fold variation) (Arrizubieta *et al.*, 2014). The HearNPV was reported to cause 90–100% larval mortality against neonate and 2nd instar *H. armigera* larva (Ginting *et al.*, 2018). The HearNPV was more effective against early instar larva of *H. zea* and caused 99% larval mortality in 1st–3rd larval instars in 4–6 days and only 35% larval mortality in 4th and 5th larval instars (Black *et al.*, 2022).

E. Field evaluation of adjuvants on enhancing the bioefficacy of HearNPV against Helicoverpa armigera on pigeonpea

There were no significant differences in the larval count, which was recorded before the spray was taken in irrespective of the plots.

The maximum larval reduction was observed in the T_1 (1.70), followed by T_5 (2.58). The chemical-treated plots. The other treatments, the larval count was T_2 (1.97), T_3 (2.16), followed by T_4 (2.39), which were on par with each other. In the unsprayed plot, there was less reduction of larval count compared to all the HearNPV-treated plots and the chemical control plot.

The highest yield was obtained in the T_1(20.35 q/ha), which was on par with the chemical-treated plot T_6 (18.33 q/ha). The significant differences in the yield were observed when compared to the untreated control plots. The HearNPV alone treated plot T_5 (10.33 q/ha) also resulted in moderate yields. Whereas, T_2 (17.33 q/ha), T_3 (15.30 q/ha), and T_4 (14.95 q/ha) yields were on par with each other. The lowest yield was obtained in the unsprayed plot T_7 (9.33 q/ha), which showed significant differences with the other treated plots (Table 5).

The present findings corroborate with earlier findings of (Navdisha *et al.*, 2024) who an increase in potency of HzNPV when applied with boric acid (0.1%) under simulated sunlight. in yields. Tinopal @1% provided the best protection from sunlight and retained viral efficacy up to 68.75 and 66.75% in SpltNPV (native) and SpltNPV (NIPHM), respectively, against third instar larvae of *Spodoptera litura* (F).

Table 5: Field evaluation of adjuvants on the bioefficacy of HearNPV against *Helicoverpa armigera* in pigeonpea.

Treatments	No. of larva(e)/ plant											Yield (q/ha)
	Before Spray	First spray			Second spray			Third spray			Pool over periods and spray	
		5DAS	10DAS	Pooled	5DAS	10DAS	Pooled	5DAS	10DAS	Pooled		
T1: HearNPV+Glycerol (0.1%)+jaggery(1%)+teepol (0.01%)	2.90 (7.91)	1.58 (2.00)	1.87 (3.00)	1.72 (2.46)	1.46 (1.63)	1.76 (2.60)	1.61 (2.09)	1.58 (2.00)	1.94 (3.26)	1.76 (2.60)	1.70 (2.39)	20.35
T2: HearNPV+Tinopal (0.1%)+jaggery(1%)+teepol (0.01%)	3.02 (8.62)	1.76 (2.60)	2.11 (3.95)	1.94 (3.26)	1.86 (2.96)	2.08 (3.83)	1.97 (3.38)	1.76 (2.60)	2.26 (4.61)	2.01 (3.54)	1.97 (3.38)	17.33
T3: HearNPV+Robin blue®(0.1%)+jaggery(1%)+teepol (0.01%)	2.79 (7.28)	1.94 (3.26)	2.33 (4.93)	2.13 (4.04)	2.10 (3.91)	2.41 (5.31)	2.25 (4.56)	1.94 (3.26)	2.26 (4.61)	2.10 (3.91)	2.16 (4.17)	15.3
T4: HearNPV+Boric acid (0.1%)+jaggery(1%)+teepol (0.01%)	2.90 (7.91)	2.26 (4.61)	2.61 (6.31)	2.44 (5.45)	2.20 (4.34)	2.53 (5.90)	2.36 (5.07)	2.33 (4.93)	2.41 (5.31)	2.37 (5.12)	2.39 (5.21)	14.95
T5: HearNPV alone (1x10^9 ml 5 ml/ lit.)]	2.79 (7.28)	2.41 (5.31)	2.91 (7.97)	2.66 (6.58)	2.53 (5.90)	2.68 (6.68)	2.60 (6.26)	2.39 (5.21)	2.55 (6.00)	2.47 (5.60)	2.58 (6.16)	10.33
T6: Emamectin benzoate @ 0.4 g/l [Chemical control]	2.90 (7.91)	1.68 (2.32)	1.95 (3.30)	1.82 (2.81)	1.56 (1.93)	1.76 (2.60)	1.66 (2.26)	1.58 (2.00)	2.12 (3.99)	1.85 (2.92)	1.78 (2.67)	18.33
T7: [Untreated control]	2.90 (7.91)	3.13 (9.30)	3.29 (10.32)	3.21 (9.80)	3.38 (10.92)	3.58 (12.32)	3.48 (11.61)	3.62 (12.60)	3.62 (12.60)	3.62 (12.60)	3.44 (11.33)	9.33
SEM.±	0.15	0.13	0.14	0.09	0.16	0.17	0.11	0.17	0.14	0.11	0.06	0.91
C.D.	NS	0.4	0.42	0.25	0.5	0.52	0.31	0.54	0.43	0.32	0.18	2.81
CV (%)	9.08	10.65	9.61	10.09	13.08	12.21	12.62	13.87	9.81	11.79	11.62	10.61

Note: Values are mean of four replications. Figures in parentheses are $\sqrt{x+1}$ transformed values.

F. Field evaluation of adjuvants on enhancing the bioefficacy of HearNPV against Helicoverpa armigera on field bean

The larval count before the spraying of HearNPV in all the treatments there was no significant differences among the treatments in larval population. From the first spray the larval count reduced in all the treated plots (2.41) and the larval population was almost reduced to (1.43) The highest reduction of larval count was observed in the chemical treated plots (1.23) which showed high significant difference with the other treatments and was on par with the T1 (1.43).

The larval count in the treatment T2 (1.91) was on par with the treatment T3 (1.72). The T4 and T5 treatment were on par with each other (2.07) and (2.10) respectively. HearNPV alone showed the larval reduction which was almost on par with the other NPV treated plots, the lowest reduction in the larval count was observed in the untreated plots T7 (2.51) which differed significantly with the treated plots, showing the HearNPV could control the *H. armigera* with the different interval of spray.

The spraying of HearNPV on the crops during the pest infection resulted in lowering the damage of the crop which inturn increased the yield of the crop significantly compared to the untreated control plots (Table 6).

The larval population reduced on the second infection due to the residual effect of the first spray and the last spray of HearNPV observed drastic reduction. The highest yield among all the treatments was found in the T1 treatment (12.95 q/ha) that was almost on par with the chemical treated plot T6 (13.67 q/ha). The second highest yield of grains was obtained in T2 (12.00 q/ha) which was on par with the T3 (12.38 q/ha) and T4 (11.33q/ha)/ The T5: HearNPV alone treated plots also showed drastic yield losses and resulted in the higher yield of (11.00 q/ha) which was on par with the other HearNPV treated plots. The lowest yield was recorded in the untreated control plots (7.33q/ha) indicates that the repeated spray of a HearNPV or chemical in the *H. armigera* infested fields would result in better control and significantly improve the ability of the plant to overcome the damage and result in the good yield compared to the control plot. The combination of adjuvants with the HearNPV also known to enhance the efficacy by protecting against the sunlight and also by boric acid which is known to buffer the pH and increase the HearNPV uptake by the larva.

Likewise, Mehrvar *et al.* (2008) opined that, combination of three adjuvants *viz.*, egg white (5%) + Tinopal (0.2%) + lampblack (0.1%) showed the highest larval mortality (94.2%) with lowest LT_{50} values (99.6 hr) in tomato plants under simulated sunlight.

Table 6: Field evaluation of adjuvants on the bioefficacy of HearNPV against *H. armigera* in field bean.

Field bean												
Treatments	No. of larva(e)/ plant											Yield (q/ha)
	Before Spray	First spray			Second spray			Third spray			Pool over periods and spray	
		5DAS	10DAS	Pooled	5DAS	10DAS	Pooled	5DAS	10DAS	Pooled		
T1: HearNPV+Glycerol(0.1%)+jaggery(1%)+teepol (0.01%)	2.58 (6.16)	1.46 (1.63)	1.87 (3.00)	1.66 (2.26)	0.88 (0.27)	1.58 (2.00)	1.23 (1.01)	1.22 (0.99)	1.56 (1.93)	1.39 (1.43)	1.43 (1.54)	12.95
T2: HearNPV+Tinopal(0.1%)+jaggery(1%)+teepol (0.01%)	2.47 (5.60)	1.68 (2.32)	2.33 (4.93)	2.00 (3.50)	1.56 (1.93)	1.95 (3.30)	1.76 (2.60)	1.77 (2.63)	2.18 (4.25)	1.98 (3.42)	1.91 (3.15)	12.00
T3: HearNPV+Robin blue® (0.1%) +jaggery(1%)+teepol (0.01%)	2.73 (6.95)	1.56 (1.93)	2.11 (3.95)	1.84 (2.89)	1.34 (1.30)	1.86 (2.96)	1.60 (2.06)	1.58 (2.00)	1.86 (2.96)	1.72 (2.46)	1.72 (2.46)	12.33
T4: HearNPV+Boric acid (0.1%) +jaggery(1%)+teepol (0.01%)	2.26 (4.61)	1.86 (2.96)	2.61 (6.31)	2.23 (4.47)	1.84 (2.89)	2.02 (3.58)	1.93 (3.22)	1.86 (2.96)	2.24 (4.52)	2.05 (3.70)	2.07 (3.78)	11.33
T5: HearNPV alone (1×10^9 OBs/ml @5 ml/ lit)	2.41 (5.31)	1.95 (3.30)	2.91 (7.97)	2.43 (5.40)	1.77 (2.63)	2.18 (4.25)	1.98 (3.42)	2.04 (3.66)	2.33 (4.93)	2.18 (4.25)	2.10 (4.34)	11.00
T6: Emamectin benzoate @ 0.4 g/l [Chemical control]	2.54 (5.95)	1.46 (1.63)	1.95 (3.30)	1.71 (2.42)	1.22 (0.99)	1.76 (2.60)	1.49 (1.72)	1.34 (1.30)	1.68 (2.32)	1.51 (1.78)	1.23 (1.96)	13.67
T7: [Untreated control]	2.35 (5.02)	2.41 (5.31)	2.60 (6.26)	2.51 (5.8)	2.20 (4.34)	2.54 (5.95)	2.37 (5.12)	2.61 (6.31)	2.68 (6.68)	2.64 (6.47)	2.51 (5.8)	7.33
SEM.±	0.16	0.12	0.14	0.10	0.13	0.15	0.10	0.08	0.15	0.08	0.06	0.90
C.D.	NS	0.38	0.42	0.28	0.41	0.46	0.28	0.26	0.46	0.23	0.16	2.76
CV (%)	11.48	11.93	10.09	10.91	15.07	12.99	13.92	8.15	12.52	10.92	12.81	13.63

Note: Values are mean of four replications. Figures in parentheses are $\sqrt{x+1}$ transformed values.

Ranvir Singh and Jagadish (2018) before the treatment imposition, percentage pod damage ranged from 3.47 to 4.73 per cent. Similar trend was observed when pooled means were compared with respect to pod damage percentage. Emamectin benzoate (3.67%) was significantly superior than other HaNPV isolates (3.83% to and untreated control (5.83%). The bioassay results of inoculated *H. armigera* nucleopolyhedrosis virus (HaNPV) with different concentrations indicate that the 4.0 g/l dosage caused maximum mortality (70.3% and 60.54%), and minimum mortality 46.83% and 44.08% was recorded in the 0.5 g/l dosage under laboratory and pot culture conditions, respectively. Singh (2001) has advocated the applications of HaNPV at 250 LE/ha for successful management of this pest in tomato. Kalita *et al.* (2017) reported HaNPV @ 1 ml/l also showed effective result which was at par with Spinosad 45 EC. Isolates with greater virulence and increased persistence in the environment are suggested as means for increasing the biopesticidal value of the viruses (Shapiro and Bell 1984). Nasution *et al.* (2015) reported 97.40 to 100 % mortality when HaNPV was administered in different formulation.

CONCLUSIONS

Although natural enemies may reduce populations of *Helicoverpa armigera* their impact is often insufficient to prevent economic losses, particularly in high-value crops. In this study HearNPV + Glycerol + jaggery + teepol (T1) is the most effective biocontrol treatment, showing substantial reduction in larval population and a high yield, comparable to the chemical control**.** The addition of adjuvants (Glycerol, Tinopal, Robin blue, Boric acid) improves NPV efficacy compared to NPV alone. Although Emamectin benzoate (T6) is more effective in pest control, T1 offers an eco-friendly, sustainable alternative with only slightly lower yield. Significant differences were observed among treatments. Treatments T1 and T6 were statistically superior to others in yield and pest control.

FUTURE SCOPE

Field Validation: Conducting large-scale multi-location field trials of T1 to validate its effectiveness under diverse agro-climatic conditions.

Mode of Action Studies: Further Investigations on the mechanism of adjuvants (e.g., glycerol, jaggery) in enhancing NPV efficacy—whether it improves adherence, ingestion, or viral persistence on leaves.

Shelf-life and Stability: Study the shelf life and formulation stability of NPV when combined with different adjuvants.

Cost-benefit Analysis: Evaluate the economic viability of the NPV + adjuvant formulations compared to chemical controls over multiple seasons.

Environmental Impact Assessment: Long-term impact of NPV formulations on non-target organisms**,** beneficial insects, and overall agroecosystem health need to be assessed.

Resistance Management: Incorporation of NPV-based biopesticides in IPM (Integrated Pest Management) programs to delay or prevent resistance development to chemical insecticides on large scale in farmers field need to be implemented.

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