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Vijay Kumar
Assistant Professor,
Department of Agriculture,
Shivalik University,
Dehradun, Uttarakhand, India

Mandedi Harivardhan Reddy
Sustainable Certification
specialist at ITC LTD, Guntur,
Andhra Pradesh, India

Dr. Navin Kumar Sharma
Senior Scientist & Head, DRI-
Krishi Vigyan Kendra,
Majhgwa, Satna, Madhya
Pradesh, India

Dr. Dina Rawat
Assistant Professor,
Department of Agriculture,
Shivalik University,
Dehradun, Uttarakhand, India

Rajneesh Pal
Assistant Professor, School of
Agriculture and
Environmental Sciences,
Shobhit University, Gangoh,
Saharanpur, Uttar Pradesh,
India

Corresponding Author:
Vijay Kumar
Assistant Professor,
Department of Agriculture,
Shivalik University,
Dehradun, Uttarakhand, India

Investigate the significance of different diets on mass production of *Helicoverpa armigera*-nuclear polyhedrosis virus

Vijay Kumar, Mandedi Harivardhan Reddy, Navin Kumar Sharma, Dina Rawat and Rajneesh Pal

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Abstract

Aim: This study the Investigate the Significance of different diets on mass production of *Helicoverpa armigera*-nuclear polyhedrosis virus during 2020-21.

Methodology: Rearing of *Helicoverpa armigera* was done at Bio control laboratory temperature was maintained at 25 ± 2 °C, $75 \pm 5\%$ relative humidity and 14-10 (L: D) h photoperiod. Fresh larvae of *Helicoverpa armigera* were collected from chickpea crop for their culture maintenance for many generations on natural chickpea leaves and pods. Two artificial diets were prepared with the change of basic ingredients including flour of chickpea (*Cicer arietinum* L.), wheat (*Triticum aestivum* L.).

Result: Our findings indicate that diet composition plays a crucial role in determining larval weight, developmental duration, and susceptibility to Ha-NPV infection. Statistical analyses revealed significant differences in Ha-NPV replication efficiency based on diet type, with optimized artificial diets showing the most promising results for large-scale viral production.

Interpretation: The study highlights the importance of selecting an optimal larval diet to enhance *Helicoverpa armigera* -nuclear polyhedrosis virus mass production efficiency, thereby improving its commercial viability as a biopesticide.

Keywords: Artificial diets, biopesticides, natural diets, nuclear polyhedrosis virus, pod borer and polyhedral inclusion body

Introduction

Legume crops are major sources of human food and animal feed, and they also aid in soil fertility management through biological nitrogen fixation, making them essential for sustainable agriculture (Maiti, 2001)^[12].

These are major sources of dietary protein for millions of people, particularly vegetarians in the Indian subcontinent and other regions of the world, due to their high protein content. In the semi-arid tropics (SAT), where one-sixth of the world's population lives, groundnut (*Arachis hypogaea* L.), chickpea (*Cicer arietinum* L.), and pigeonpea (*Cajanus cajan* L.) are major legume crops (Sharma *et al.*, 2021)^[20].

The main restrictions affecting crop productivity in the SAT regions include unpredictable weather, inadequate and inconsistent rainfall, nutrient-poor soils, and the incidence of pests and diseases. *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), and *Amsacta albistriga* (Walker) (Lepidoptera: Arctiidae) are among the insect pests that limit groundnut, pigeon pea, and chickpea production in the semi-arid tropics (SAT) (Sharma, 2005; Tay *et al.*, 2022)^[18, 24].

H. armigera, often known as the cotton bollworm or legume pod borer, is found in Africa, Australia, Southeast Asia, New Zealand, and Mediterranean Europe (Sharma, 2005)^[18]. It's a polyphagous pest that feeds on a wide range of food, fibre, oil seed, fodder, and horticultural crops from over 300 different plant species (Arora *et al.*, 2005)^[1].

It has been found in India in approximately 20 crop and 180 wild plant species (Sharma, 2005)^[18]. Understanding how different diets impact the susceptibility of *H. armigera* to HaNPV is crucial for optimizing the use of this biopesticide.

Diet can influence several factors such as the insect's immune response, developmental rate, and overall health, all of which may alter the effectiveness of HaNPV.

While research has been conducted on the effects of diet on pest resistance and survival, the specific relationship between dietary composition and the viral efficacy remains underexplored.

This study aims to investigate how different diets, varying in nutrient composition, affect the mass production of *Helicoverpa armigera* and the viral replication and pathogenicity of HaNPV. Even at the farmer level, mass production of NPV pesticide is straightforward and widespread (Raymond *et al.*, 2021)^[15].

Healthy larvae are fed a modest dosage of NPV in the lab or in the field, and the virus produced in the insect is retrieved and its concentration determined by counting polyocclusion bodies (POBs) using a light microscope equipped with a hemocytometer. Scientists, farmers, NGOs, and state agricultural and extension administrations have recently joined together in India to promote local production and use of NPV. NPV is multiplied on field collected larvae and applied to crops, despite the fact that commercial production, quality, and storage are still contested concerns. When compared to laboratory-reared larvae, multiplying NPV on field collected larvae was found to be easier and more cost effective; however, efficacy and quality may be compromised due to impurities such as bacteria and fungi (Rabindra *et al.*, 2021)^[7].

The concentration of POB, which is indicated as LE, is important for the viral insecticide's efficiency (Larval Equivalent). A basic stock preparation typically contains 1LE, or 6 10⁹ POBs/ml. Microbial pesticides, such as NPVs and GV's, have now been placed under the Central Insecticide Act of 1968.

Microbial pesticides can only be sold after being registered with the Central Insecticide Board (CIB) in India, which was established under the insecticides act to govern their manufacture and usage. The act's section 9(3b) establishes a minimum amount of active component in a formulation.

Materials and Methods

Rearing of *Helicoverpa armigera* done at Bio control laboratory, Department of Entomology, A.N.D.U.A. & T. Kumarganj, Ayodhya. Laboratory temperature was maintained at 25±2 °C, 75±5% RH and 14-10 (L: D) h photoperiod. Fresh larvae of *Helicoverpa armigera* were collected from chickpea crop for their culture maintenance for many generations on natural chickpea leaves and pods. Emerged adults were paired to get eggs and neonate larvae. Two artificial diets were prepared with the change of basic ingredients including flour of chickpea (*Cicer arietinum* L.), wheat (*Triticum aestivum* L.) and each measuring 600 g and mixed in a common mixture containing the following three parts:

Table 1: Detail of Treatments

Treatment No.	Treatments	Source
T ₁	Chickpea leaves	From field
T ₂	Chickpea pod	From field
T ₃	Chickpea flour	Local market
T ₄	Wheat flour	Local market

Table 2: Composition of semi-synthetic diet for (larvae) *H. armigera*

S. No.	Component	Quantity
1.	Chickpea flour	100g
2.	Yeast powder	60g
3.	Sucrose	60g
4.	Formaldehyde 10%	15ml
5.	Choline chloride 20%	30ml
6.	Ascorbic acid	12g
7.	Agar	45g
8.	Methyl 4 hydroxy benzoate	7.5g
9.	Sorbic acid	4.5g
10.	Streptomycine sulphate	0.1g
11.	Cholesterol	0.6g
12.	Wheat germ oil	0.6ml
13.	Vitamin mixture	0.6g
14.	Distilled water	1200ml

Table 3: Composition of semi-synthetic diet for Adult *H. armigera*.

S. No.	Component	Quantity
1.	Sucrose	50g
2.	Methyl paraben	1g
3.	Absolute alcohol	1ml
5.	Vanderzant vitamin solution (28% in distilled water)	10ml
6.	Distilled	500ml

Part A: It includes yeast powder (60 g), sucrose (60 g), formaldehyde 10% (15 ml), choline chloride 20% (30 ml), distilled water (1200 ml).

Part B: It includes ascorbic acid (12 g), methyl 4 hydroxy benzoate (7.5 g), sorbic acid (4.5 g), streptomycine sulphate (0.1 g), cholesterol (0.6g), wheat germ oil (0.6 ml) and vitamin mixture (0.6 g).

Part C: It includes agar (45 g) and distilled water (1000 ml). The vitamin mixture in part B contains nicotine acitamide (9.30 g), riboflavin (4.64 g), pyridoxine hydrochloride (2.32 g), biotin (0.18 g), vitamin B12 (0.01 g), folic acid (4.64 g) and thiamine hydrochloride (2.32 g).

Preparation of diet

Part C was boiled in distilled water and a homogenous mixture was prepared, then mixed the ingredients of part A and part B separately and blended in a grinder. Ingredients of part A was poured in to the ingredients of part B and blended and mixed in the ingredients of part C and then kept at room temperature for some time and stored at low temperature (8 °C) in refrigerator. First instar larvae were taken from the stock culture and released in all treatments including control and four artificial prepared diets, each with six replicate and each replicate was maintained ten larvae. In control larvae were fed on natural chickpea leaves and pods.

Preparation of artificial diet

Artificial diet for *H. armigera* was prepared in three steps such as follows:

Step I: Preparation of mixture A

The following ingredients were mixed with 390 ml of water: 105 gm chickpea flour + 2 gm Methyl Para Hydroxy Benzoate + 1 gm Sorbic acid + 0.25 gm Streptomycin + 2 ml of 10% formaldehyde. This combination was mixed thoroughly in a blender for two minutes.

Step II: Preparation of mixture B

12.75 gm of Agar-Agar was boiled in 390 ml of water. This gave mixture B.

Step III: Preparation of mixture C

Mixture A and B were combined and blended for 1 minute and then 10 gm Yeast + 3.25 gm Ascorbic acid + 2 Multiple Vitamin capsule + 2 Vitamin E capsules were added and was mixed again by using a blender for one minute. This provided artificial diet was poured in a flat tray and small squares were cut by knife and the diet is ready to carry out the experiments.

For maintaining the culture of the test insect, *H. armigera*, adults were collected from the unsprayed field and kept inside the wired cylindrical cage of 115 cm. length and 38 cm. dia covered with muslin cloth. Ten pairs of male and female were released in each cage by providing 10% sugar solution soaked in cotton swab and the insect was allowed for egg laying. The eggs laid on the muslin cloth were collected with the help of moist camel hair brush and then transferred to the test experimental diets.

Statistical Analysis

Daily data on parameters regarding larval duration, weight, length and percent mortality, pupal duration and weight, percent pupal recovery, male and female percentage and percent emergence were recorded. Data was statistically analyzed following Steel and Torrie (1980) and significance was tested by using DMRT and following formulae.

$$s_d = \sqrt{\frac{2s^2}{r}}$$

(t - 1) values of the shortest significant ranges are computed as

$$R_p = \frac{(r_p)(s_d)}{\sqrt{2}} \text{ for } P = 2, 3, \dots, t$$

Where,

t = Total number of treatments,

s_d = Standard error of the mean difference,

r_p = Tabular values of the significant studentized ranges obtained from table

p = Distance in rank between the pairs of treatment means to be compared, i.e. p = 2 for the 2 means with consecutive rankings and p = t for the highest and lowest means.

Results and Discussion

Yield of Polyhedral Inclusion Bodies from laboratory reared *H. armigera* larvae

The data shown in Tables 4 and 5 makes it evident how various *Helicoverpa armigera* nucleopolyhedrovirus (Ha-NPV) inoculum doses affected larval mortality, pupation, polyhedral inclusion body (PIB) yield, and production ratio in the years 2019-20 and 2020-21.

Effect of inoculum dose on larval mortality

During both years of investigation, a steady increase in larval mortality was noted as the inoculum dose of Ha-NPV was increased. Cumulative mortality varied from 62.66% at 0.5 LE to 86.00% at 3.0 LE in 2019-20 and from 61.33% to 87.33% at the same doses in 2020-21. Ha-NPV's dose-dependent toxicity is confirmed by the fatality rate gradually rising as the virus dose rises.

At lower doses, early larval mortality was very modest; nevertheless, after 8-10 days following treatment, greater doses led to quicker mortality. Kumar *et al.* (2021)^[11] and Gupta and Bhattacharya (2023)^[6] showed similar dose-dependent mortality responses of *H. armigera* larvae to NPV infection, noting that higher viral doses speed up infection but may negatively impact virus multiplication due to premature host death.

Effect on larval pupation

The inoculum dose and larval pupation had an inverse relationship. In both years, the pupation percentage was highest at 0.5 LE (35.33% in 2019-20 and 36.66% in 2020-21), while it was lowest at 3.0 LE (12.66% and 12.00%, respectively). Reduced pupation at higher doses is a sign of successful viral infection and host death prior to larval development.

These results are consistent with the findings of Patel *et al.* (2022)^[14], who found that rapid systemic infection and tissue disintegration in host larvae caused by increased concentrations of NPV greatly decrease pupation.

Yield of polyhedral inclusion bodies (PIBs)

Among the various inoculum doses, there were notable differences in the overall and average yield of PIBs. It's interesting to note that in both years, the greatest PIB yield was 1.0 LE, yielding 229.32 × 10² PIBs (2019-20) and 220.64 × 10² PIBs (2020-21). Higher doses (2.0 and 3.0 LE) increased larval mortality but did not improve PIB output in proportion.

Accelerated host mortality, which inhibits the virus's reproduction cycle and polyhedra formation, is the reason

for this decrease in PIB production at higher doses. OEPP/EPPO (2022) and Sharma *et al.* (2021)^[20] came to similar conclusions, emphasising that optimal viral generation happens at moderate infection levels rather than at maximum lethal dosages.

Production ratio (PR)

When producing Ha-NPV on a big scale, the production ratio is an important economic factor. In both years, 1.0 LE was consistently the optimal production ratio (1:591).

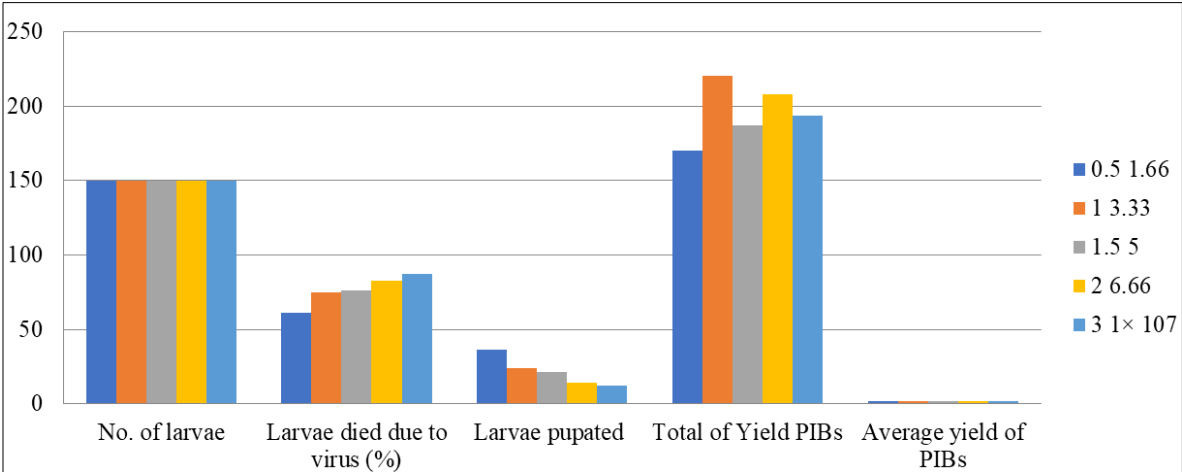
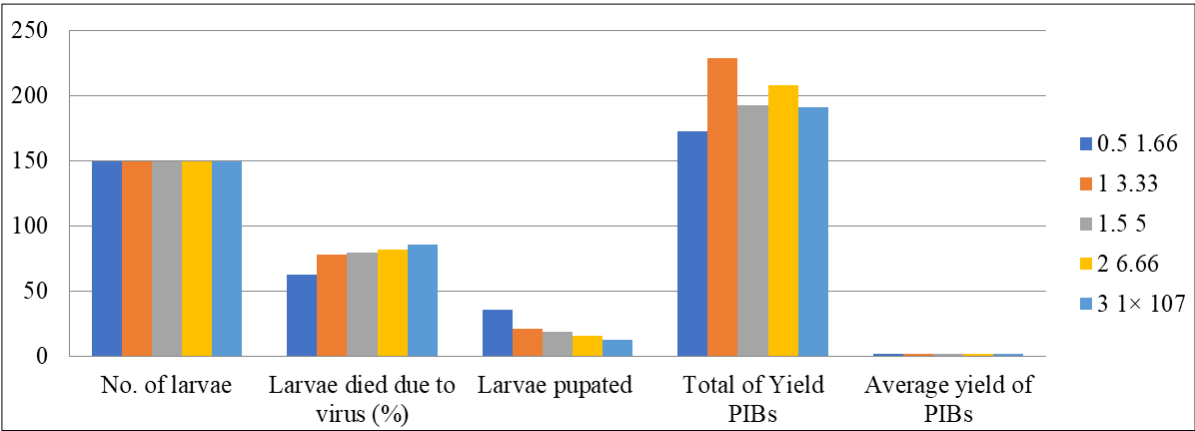
Higher doses raised production costs since they produced less PIB per larva, whereas lower doses produced poor viral yield. Higher inoculum concentrations are biologically effective but economically inefficient, as seen by the dramatic drop in PR at doses above 1.0 LE. The findings of Jayanthi and Rabindra (2021)^[7] and Koul *et al.* (2024)^[10], who suggested moderate inoculum doses for commercial NPV production systems, are substantially supported by these data.

Table 4: Optimizing the inoculums dose of Ha-NPV for mass production of virus during 2019-20.

Sr. No.	NPV (LE)	Dose of PIBs/Larvae	No. of Larvae	Larvae died due to virus		Cumulative per cent mortality (Days after treatment)						Larvae pupated		Yield of PIBs		Production ratio (PR)
				No.	%	4	6	8	10	12	14	No.	%	Total	Average	
1.	0.50	1.66×10 ⁶	150	94	62.66	4.33	10.66	25.33	52.00	57.33	62.66	53	35.33	172.96×10 ⁹	1.84×10 ⁹	1: 1108
2.	1.00	3.33×10 ⁶	150	117	78.00	8.66	22.33	49.66	67.33	73.66	78.00	31	20.66	229.32×10 ⁹	1.96×10 ⁹	1: 591
3.	1.50	5.00×10 ⁶	150	119	79.33	8.33	22.33	52.66	62.33	76.00	79.33	28	18.66	192.78×10 ⁹	1.62×10 ⁹	1: 324
4.	2.00	6.66×10 ⁶	150	123	82.00	9.66	26.00	61.66	78.33	82.00	----	24	16.00	207.87×10 ⁹	1.69×10 ⁹	1: 253
5.	3.00	1.00×10 ⁷	150	129	86.00	9.33	29.66	69.33	86.00	----	----	19	12.66	190.92×10 ⁹	1.48×10 ⁹	1: 148

Table 5: Optimizing the inoculums dose of Ha-NPV for mass production of virus during 2020-21.

Sr. No.	NPV (LE)	Dose of PIBs/Larvae	No. of Larvae	Larvae died due to virus		Cumulative per cent mortality (Days after treatment)						Larvae pupated		Yield of PIBs		Production ratio (PR)
				No.	%	4	6	8	10	12	14	No.	%	Total	Average	
1.	0.50	1.66×10 ⁶	150	92	61.33	3.66	11.66	24.33	53.00	58.33	61.33	55	36.66	170.20×10 ⁹	1.85×10 ⁹	1: 1114
2.	1.00	3.33×10 ⁶	150	112	74.66	7.66	21.33	51.66	65.33	69.00	74.66	36	24.00	220.64×10 ⁹	1.97×10 ⁹	1: 591
3.	1.50	5.00×10 ⁶	150	114	76.00	8.66	23.33	55.66	59.33	68.00	76.00	32	21.33	186.96×10 ⁹	1.64×10 ⁹	1: 328
4.	2.00	6.66×10 ⁶	150	124	82.66	9.00	27.33	63.66	76.33	82.66	----	21	14.00	208.32×10 ⁹	1.68×10 ⁹	1: 252
5.	3.00	1.00×10 ⁷	150	131	87.33	9.66	28.66	73.33	87.33	----	----	18	12.00	193.88×10 ⁹	1.49×10 ⁹	1: 149



Conclusion

The best inoculum dose for mass production of Ha-NPV was determined to be 1.0 LE (3.33×10^2 PIBs per larva) based on larval mortality, pupation, PIB yield, and production ratio. Higher doses increased mortality, but they were less cost-effective and efficient in terms of virus growth. For the commercial production of Ha-NPV that is both affordable and sustainable, 1.0 LE is advised.

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