Field Efficacy of *Helicoverpa armigera* Nucleopolyhedrovirus Isolates against *H. armigera* (Hübner) (Lepidoptera: Noctuidae) on Cotton and Chickpea

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Abstract

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Field efficacy of seven geographical isolates of *Helicoverpa armigera* nucleopolyhedrovirus (HaNPV) along with an insecticide control was evaluated against *H. armigera* on cotton and chickpea in the Coimbatore district of Tamil Nadu, India. Among the HaNPV isolates, CBE I (Coimbatore) and NEG (Negamum) applied at 3.0×10^{12} POB/ha to cotton and 1.5×10^{12} POB/ha to chickpea with an adjuvant, crude sugar, significantly reduced the *H. armigera* larval population and increased the yield. CBE I and NEG recorded the highest yield of 2038 kg/ha and 2033 kg/ha, which was on a par with endosulfan (2026.7 kg/ha) with cost/benefit ratios of 1:2.32, 1:2.48, and 1:1.12, respectively, on cotton. In chickpea grain yields of 980, 983, and 973.3 kg/ha and cost/benefit ratios of 1:1.36, 1:1.48 and 1:0.87, respectively, in CBE I, NEG and endosulfan treated plots were obtained. The isolate RAJ (Rajasthan) recorded the lowest yield comparable to that of the untreated control in both crops.

Keywords: *Helicoverpa armigera*; nucleopolyhedrovirus; geographical isolates; endosulfan

The cotton bollworm, *Helicoverpa armigera* (Hübner), is a pest of major importance in India in most agroecological zones ranging from Andaman Nicobar Islands to Jammu and Kashmir (Singh *et al.* 2002). Crop losses of 75–100% in chickpea (Lal 1996) and 57–80% in cotton (Gupta 1999) have been recorded. The estimated monetary loss in Tamil Nadu was Rs.20.12 million USD on different crops (Jayaraj 1990). In Punjab, Haryana and Rajasthan the damage due to the pest on cotton was estimated at Rs.296.93 million USD (Harish 2002). The ability of this pest to adopt transient habitats in a short span of time accelerated the excessive use of pesticides resulting in development of resistance to various classes of insecticides (Armes *et al.* 1992) and outbreaks in several areas since 1983–1984 (Singh *et al.* 2002). This necessitated the search for ecofriendly alternatives, especially microbial insecticides in view of their high specificity, potential activity and environmental safety. Biopesticides based on the baculovirus group, the nucleopolyhedrovirus (NPV), offer a great scope against *H. armigera*. Successful utilisation of *H. armigera* NPV (HaNPV) under field conditions was reported on chickpea (Rabindra *et al.* 1989) and cotton (Sathiah & Rabindra 2001).

Isolates with greater virulence and increased persistence in the environment are suggested as means for increasing the biopesticidal value of the viruses (Shapiro & Bell 1984). Isolates with enhanced virulence have been identified under laboratory conditions (Geetha & Rabindra 2000; Gopali & Lingappa 2001). However, their assessment in the field is missing. Keeping these facts in mind, field trials were conducted on cotton and chickpea to find out the useful isolate of HaNPV against *H. armigera* for successful field utilisation.

MATERIALS AND METHODS

HaNPV isolates maintenance. The HaNPV isolates Coimbatore (CBE I), Negamum (NEG), Bangalore (BAN I), Hyderabad (HYD), Maharastra (MAH I), Rahuri (RAH), Rajasthan (RAJ) used in this study were maintained in the Department of Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The viruses were propagated *in vivo* with utmost care by a diet surface contamination method (Shorey & Hale 1965) and purified by adopting the standard procedure. The polyhedral occlusion body (POB) strength was assessed using a haemocytometer (Weber, England) (Evans & Shapiro 1997). The stock suspensions were stored at –20°C for further studies.

Evaluation of HaNPV isolates on cotton and chickpea. Two field trials, one on cotton (cv. Surabhi) at Puthur village, Coimbatore district, and the other on chickpea (cv. Shoba) at Kurumbapalayam village, Coimbatore district, were conducted in a randomised block design. Each treatment was replicated three times with plot sizes of 10 × 4 m. Seven HaNPV isolates of Indian origin were evaluated in comparison with the insecticide endosulfan 35 EC and untreated control.

Six rounds of spray in teepol 0.1% were applied with a backpack hydraulic sprayer. Crude cane sugar at 2.5 kg/ha was added as adjuvant for all the virus treatments. The crude cane sugar was added to the virus treatment as phagostimulant. Untreated control plots were sprayed with teepol 0.1% and crude cane sugar at 2.5 kg/ha without virus. The commercial formulation of endosulfan 35 EC available in the market was also included as one of the treatments at 700g/ha for comparison and was applied using a hand-operated knapsack sprayer. The pest incidence was monitored visually and the treatments were started when the pest incidence was noticed and thereafter at 7-day intervals. Observations on the number of live larvae in cotton and chickpea were recorded seven days after each spray on ten plants per treatment per replication selected at random. In chickpea, the pod damage was assessed by counting the number of total pods and the affected pods in 10 plants selected at random. At the time of harvest, grain yield/plot was recorded. In cotton, damage to squares and bolls was assessed by counting the total number of squares and bolls as well as the affected ones and the pooled mean and per cent reduction over control were worked out. The yield was recorded at harvest.

Statistical analysis, The larval counts in the field experiments were transformed to values as per the method developed by Poisson for statistical analysis (Snedecor & Cochran 1967). The analysis of variance in different experiments was carried out in IRRISTAT ver. 3.1. (Biometric unit, IRRI, Philippines) and the means were separated by Duncan's new Multiple Range Test (DMRT) (Duncan 1966) available in the package. The cost/benefit ratio was calculated from the net income

Table 1. Cost of treatment with HaNPV isolates

Isolate	Yield/100 inoculated larvae ($\times 10^{11}$ POB)	Number. of larvae required for producing		Cost of production (Rs.)		Cost of protection (Rs./spray/ha)*	
		1.5×10^{12} POB	3.0×10^{12} POB	1.5×10^{12} POB	3.0×10^{12} POB	chickpea	cotton
CBE I	5.52	272	544	150	300	405	555
NEG	6.08	247	494	135	270	390	525
BAN I	5.10	294	588	160	320	415	575
HYD	4.50	333	666	180	360	435	615
MAH I	1.68	893	1786	490	980	745	1235
RHI	1.31	1145	2290	630	1260	885	1515
RAJ	0.47	3191	6382	1755	3510	2010	3765

*Includes adjuvant @ Rs.40/spray/ha; teepol @ Rs.65/spray/ha; cost of application @ Rs.150/spray/ha and cost of protection. The cost of production (calculated on the basis of consumable cost, labour cost and overheads) for the different isolates derived from an earlier experiment (Jeyarani 2004) was used to calculate the cost/benefit ratio (C/B ratio) (Table 1).

RESULTS

Cotton

Observations recorded on the larval population prior to treatments showed that the differences were not significant. After the second round of treatment onwards, significant differences in the larval population could be recorded fully. Isolates CBE I, NEG, BAN I and HYD were highly effective and comparable to endosulfan after six rounds of treatment. They were either on a par or better than MAH I and RHI after different rounds of spray. Of all isolates evaluated RAJ was found to be the least effective in reducing the larval population and recorded a pooled mean of 8.33 larvae per 10 plants with only 19.78% reduction compared to the control. The trend was similar for square and boll damage. CBE I and NEG treated plots recorded 61.09 and 61.01 percent reduction in boll damage compared to the control, followed by endosulfan treated plots (Table 2).

Observations on the yield of seed cotton showed that the isolates CBE I and NEG along with adjuvant recorded the significantly maximum yield of 2038 and 2033 kg/ha, respectively, and were equal to endosulfan (2026.67 kg/ha) followed by BAN I, HYD and RHI in the order of effectiveness. However, the yields in RAJ isolate treated plots (1601.67 kg/ha) and untreated control (1595 kg/ha) were the lowest and on a par with each other (Table 3).

The net income and C/B ratio were maximum in NEG (1:2.48) and CBE I (1:2.32) treated plots. However, the net income and the C/B ratio were on negative side for the isolates RAJ, RHI and MAH I (Table 3) due to the increased cost of production of the isolates (Table 1).

Chickpea

Observations on the larval population before the application of respective virus isolates showed

Table 2. Field efficacy of HaNPV isolates against *H. armigera* on cotton (cv. Surabhi) (Puthur, Coimbatore)

Treatment*	Number of larvae per 10 plants		Reduction over control (%)	Square damage (%) (pooled mean)**	Reduction over control (%)	Boll damage (%) (pooled mean)**	Reduction over control (%)
	pre-treatment count	pooled mean**					
CBE I	9.00	3.78^{a}	63.63	9.71^{a}	45.04	5.87^{a}	61.09
NEG	9.33	4.00^{a}	61.50	10.84^{ab}	38.65	5.89^{a}	61.01
BAN I	10.00	4.17^{a}	59.91	11.33^{cd}	35.91	7.11^{b}	52.92
HYD	10.00	4.34^{a}	58.27	12.11^{bcd}	31.50	7.14^{b}	52.71
MAH I	10.33	5.22^{b}	49.75	14.32^{de}	18.98	10.58^{c}	29.93
RHI	10.33	5.78^{b}	44.38	14.76^{e}	16.48	10.41^{c}	31.04
RAJ	9.67	8.33^{c}	19.78	15.79^{f}	10.63	11.36^{d}	24.71
Endosulfan 35 EC (700 g/ha)	10.67	4.39^{a}	57.74	11.24^{abc}	36.42	7.05^{b}	53.27
Untreated control	10.33	10.39^{d}	–	17.67^{g}	–	15.09^{e}	–

*NPV was applied @ 3.0×10^{12} POB/ha in teepol 0.1%; crude sugar @ 2.5 kg/ha was used as adjuvant

**Pooled mean after six rounds of spray

In a column means followed by similar letters are not statistically different by DMRT ($P < 0.05$). ANOVA statistics – number of larvae $F = 50.41$, df = 40, $P < 0.001$; square damage $F = 46.93$, df = 40, $P < 0.001$; boll damage $F = 167.53$, df = 24, $P < 0.001$

Table 3. Yield and economics of HaNPV isolates in the control of *H. armigera* on cotton (cv. Surabhi) (Puthur, Coimbatore)

Treatments*	Yield** (kg/ha)	Yield increase over untreated control (kg/ha)	Value (additional yield) (Rs./ha)	Cost of protection$ (Rs./ha)	Net income (Rs./ha)	Cost/benefit ratio
CBE I	2038.00[a]	443.00	11 075.00	3 330.00	7 745.00	1:2.32
NEG	2033.00[a]	438.00	10 950.00	3 150.00	7 800.00	1:2.48
BAN I	1961.67[ab]	366.67	9 166.75	3 450.00	5 716.75	1:1.66
HYD	1903.33[b]	308.33	7 708.25	3 690.00	4 018.25	1:1.09
MAH I	1815.00[c]	220.00	5 500.00	7 410.00	–1 910.00	1: –0.26
RHI	1768.33[c]	173.33	4 333.25	9 090.00	–4 756.75	1: –0.52
RAJ	1601.67[d]	6.67	166.75	22 590.00	–22 423.25	1: –0.99
Endosulfan 35 EC (700 g/ha)	2026.67[a]	431.67	10 791.75	5 100.00	5691.75	1:1.12
Untreated control	1595.00[d]	–	–	–	–	–

*NPV was applied @ 3.0 × 10^{12} POB/ha in teepol 0.1%; crude sugar @ 2.5 kg/ha was used as adjuvant

**In a column means followed by similar letters are not significantly different by DMRT (P < 0.05). ANOVA statistics – yield F = 47.29, df = 16, P < 0.001

$Cost of virus treatment – Table 1; Cost of endosulfan treatment – Rs. 850/spray/ha

insignificant differences among the treatments. In all the observations on both the larval population and the pod damage, isolates CBE I and NEG caused a significant reduction in the larval population throughout and recorded a pooled mean of 5.17 and 5.50 larvae per 10 plants, respectively, with 61.88% and 59.42% reduction over control after six rounds of spray. In at least four of the six

Table 4. Field efficacy of HaNPV isolates against *H. armigera* on chickpea (cv. Shoba) (Kurumbapalayam, Coimbatore)

Treatment*	Number of larvae per 10 plant		Reduction over control (%)	Pod damage (%) (pooled mean)**	Reduction over control (%)
	pre-treatment count	pooled mean**			
CBE I	9.33	5.17[a]	61.88	9.72[ab]	54.52
NEG	10.33	5.50[ab]	59.42	9.26[a]	56.67
BAN I	9.67	6.50[cd]	52.03	11.11[c]	48.01
HYD	10.00	6.33[bcd]	53.28	10.80[bc]	49.44
MAH I	9.67	7.11[de]	47.53	12.48[d]	41.59
RHI	10.33	7.56[e]	44.26	13.22[d]	38.11
RAJ	9.67	9.33[f]	31.14	17.98[e]	15.86
Endosulfan 35 EC (700 g/ha)	9.00	6.00[abc]	55.75	10.48[bc]	50.96
Untreated control	10.33	13.56[g]	–	21.37[f]	–

*NPV was applied @ 1.5 × 10^{12} POB/ha in teepol 0.1%; crude sugar @ 2.5 kg/ha was used as adjuvant

**Pooled mean after six rounds of spray

In a column means followed by similar letters are not statistically different by DMRT (P < 0.05). ANOVA statistics – number of larvae F = 46.25, df = 40, P < 0.001; pod damage F = 89.30, df = 24, P < 0.001

Table 5. Yield and economics of HaNPV isolates in the control of *H. armigera* on chickpea (cv. Shoba) (Kurumbapalayam Coimbatore)

Treatments*	Yield** (kg/ha)	Yield increase over untreated control (kg/ha)	Value (additional yield) (Rs./ha)	Cost of protection$ (Rs./ha)	Net income (Rs./ha)	Cost/benefit ratio
CBE I	980.00[a]	286.67	5733.40	2 430.00	3 303.40	1:1.36
NEG	983.67[a]	290.34	5806.80	2 340.00	3 466.80	1:1.48
BAN I	886.67[b]	193.34	3866.80	2 490.00	1 376.80	1:0.55
HYD	871.67[b]	178.34	3566.80	2 610.00	956.80	1:0.37
MAH I	805.00[c]	111.67	2233.40	4 470.00	–2 236.60	1: –0.50
RHI	783.33[c]	90.00	1800.00	5 310.00	–3 510.00	1: –0.66
RAJ	718.33[d]	25.00	500.00	12 060.00	–11 560.00	1: –0.96
Endosulfan 35 EC (350 g/ha)	973.33[a]	280.00	5600.00	3 000.00	2600.00	1:0.87
Untreated control	693.33 [d]	–	–	–	–	–

*NPV was applied @ 1.5 x 10^{12} POB/ha in teepol 0.1%; crude sugar @ 2.5 kg/ha was used as adjuvant

**In a column means followed by similar letters are not significantly different by DMRT ($P \leq 0.05$). ANOVA statistics – yield $F = 73.32$, $df = 16$, $P < 0.001$

$Cost of virus treatment – Table 1; Cost of endosulfan treatment – Rs. 500/spray/ha

observations, the CBE I and NEG isolates along with adjuvant were found to be significantly more effective than endosulfan treatment. Like in the cotton experiment, the isolate RAJ did not exert any influence on the larval population on chickpea. Data on pod damage also showed significant differences among the treatments over control. Isolates NEG (56.67%) and CBE I (54.52%) recorded the maximum per cent reduction in pod damage over control (Table 4).

The highest yields were recorded in plots treated with NEG (983.7 kg/ha) and CBE I (980.0 kg/ha) and were comparable to those of endosulfan (973.3 kg/ha). The C/B ratio revealed that a maximum benefit was obtained in treatment with NEG (1:1.48) isolate and the isolate CBE I was placed next receiving the C/B ratio of 1:1.36. Though the other treatments recorded increased yields over untreated control, the net income and the C/B ratio were lower or negative due to the increased cost of production (Table 5).

DISCUSSION

Field efficacy of the selected isolates indicated a possibility of the selection of strains with higher virulence. Of all the isolates tested, NEG and CBE I applied along with adjuvant significantly reduced the population of *H. armigera* and pod damage compared to the other isolates evaluated. The isolate RAJ recorded the lowest yield comparable to that of the untreated control on both crops.

Limited reports are available on the field utility of various isolates of different insects. Rabindra *et al.* (1998) evaluated the efficacy of CBE I and NEG on chickpea under Coimbatore conditions in comparison with endosulfan and *B. thuringiensis*. Both isolates were found to control the pests, reduce the damage and increase the yield. Gopali & Lingappa (2001) reported that the application of Gulbarga and Coimbatore isolates to pigeon pea resulted in lower pod damage and higher grain yield compared to other isolates. Gulbarga isolates maintained their superiority and were highly cost effective.

While Rabindra *et al.* (1998) could not observe any significant differences between the two virulent isolates, CBE I and NEG, in the present investigations the differences in virulence under laboratory conditions were also reflected in the field. The study also brought to light that the higher cost of production of less virulent isolates (Tables 1–5) can adversely affect the C/B ratio. The isolates CBE I and NEG netted the higher C/B ratio of 1:2.32 and 1:2.48 on cotton and 1:1.36 and 1:1.48 on chickpea, respectively, than endosulfan which recorded 1:1.12 and 1:0.87 on cotton and chickpea, respectively.

The report of Gopali and Lingappa (2001) indicated the superiority of Gulbarga isolate over CBE at Dharwad District of Karnataka. It has been noted by several authors that the stabilization of virus occurs upon continuous serial passage (Teakle & Byrne 1989). The virulence of CBE I is believed to have reached the maximum as the isolates CBE I were put to test and serially passed over several decades since the work of Narayanan (1980). The reason for the Gulbarga isolate expressing superiority over CBE is not known. If conclusions are to be drawn that isolates have to be recommended based on the geographical regions of origin, from the commercial aspect and against the background of registration requirements in India, the investment cost is bound to increase. Moreover, Muthiah (1988) reported that the CBE I isolate expression was uniformly similar in several geographical populations of Tamil Nadu. Hence, the superiority of an isolate can be tested and verified under multilocation conditions before its large-scale release. The results of the investigations indicate the need for selection of isolates that give higher productivity *in vivo*, maintain increased virulence in the laboratory and exert a maximum influence on reducing the population, reducing the damage and increasing the yield under field conditions.

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