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Safety of UV-selected *Helicoverpa armigera* nucleopolyhedrovirus to non-target beneficial organisms

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ABSTRACT: **The safety of the UV-selected Coimbatore isolate of *Helicoverpa armigera* nucleopolyhedrovirus (*Ha*NPV-UVT-CBE1) was tested against the non-target organisms *viz.*, *Trichogramma chilonis*, *Chrysoperla carnea*, honey bee species and *Bombyx mori* to find out whether the cyclical exposure of *Ha*NPV to ultra-violet (UV) radiation could cause adverse effect on the above organisms. The results showed that the *Ha*NPV-UVT-CBE1 had no adverse effect on the growth parameters, survival and production parameters of the organisms tested in comparison with the UV shielded *Ha*NPV (*Ha*NPV-UVS-CBE1). The cyclical exposure of *Ha*NPV to UV radiation for the selection of UV tolerant strain did not have any deleterious effect on the safety of the virus to the non-target organisms.**

KEY WORDS: *Apis* sp., *Bombyx mori*, *Chrysoperla carnea*, *Helicoverpa armigera*, nucleopolyhedrovirus, safety, *Trichogramma chilonis*

INTRODUCTION

American bollworm, *Helicoverpa armigera* (Hubner) is a serious pest of legumes, cotton and vegetables in the Indian subcontinent and South East Asia (King, 1994). Changing scenario in the management of *H. armigera* embodies the nuclear polyhedrovirus (*Ha*NPV) as an important biocontrol agent. However, the use of baculoviruses as biological control agents is hampered by their susceptibility to inactivation by ultraviolet (UV) light. Hence artificial selection for UV tolerance was attempted in baculoviruses *viz.*, *Heliothis zea* (Boddie), *Heliothis virescens* (Fabricius) NPV (Bullock *et al.*, 1970) and *Cydia pomenella* (L.) granulovirus (*Cp*GV) (Brassel and Benz, 1979; Krieg *et al.*, 1980). Selection for UV tolerance was also attempted in *H. armigera* NPV. The UV selected *Ha*NPV strain was proved to have increased persistence with higher C/B ratio under field conditions on cotton (1: 3.25) and chickpea (1: 1.82) in comparison with unselected *Ha*NPV (Jeyarani, 2004). Utilization of baculoviruses as biopesticides requires proof that they are safe to non-target species, though they are isolated from naturally infected diseased insects. Several nucleopolyhedroviruses (NPVs) have been tested against beneficial organisms and natural enemies of insect pests (Maheshbabu, 1990; Heinz *et al.*, 1995; Geetha, 1997; Parthasarathy, 2002). Though it was reported that both prokaryotic and eukaryotic organisms including baculoviruses have evolved pathways to repair UV induced damage (Petrik *et al.*, 2003), the effect of UV selection of NPVs on the

safety of non-target organisms was not reported so far. It is also important to verify the safety of any organisms that are subjected to genetic improvement. Hence, keeping this in view, the present investigations were made on the safety of UV-selected/tolerant *Ha*NPV (*Ha*NPV-UVT-CBE I) to non-target organisms.

MATERIALS AND METHODS

Insect cultures

The parasitoids and predators, used in this study were obtained from the Biocontrol laboratory, honeybees from an Apiary and silkworms from Department of Sericulture, Tamil Nadu Agricultural University (TNAU), Coimbatore. The insects, *viz.*, *Trichogramma chilonis*, *Chrsyoperla carnea* and *Bombyx mori* were maintained at a constant temperature of 25.0 ± 2.0°C, honey bee species were maintained in an artificial hive and were utilized for the experiments at appropriate stages.

Virus inoculum

The Coimbatore isolate-I of *H. armigera* nucleopolyhedrovirus (*Ha*NPV-CBE1) maintained at the Biocontrol laboratory, TNAU was used in the present study. The inoculum was propagated in the early fifth instar *H. armigera* larvae by diet surface treatment method (Sathiah, 2001) and the concentration was standardized using Neuber Haemocytometer (Weber, England) (Evans and Shapiro, 1997). The *Ha*NPV–CBE1 was selected for UV tolerance by seven cyclical exposure to UV-B at a cumulative energy level of 11340 KJ / m^2 and at an irradiance level of 300 watts / m^2 in a weathering system (Sun Test CPS$^+$, Atlas GmbH Germany that generated UV-B from xenon arc lamp source). The *Ha*NPV-UVT–CBE I strain selected for UV tolerance was compared with the UV shielded strain (*Ha*NPV-UVS-CBE I, not selected for UV tolerance) for their safety to the non-target organisms.

Safety studies

The safety of *Ha*NPV-UVT-CBE1 strain was tested against *T. chilonis*, *C. carnea*, *Apis mellifera*, *A. cerana indica*, *Trigona iridipennis* and *B. mori*. The treatments for the safety tests included *Ha*NPV-UVT–CBE1 @ 2x10^7 POB ml^{-1}, *Ha*NPV-UVS–CBE1 @ 2x10^7 POB ml^{-1}, endosulfan 35EC @ 0.07% and control with water spray. Against silkworm, the insecticide treatment was not included as it is a known fact that they are highly sensitive to chemicals (Kariappa and Narasimhanna, 1981; Bhosale *et al.*, 1988).

T. chilonis

Newly emerged *T. chilonis* adults were anaesthetized using etherized carbon dioxide for 15 seconds (Tiwari and Khan, 2004) and were released @ 50 per treatment on the treated egg cards in polybags. Parasitization was allowed for two days after which the egg cards were transferred to fresh polybags. Observations on per cent parasitism, parasitoid emergence, adult longevity and total life cycle were recorded. The parasitoids emerged in the respective treatments in the first generation were counted by anaesthetizing with CO_2 and utilized for second generation studies and the procedure repeated.

C. carnea

Chrysoperla carnea first instar grubs emerging from previously treated *C. carnea* eggs were released @ 1: 100 (Grub: Eggs) per treatment to the UVT-CBE1, UVS-CBE I and endosulfan treated *H. armigera* eggs. Each treatment had 50 grubs in five replicates. The grubs were confined in test tubes covered with muslin cloth and secured tightly with a rubber band. The grubs were daily fed with treated one day old *H. armigera* eggs till pupation. After pupation, they were separated and transferred to plastic containers (20cm ht, and 8cm dia) for adult emergence. The adults were allowed in plastic jars and fed with a mixture of honey, protein hydrolysate, fructose, yeast and (1: 1: 1: 1: 1). Observations on hatching, larval duration, larval mortality, pupal period and adult longevity were recorded.

Honey bees

The safety of *Ha*NPV-UVT-CBE I and UVS-CBE I was tested with three honey bee species *viz.*,

A. cerana indica, A. mellifera and *T. iridipennis*. One day old worker bees were used @ 30 per treatment in five replicates. The bees were caged (30x30x30 cm) and were fed with 50 per cent sucrose solution containing respective viruses @ $2x10^7$ POB ml^{-1}. Similarly, endosulfan @ 0.07% was mixed in 50 per cent sucrose solution (50%) and fed for 24h. Afterwards, 50 per cent sucrose alone was provided till the bees died. In control, bees were fed with sucrose solution alone. The mortality of bees was observed daily until all the bees died. The bees were observed for the presence of viral particles in the tissues.

B. mori

Third instar larvae of hybrid race, PM x NB_4D_2 were fed with chopped mulberry leaves treated with HaNPV (UVT and UVS) @ $2x10^7$ POB ml^{-1} for 24h. Subsequently, fresh untreated leaf bits were provided at 12 h interval. Each treatment had 20 larvae replicated five times. A check without virus was maintained by feeding the larvae with leaves dipped in distilled water. Observations on the larval weight, mortality, cocoon weight, pupal period, shell weight and adult emergence were recorded.

Statistical analysis

Data were analyzed for significance using analysis of variance (ANOVA) and the means were separated using Duncan multiple range test (Duncan, 1966).

RESULTS AND DISCUSSION

Safety to *T. chilonis*

Exposure of *T. chilonis* to UVT-*Ha*NPV by egg treatment did not show any deleterious effect on parasitism and parasitoid emergence, however, the per cent parasitism, adult emergence was significantly lower in endosulfan treated eggs in the first generation (Table 1). Similar trend was also observed in the second generation. The parasitoid had a total life cycle of 9.2 and 9 days in UVS-CBE I treatment and 9 and 9.4 days in UVT-CBE1 treatment and the adult longevity was 3.2 and 3 days in UVS-CBE I and was 3 and 3.2 days in UVT-CBE1 both in the first and second generation, respectively which were on par with control. Safety of *Ha*NPV to *T. chilonis* has been reported by Muthiah and Rabindra (1991) and Boomathi *et al.* (2005).

Table 1. Safety of *Ha*NPV - UVT – CBE1 to *T. chilonis* by egg treatment method

a. Generation I

Treatment	Parasitization *(%)	Parasitoid emergence * (%)	Period (days) ^{s}	
			Adult longevity	Total life cycle
UVS – CBE1	81.20 ± 1.14[a]	91.60 ± 0.93[a]	3.20 ± 0.37	9.20 ± 0.20
UVT – CBE1	80.40 ± 1.21[a]	91.60 ± 0.68[a]	3.00 ± 0.32	9.00 ± 0.32
Endosulfan 35EC	77.60 ± 1.08[b]	89.20 ± 0.86[b]	2.60 ± 0.40	8.80 ± 0.37
Control	81.60 ± 1.28[a]	93.20 ± 0.89[a]	3.20 ± 0.20	9.40 ± 0.24

^{s} Differences between the means are not significant (P = 0.05) by DMRT; *in a column means followed by similar letters are not significantly different (P = 0.05) by DMRT

a. Generation II

Treatment	Parasitization *(%)	Parasitoid emergence * (%)	Period (days) ^{s}	
			Adult longevity	Total life cycle
UVS – CBE1	80.80 ± 0.58[a]	90.80 ± 0.58[a]	3.00 ± 0.45	9.00 ± 0.32
UVT – CBE1	80.40 ± 1.21[a]	91.20 ± 0.71[a]	3.20 ± 0.20	9.40 ± 0.24
Endosulfan 35EC	78.00 ± 0.71[b]	88.40 ± 0.93[b]	3.00 ± 0.32	9.00 ± 0.32
Control	82.00 ± 0.84[a]	91.60 ± 0.68[a]	3.20 ± 0.20	9.20 ± 0.20

^{s} Differences between the means are not significant (P = 0.05) by DMRT; *in a column means followed by similar letters are not significantly different (P = 0.05) by DMRT

Table 2. Safety of *Ha*NPV - UVT – CBE I to *C. carnea*

Parameter	Treatment			
	UVS – CBEII	UVT – CBEII	Endosulfan	Control
Hatchability (%) ^{s}	92.80±0.86	93.20±0.66	91.20±0.55	94.00±0.32
Larval period (days) ^{s}	10.40±0.24	10.40±0.24	9.80±0.20	10.20±0.20
Pupation (%) *	81.20±0.68 ^{a}	82.00±0.71 ^{a}	79.20±0.58 ^{b}	81.20±0.58 ^{a}
Pupal period (days) ^{s}	10.40±0.24	10.20±0.20	10.80±0.20	10.20±0.20
Adult emergence (%) *	89.60±0.37 ^{ab}	90.40±0.68 ^{a}	88.40±0.40 ^{b}	90.40±0.51 ^{a}
Adult longevity (days) ^{s}	37.00±0.45	36.40±0.81	35.60±0.60	37.40±0.40
Total life cycle (days) *	59.80±0.37 ^{a}	60.00±0.55 ^{a}	58.20±0.20 ^{b}	59.80±0.37 ^{a}
Larval mortality (%) *	8.80±0.37^{a}	9.20±0.32 ^{a}	10.40±0.20^{b}	8.40±0.40 ^{a}

^{s} Differences between the means are not significant (P = 0.05) by DMRT; *in a column means followed by similar letters are not significantly different (P = 0.05) by DMRT

Table 3. Safety of *Ha*NPV - UVT – CBE I to different species of honey bees

Species	Treatment	Per cent Honey bee mortality (days after feeding)				Mean number of days survived *
		5 *	10	15	20	
Apis cerana indica	UVS – CBE1	43.33^{a}	66.67	96.67	100.00	11.00 ± 0.41 ^{a}
	UVT – CBE1	46.67^{b}	66.67	93.33	100.00	11.50 ± 0.29 ^{a}
	Endosulfan	100.00 ^{c}	-	-	-	0.75 ± 0.25 ^{b}
	Control	43.33 ^{a}	63.33	96.67	100.00	11.75 ± 0.48 ^{a}
Apis mellifera	UVS – CBE1	20.00 ^{a}	50.00	86.67	100.00	16.75 ± 0.48 ^{a}
	UVT – CBE1	23.33 ^{b}	46.67	83.33	96.67	16.25 ± 0.25 ^{a}
	Endosulfan	100.00 ^{c}	-	-	-	1.25 ± 0.25 ^{b}
	Control	20.00 ^{a}	46.67	86.67	96.67	17.25 ± 0.25 ^{a}
Trigona iridipennis	UVS – CBE1	53.33 ^{a}	93.33	100.00	-	8.50 ± 0.29 ^{a}
	UVT – CBE1	56.67 ^{b}	93.33	100.00	-	8.75 ± 0.25 ^{a}
	Endosulfan	100.00 ^{c}	-	-	-	0.50 ± 0.29 ^{b}
	Control	56.67 ^{a}	96.67	100.00	-	9.00 ± 0.41 ^{a}

* In a column means followed by similar letters are not significantly different (P = 0.05) by DMRT

Safety to *C. carnea*

Chrysoperla carnea exposed to *Ha*NPV-UVT-CBE1 isolate showed no adverse effects on per cent hatchability, larval period, per cent pupation, pupal period, adult emergence, adult longevity, total life cycle and larval mortality in comparison with UVS-CBE1 and control. Endosulfan was found to be toxic compared to NPV and control. Larval mortality of 8.8 and 9.2 per cent in UVS-CBE1 and UVT-CBE1 indicated that larval mortality in the virus treatment was due to natural causes only and was on par with control (8.4%). Application of UVS and UVT-CBE I had no adverse effect on *C. carnea* with reference to pupation (81.2 and 82%), adult emergence (89.6 and 90.4%) and total life cycle (59.8 and 60 days), respectively (Table 2). Safety of baculoviruses to *C. carnea* has been reported by many workers (Heinz *et al.*, 1995; Subramanian, 2003) and the present investigation also proved the safety of UV selected *Ha*NPV. This shows that

Table 4. Safety of *Ha*NPV - UVT – CBE I to *B. mori*

Parameter	Treatment*		
	UVS – CBEII	UVT – CBEII	Control
Larval weight (g) at instar III	0.414±0.005	0.412±0.005	0.427±0.013
Larval weight (g) at instar IV	1.502±0.025	1.492±0.031	1.553±0.026
Larval weight (g) at instar V	3.224±0.056	3.185±0.044	3.273±0.021
Larval mortality (%)	12.143±1.010	11.428±0.922	10.714±0.714
Pupation (%)	82.143±1.056	81.714±0.565	83.571±0.481
Pupal period (days)	10.571±0.297	10.857±0.261	10.714±0.184
Adult emergence (%)	90.000±0.488	90.143±0.459	91.286±0.421
Cocoon weight (g)	1.683±0.020	1.666±0.021	1.715±0.012
Shell weight (g)	0.361±0.007	0.357±0.010	0.358±0.008

the *Ha*NPV on UV exposure had not undergone any modification that could cause interference with the entomophages activity. However exposure to endosulfan treated eggs caused significant variation in the biological parameters like egg hatchability, larval period, pupal period and adult longevity of *C. carnea*.

Safety to honey bees

Results indicated no observable difference in the behaviour of caged bees during this test. Longevity of *A. cerana indica* was 11 and 11.5 days in UVS-CBE1 and UVT-CBE1, respectively, and was on par with control (11.75 days), while it was 16.75, 16.25 and 17.25 days in *A. mellifera* and 8.5, 8.75 and 9 days in *T. iridipennis* for UVS-CBE1, UVT-CBE1 and control, respectively (Table 3). Microscopical examination of the tissue smears revealed no evidence of infection or other pathological manifestations in the bees, perhaps because of cage effect mortalities were also noted in control. However, honeybees fed with endosulfan (0.07%) through sucrose solution caused 100 per cent mortality within five days. The safety of viruses to honey bees was reported earlier by Dhaduti and Mathad (1980), Santharam *et al.* (1982) and Parthasarathy (2002).

Safety to *B. mori*

Results on the larval and cocoon parameters revealed that they were not affected by the virus treatments in comparison with control. UVT-CBE1 and UVS-CBE1 recorded the shell weight of 0.357g and 0.361g, respectively which was on par with control (0.358g) (Table 4). Dhaduti and Mathad (1979) reported that *Mythimna separata* (Walker) NPV was innocuous to *B. mori*. No signs of abnormality in *B. mori* treated with *Ha*NPV were reported earlier (Bijjur *et al.*, 1991; Muthiah and Rabindra, 1991). Safety of other baculoviruses were also established previously (Parthasarathy, 2002; Subramanian, 2003).

The findings revealed the relative safety of UV selected *Ha*NPV to the beneficial organisms and natural enemies that are often encountered along with the *H. armigera* under field conditions.

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