

Original Research Article

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Safety Studies of DpNPV (*Diaphania pulverulentalis* Nuclearpolyhedrosis Virus) Suspension and its Formulation on Non-Target Organisms in Mulberry Ecosystem

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ABSTRACT

Laboratory studies were carried out to explore the potential of *Diaphania pulverulentalis* (Hampson.) nucleopolyhedrosis virus (*DpNPV*) suspension and its formulations against non-target organisms viz., *Trichogramma chilonis* Ishii, *Chrysoper lacarnea* (Stephens), *Bombyx mori* (L.), *Apis cerena indica* (Fab.), *Apis mellifera* (L.) and *Apis florea*. The per cent parasitization, adult emergence of *T. chilonis* exposed to *DpNPV* suspension did not differ significantly with that of *DpNPV* formulations and control, while, the per cent parasitization and adult emergence were significantly lower in dichlorvos treated eggs. *C. carnea* exposed to *DpNPV* suspension showed no adverse effects on per cent hatchability, larval period, per cent pupation, pupal period, adult emergence, adult longevity, total life cycle and grub mortality in comparison with *DpNPV* formulations and control. Whereas, dichlorvos was found to be hazardous compared to NPV and control. The larval weight of third, fourth and fifth instar of *B. mori*, per cent larval mortality, pupation, pupal period, adult emergence cocoon weight and shell weight exhibited no significant differences between the virus treated and control indicating the safety of the *DpNPV* suspension and its formulation. The survival period of bees in virus treatments was on par with control, while the survival period of bees was significantly low (1-2 days only) in dichlorvos. Results indicated no evidence of infection or other pathological manifestations in the tissues of bees.

Keywords

DpNPV,
*Diaphania
pulverulentalis*,
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Introduction

Regular usage of toxic chemicals in mulberry garden to control the pests cause pollution and are detrimental to human beings and beneficial organisms including silkworms. Further, the pests develop resistance to the chemical insecticides with indiscriminate use and result in sudden outbreak. In view of these, pest management using non-chemical methods have gained importance including

biological control. Among bio control agents, baculovirus are very important as they are arthropod specific pathogens. Higher host specificity and amenability for formulation as that of chemical pesticides make baculoviruses particularly attractive as biological control agents (Dent and Jenkins, 2000). The Baculoviridae is a promising family of viruses that might provide active

agents for successful biopesticides because members of two groups, the nucleopolyhedroviruses (NPVs) and the granulosis viruses (GV), infect many important insect pests (Blissard *et al.*, 2000; Fauquet *et al.*, 2004). NPV based biopesticides, along with the use of feeding stimulants that encourage phytophagous larvae to consume foliage contaminated with viral occlusion bodies (OBs) could result in an increased prevalence of infection and improved pest control (Lasa *et al.*, 2007).

The biopesticidal potential of baculoviruses has not been completely realized due to lack of information about particular properties of the virus that are involved in virulence. Considerable effort has been made to understand more about the molecular biology of baculoviruses and how they interact with host insect at the molecular level with the expectation that this information would aid in improving virus efficacy (Burand and Park, 1992).

One of the major drawbacks of using entomopathogens as biopesticides is their lack of persistence in the environment. Their infectivity is affected by environmental factors such as sunlight radiation, temperature, moisture and pH. These factors limit the field application and subsequent commercialization of many entomopathogens, including baculoviruses (Rabindra and Jayaraj, 2005).

Baculoviruses, particularly, nucleopolyhedroviruses (NPV), are deleteriously affected by sunlight radiation (Priyadharshini, 2009). Formulation of NPV based biopesticides could improve their efficacy to achieve acceptable levels of pest control with low doses of pathogen, representing an important reduction in the cost of each application (Lasa *et al.*, 2009). Inactivation of viruses on foliage has been a major problem in the development

of formulations of viral insecticides for use in insect management systems. Use of adjuvants has been found to increase the persistence of the virus in the environment (Mehrvar *et al.*, 2008). The incorporation of adjuvants with microbial insecticides to preserve the virus activity is commonly followed (Muthuswami *et al.*, 1994; Rabindra and Jayaraj, 1995). This is usually attributed to the improved field persistence of the virus due to increased consumption of the virus by the pest (Arivudainambi *et al.*, 2000; Amin *et al.*, 2005). Being obligate pathogens, viruses cannot multiply outside the environment of the host insect and have to remain in a viable state before they are ingested by the host insect.

A number of materials tested for use as adjuvants to protect the baculoviruses from sunlight inactivation, enhance activation over the foliage and effective intake by lepidopteran larvae (Sajap *et al.*, 2007). Tinopal, sugars such as sucrose, fructose and sorbitol have been proved to increase the efficacy of NPV formulation (Sajap *et al.*, 2009).

The use of pesticide on mulberry is discouraged due to its broad spectrum nature and therefore, could have a great fatal effect on the predators of leaf webber. Though pesticides could give quick relief to the pest problem owing to their knock down effect, these strategies cannot be employed on the pest when the silkworm rearing is under progress, because of detrimental effect of the chemical on silkworm.

Baculoviruses are used as an excellent biological insecticide due to its restricted host specificity and non-infectivity to beneficial insects, with this background the present investigation on the pathogenicity, development of formulation and its safety to non-target organisms was carried out.

Materials and Methods

The safety of *DpNPV* suspension and its formulations to the following non-target organisms viz., Egg parasitoid-*Trichogramma chilonis* Ishii, Predator - *Chrysoperla carnea* (Stephens), Silkworm - *Bombyx mori* (L.), Honey bees species like *Apis cerana indica* (Fab.), *Apis mellifera* (L.) and *Apis florea* were tested with the following treatments viz., T1-*DpNPV** + Starch 10% + Tinopal 0.2% + Tween 80 1%, T2- *DpNPV** + Sucrose 10% + Tinopal 0.2% + Tween80 1%, T3- *DpNPV** + Glycerol 10% + Tinopal 0.2% + Tween80 1%, T4- *DpNPV* @ 1×10^9 POBs/ml, T5- Dichlorvos @ 0.2 ml/lit and T6- Control with water spray. The dosage of virus which used in the treatments is **DpNPV* @ 1×10^9 POBs/ml, the above treatments with five replications included for safety tests.

Trichogramma chilonis

Freshly laid *D. pulverulentalis* eggs on cloth strips were used for the study of parasitism and parasitoid emergence. Each cloth strip was cut into pieces of size 2x2 cm with egg density ca. 200 in five replicates for each treatment. The pieces were stapled firmly to 144 gsm paper and exposed to UV for 20 min to kill the developing embryo. The treatments were applied on the egg cards with the help of an atomizer using a spray fluid volume of 2 ml. The cards were allowed to shade-dry for 30 min and transferred to poly bags.

Newly emerged parasitoids of *T. chilonis* were anaesthetized with CO₂ and 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 and parasitoid emergence were recorded. Parasitoids that emerged in the respective treatments in the first generation were counted by anaesthetizing with CO₂ and

utilized for second generation studies and the procedure was repeated.

Chrysoperla carnea

Dry film method was adopted to access the *DpNPV* to *C. carnea*, though the virus and dichlorvos treated *D. pulverulentalis* eggs previously exposed to UV, *C. carnea* first instar grubs emerging from previously treated *C. carnea* eggs were released @ 1:100. 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 *D. pulverulentalis* eggs till pupation. After pupation, they were separated and transferred to plastic jars (20 cm ht, and 8 cm dia) for adult emergence. The adults were allowed in plastic jars and fed with a mixture of honey, protein hydrolysate, fructose, yeast and water in the ratio 1:1:1:1. Observations on grub mortality, per cent pupation, hatching and adult longevity were recorded.

Bombyx mori

Larvae of III, IV, V instars of double hybrid DH₁ silkworm were fed with chopped mulberry leaves treated with *DpNPV* suspension @ 1×10^9 POBs/ml *DpNPV* formulation (*DpNPV** + Starch 10% + Tinopal 0.2% + Tween 80 1%) for 24 h. Subsequently, fresh untreated leaf bits were provided at 12 h interval. Control with water spray was maintained. Each treatment was replicated five times with 20 larvae. A check without virus was maintained by feeding the larvae with leaves dipped in distilled water. Observations on the weight of larvae, mortality of larvae, fresh weight of cocoon, shell weight and adult emergence were recorded.

Honey bees

Safety of *DpNPV* was tested for honey bees viz., *A. cerana indica*, *A. mellifera* and *A.*

florea. Day old worker bees were caged (30x30x30 cm) at the rate of 30 without the queen and fed with 50 per cent sucrose solution containing *DpNPV* virus @ 1×10^9 POB/ml. Similarly, different *DpNPV* formulations and dichlorvos 0.2% were mixed in 50 per cent sucrose solution and fed for 24 h. 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.

Results and Discussion

The results of the experiments on safety of *DpNPV* suspension and its formulation to non- target organisms, laboratory experiments conducted to assess the bioefficacy of different *DpNPV* formulations against mulberry leaf webber – *D. pulverulentalis* in mulberry and safety to non- target organisms are presented below.

Safety tests on non-target organisms

T. chilonis

In the present study, *DpNPV* was found to be safe to *T. chilonis* when it was treated on egg, further it did not show any deleterious effect on parasitization and parasitoid emergence (Fig. 1). HaGV and PxGV were found to be safe to *T. chilonis* (Kuppusamy, 1994; Sairabanu, 2000). Safety of baculoviruses to *T. chilonis* was reported earlier by Ethiraju (1986), Muthiah and Rabindra (1991), Maheshbabu (1991) and Geetha (1997).

HaNPV was not found to be pathogenic to *T. chilonis* (Balasubramanian *et al.*, 2001). Boomathi *et al.*, (2005) reported that the use of *HaNPV* @ 3×10^{12} POBs ha^{-1} and Spicturin (commercial *Bt* formulation) @ 2.0 L ha^{-1} found to be safe to the egg parasitoid (*T. chilonis*). In laboratory studies, a UV-

selected strain of *HaNPV* was found to be safer to *T. chilonis*, *Chrysoper lacarnea* (Stephen), honeybee and *Bombyx mori* L. (Jeyarani *et al.*, 2008).

Reduction of *H. armigera* parasitoid, *Camponotus chloridiae* Uchida and other natural enemies was lower in the *HaNPV* sprayed plots (3%) as compared to 60 per cent reduction in the endosulfan treated plots in chickpea. *HaNPV*@ 250 LE ha^{-1} application on chickpea resulted in a reduction of aerial and soil inhabiting natural enemies by 15 and 22 per cent respectively, over the control plots, while the reduction in the endosulfan sprayed plots was 52.4 and 63.1 per cent, respectively (Ranga Rao *et al.*, 2008).

C. carnea

In the present study, *DpNPV* was found to be safe to *C. carnea* with reference to pupation, adult emergence and total life cycle (Table 1 and Fig. 2). Safety of baculoviruses to *C. carnea* has been reported by many workers in *HaGV* (Kuppusamy, 1994) and *PxGV* (Sairabanu, 2000) to *C. carnea*. Safety of other baculoviruses to *C. carnea* has been reported by many workers (Maheshbabu, 1991; Heinz *et al.*, 1995; Thennarasan, 1997; Geetha, 1997; Subramanian, 1998).

However, exposure to endosulfan treated eggs caused significant variation in the biological parameters of *C. carnea*. It could be inferred that NPV is compatible with *C. carnea* and could be utilized in IPM.

B. mori

In the present studies, application of *DpNPV* and its formulations was found to be safe to *B. mori*. The larval and cocoon parameters were not affected by the virus treatments (Table 2). Application of *DpNPV* had no adverse effects on *B. mori*. In earlier

investigations, the granulosis viruses of *Chiloinfus catellus*, *Chilosaccharipha gusindicus*, *Adalia bipunctata*, *Helicoverpa armigera* and *Spodoptera exigua* NPV were not infective to *B. mori*, *T. chilonis* and *C. carnea* (Easwaramoorthy and Jayaraj, 1988; Chen *et al.*,

1992; Kuppusamy, 1994; Kondo *et al.*, 1994). Mahiba Helen *et al.*, (2012) proved the safety of *HpNPV* to *B. mori* larvae. This indicated that the viruses could be effectively utilized in mulberry ecosystem where sericulture is practiced.

Table.1 Selective toxicity of *DpNPV* and its formulation to adults of *Chrysoperla carnea*

S.No	Treatments	Mortality (%)		
		12 HAT	24 HAT	48 HAT
1.	<i>DpNPV</i> *+ Starch 10% +Tinopal 0.2% + Tween80 1%	0.00 (0.19) ^a	3.33 (10.25) ^b	3.33 (10.25) ^a
2.	<i>DpNPV</i> *+ Sucrose 10% + Tinopal 0.2% + Tween80 1%	6.67 (14.70) ^b	6.67 (14.70) ^c	6.67 (14.70) ^b
3.	<i>DpNPV</i> *+ Glycerol + Tinopal 0.2% +Tween80 1%	6.67 (14.96) ^b	10.00 (18.43) ^c	13.33 (21.41) ^c
4.	<i>DpNPV</i> *	13.33 (21.39) ^c	33.33 (35.26) ^d	36.67 (37.27) ^d
5.	Dichlorvos76EC 0.2%	33.33 (35.26) ^d	56.67 (48.83) ^e	63.33 (52.73) ^e
6.	Untreated check	0.00 (0.19) ^a	0.00 (0.19) ^a	3.33 (10.32) ^a

**DpNPV* @ 1x10⁹ POB/ml, Mean of three observations,

In a column, means followed by a common letter are not significantly different (P = 0.05) by DMRT

Figures in parentheses are arcsine transformed values

HAT – Hour after treatment

Table.2 Safety of *DpNPV* formulation and suspension on *Bombyx mori*

S.No	Parameters	Treatments**		
		<i>DpNPV</i> * Formulation	<i>DpNPV</i> * Suspension	Control
1.	Larval weight (g) at instar III	0.414 ± 0.005	0.412 ± 0.005	0.427 ± 0.013
2.	Larval weight (g) at instar IV	1.502 ± 0.025	1.492 ± 0.031	1.553 ± 0.026
3.	Larval weight (g) at instar V	3.224 ± 0.056	3.185 ± 0.044	3.273 ± 0.021
4.	Larval mortality (%)	12.143 ± 1.010	11.428 ± 0.922	10.714 ± 0.714
5.	Pupation (%)	82.143 ± 1.056	81.714 ± 0.565	83.571 ± 0.481
6.	Pupal period (days)	10.571 ± 0.297	10.857 ± 0.261	10.714 ± 0.184
7.	Adult emergence (%)	90.000 ± 0.488	90.143 ± 0.459	91.286 ± 0.421
8.	Cocoon weight (g)	1.683 ± 0.020	1.666 ± 0.021	1.715 ± 0.012
9.	Shell weight (g)	0.361 ± 0.007	0.357 ± 0.010	0.358 ± 0.008

**DpNPV*@ 1x10⁹ POB/ml; ** Differences between means were not significant (P=0.05) by DMRT

Table.3 Safety of *DpNPV* and its formulations to honey bee – *Apis cerana indica*

Species	Treatments	Per cent bee mortality (days after feeding)				Mean number of days survived *
		5 *	10	15	20	
<i>Apis cerana indica</i>	<i>DpNPV</i> * + Starch 10% +Tinopal 0.2% + Tween80 (1%)	46.67 ^b	66.67	93.33	100.0	11.50± 0.29 ^a
	<i>DpNPV</i> * + Sucrose 10% + Tinopal 0.2% + Tween80 (1%)	43.33 ^a	66.67	96.67	100.0	11.00± 0.41 ^a
	<i>DpNPV</i> * + Glycerol 10%+Tinopal 0.2% +Tween80 (1%)	40.42 ^a	62.47	96.77	100.0	11.00± 0.41 ^a
	<i>DpNPV</i> *	43.93 ^a	65.67	96.07	100.0	11.00± 0.41 ^a
	Dichlorvos76EC 0.2%	100.0 ^c	-	-	-	0.75± 0.25 ^b
	Control	43.33 ^a	63.33	96.67	100.0	11.75 ± 0.48 ^a

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

**DpNPV*@ 1x10⁹ POB/ml

Table.4 Safety of *DpNPV* and its formulations to honey bee – *Apis mellifera*

Species	Treatments	Per cent bee mortality (days after feeding)				Mean number of days survived *
		5 *	10	15	20	
<i>Apismellif era</i>	<i>DpNPV</i> * + Starch 10% +Tinopal 0.2% + Tween80 (1%)	23.33 ^b	46.67	83.33	96.67	16.25 ± 0.25 ^a
	<i>DpNPV</i> * + Sucrose 10% + Tinopal 0.2% + Tween80 (1%)	20.00 ^a	50.00	86.67	100.0	16.75 ± 0.48 ^a
	<i>DpNPV</i> * + Glycerol 10%+Tinopal 0.2% +Tween80 (1%)	19.50 ^a	51.00	87.67	100.0	16.75 ± 0.48 ^a
	<i>DpNPV</i> *	19.50 ^a	51.00	87.67	100.0	16.75 ± 0.48 ^a
	Dichlorvos76EC 0.2%	100.0 ^c	-	-	-	1.25 ± 0.25 ^b
	Control	20.00 ^a	46.67	86.67	96.67	17.25 ± 0.25 ^a

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

**DpNPV*@ 1x10⁹ POB/ml

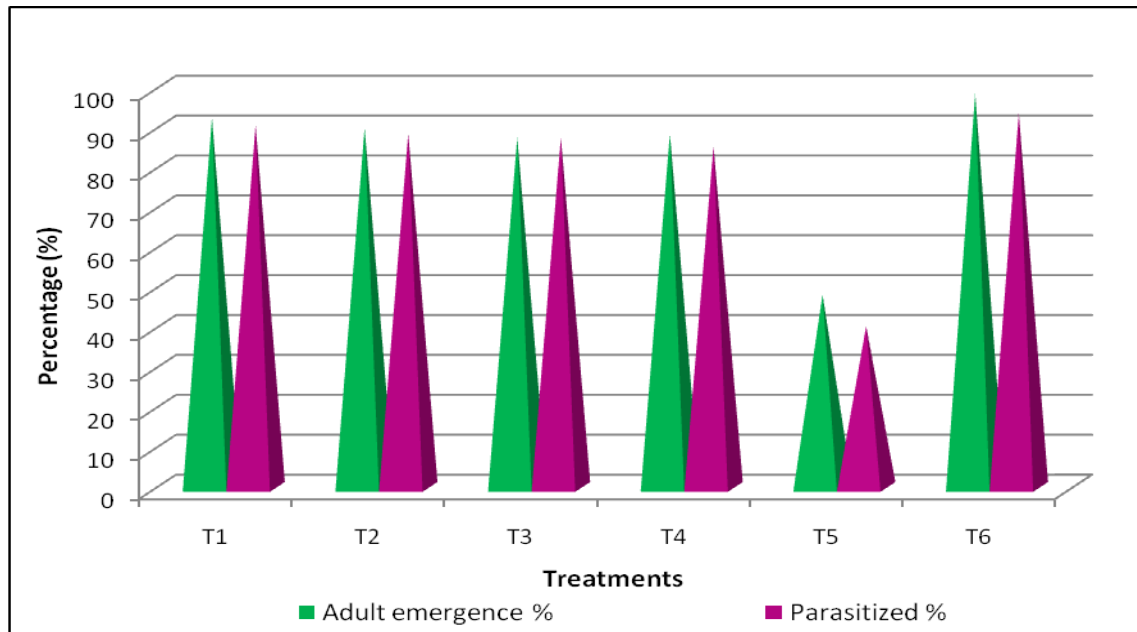
Table.5 Safety of *Dp*NPV and its formulations to honey bee – *Apis florea*

Species	Treatments	Per cent bee mortality (days after feeding)				Mean number of days survived
		5 *	10	15	20	
<i>Apis florea</i>	<i>Dp</i> NPV* + Starch 10% +Tinopal 0.2% + Tween80 (1%)	56.67 ^b	93.33	100.0	-	8.75 ± 0.25 ^a
	<i>Dp</i> NPV* + Sucrose 10% + Tinopal 0.2% + Tween80 (1%)	53.33 ^a	93.33	100.0	-	8.50 ± 0.29 ^a
	<i>Dp</i> NPV* + Glycerol 10%+Tinopal 0.2% +Tween80 (1%)	52.33 ^a	93.33	100.0	-	8.50 ± 0.29 ^a
	<i>Dp</i> NPV*	52.70 ^a	94.0	100.0	-	8.50 ± 0.29 ^a
	Dichlorvos76EC 0.2%	100.0 ^c	-	-	-	0.50 ± 0.29 ^b
	Control	56.67 ^a	96.67	100.0	-	9.00 ± 0.41 ^a

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

**Dp*NPV@ 1x10⁹ POB/ml

Fig.1 Safety of *Dp*NPV and its formulation on *Trichogramma chilonis* Ishii



T1- *Dp*NPV 1x10⁹ POB/ml + Starch 10% + Tinopal 0.2% + Tween80 1%

T2- *Dp*NPV 1x10⁹ POB/ml + Sucrose 10% + Tinopal 0.2% + Tween80 1%

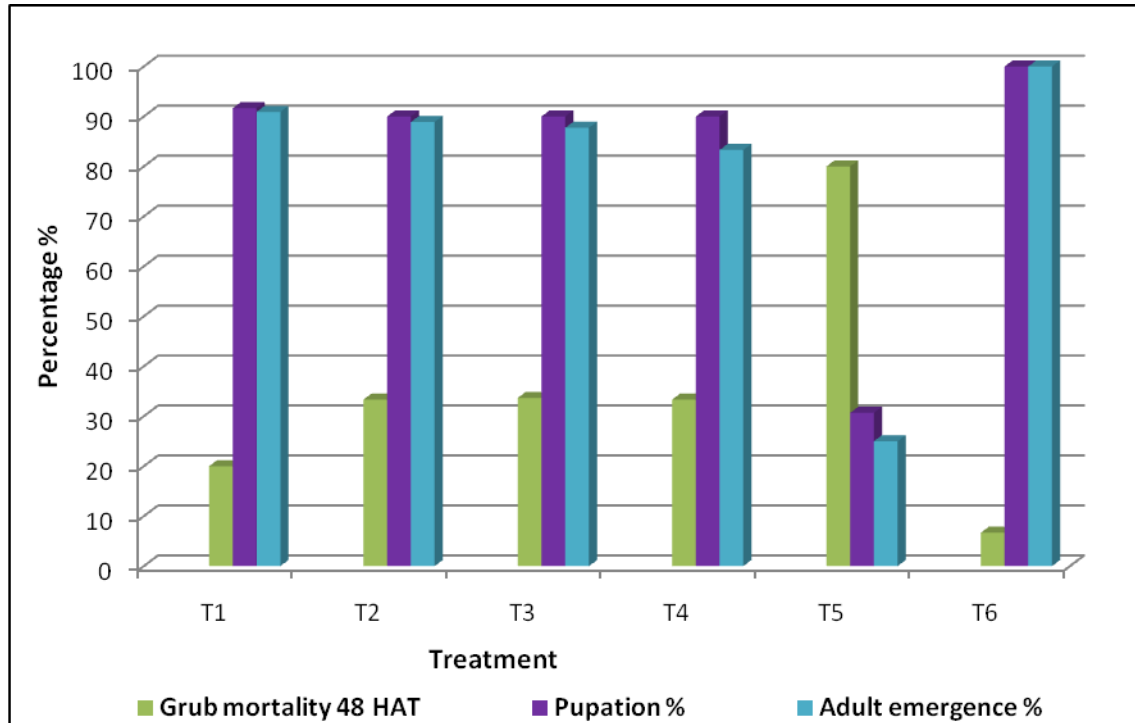
T3- *Dp*NPV1x10⁹ POB/ml + Glycerol 10% + Tinopal 0.2% + Tween80 1%

T4- *Dp*NPV 1x10⁹ POB/ml

T5- Dichlorvos 76EC 0.2%

T6- Untreated check

Fig.2 Safety of DpNPV and its formulation to *Chrysoperla carnea* Stephens



T1- *DpNPV* 1×10^9 POB/ml + Starch 10% + Tinopal 0.2% + Tween80 1%

T2- *DpNPV* 1×10^9 POB/ml + Sucrose 10% + Tinopal 0.2% + Tween80 1%

T3- *DpNPV* 1×10^9 POB/ml + Glycerol 10% + Tinopal 0.2% + Tween80 1%

T4- *DpNPV* 1×10^9 POB/ml

T5- Dichlorvos 76EC 0.2%

T6- Untreated check

Honeybees

The *DpNPV* and its formulations did not show any harmful effect in terms of longevity of various honey bee species (Tables 3, 4 and 5). No changes in the behaviour of the bees were noticed in the adults fed with NPV in sugar solution. While, honey bees fed with dichlorvos in sugar solution caused 100 per cent mortality of the bees within two days. The safety of viruses to honey bees was reported earlier by Dhaduti and Mathad (1980), Santharam *et al.*, (1982), Muthiah (1988) and Parthasarathy (2002). The NPV of *Mamestra brassicae* had no harmful effect on the honeybees (Groner *et al.*,

1978). *Nomuraea rileyi* (Farlow) Samson was found to be safe to the larval parasitoid, *Microplitis maculipennis* Szep and honey bee, *Apis cerana indica* Fab., in the laboratory studies (Mulimani and Kulkarni, 2004). No significant changes in the behaviour of the caged bees were observed both in treated and untreated adults. Results of the present investigations revealed the safety of *DpNPV* and its formulations to *B. mori* and honey bees and hence could be integrated in bio-intensive pest management programmes.

In conclusion, studies undertaken revealed that formulated *DpNPV* showed effective control of mulberry leaf webber compared to

DpNPV suspension under laboratory condition and also exhibited safety to non-target organisms viz., *T. chilonis*, *C. carnea*, *B. mori* and *A. indica*, *A. mellifera* and *A. florea* and it could be used as a safe biopesticide in mulberry ecosystem.

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