IN VITRO EVALUATION OF *DP*NPV (NUCLEAR POLYHEDROSIS VIRUS) FORMULATIONS AGAINST MULBERRY LEAF WEBBER, *DIAPHANIA PULVERULENTALIS* (Hampson)

S. PRABHU*
P.PRIYADHARSHINI**
C. A. MAHALINGAM***

*Teaching Assistant, Department of Sericulture, Forest College & Research Institute, Mettupalayam, Tamil Nadu, India
**Assistant Professor, Department of Sericulture, Forest College & Research Institute, Mettupalayam, Tamil Nadu, India
***Professor, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Abstract

Bioassay under *in vitro* condition using early third in star larvae of *Diaphania pulverulentalis* with different *Dp*NPV formulations showed subtle variations in larval mortality based on their combinations of *Dp*NPV formulations. Among the various formulations tested, the phagostimulants *i.e.*, starch, sucrose and glycerol at 10 per cent concentration with the combination of UV protectant tinopal 0.2 per cent and virus *Dp*NPV@ $1x10^9$ POB showed maximum mortality of 82.33, 81.44 and 80.88 per cent mortality at 12 DAS (Days After Spraying) respectively, which was followed by the treatments *viz.*, T_4, T_8, T_{12}, With the above combination of phagostimulants at 10 per cent concentration with UV protectant congo red 1 per cent showed mortality of 76.55, 75.44 and 76.99 per cent respectively and were on par were recorded. With the combination of phagostimulants at 5 per cent concentration and tinopal 0.2 per cent recorded mortality of 70.03, 69.99, 69.66 per cent which was followed by T_3 (62.22), T_7 (62.88), T_{11}(61.11) showed which were on par. When virus alone *Dp*NPV@ $1x10^9$ POB/ml were used, it showed, minimum mortality of 55.01 per cent at 12 DAS which was followed by untreated control (4.88 %). Dichlorvos 76 EC @ 0.2 per cent which was used as an insecticide check showed 100 per cent mortality at 5 DAS. The present study clearly showed that the *Dp*NPV formulations possess larvicidal activity and could be used for the management of *D. pulverulentalis*.

Keywords: *Dp*NPV, Formulations, Phagostimulants

Introduction

Mulberry (*Morus* sp.) is the sole food source for silkworm *Bombyx mori* L. It is a perennial crop cultivated in all types of soils under both rainfed and irrigated conditions. It is prone to depredation by diverse organisms because of its fast growth, nutritional abundance and possession of green foliage throughout the year. So far, more than

300 insect and non-insect species are known to inhabit the mulberry cropping system in varying intensities during different stages of crop and season (Narayanaswamy *et al.*, 1996).

Mulberry leaf webber (*Diaphania pulverulentalis* H.) is a serious pest of mulberry, it attacks the tender leaves of the host causing considerable damage which alters the leaf quality. The incidence of the pest occurs during June - February months and causes severe damage to young plantation and it affects severe loss in tender chawki leaves, which is very much essential for young age silkworm larvae. The incidence of *D. pulverulentalis* was found to be 22 per cent during June and increased to 85 and 100 per cent during September and December months respectively. (Rajadurai *et al*., 2003).

Timely management of insect pests is essential to avoid economic loss to farmers. However, the regular usage of toxic chemicals in mulberry garden to control the pests caused pollution and was detrimental to silkworms. Further, the pests developed resistance to the chemical insecticides with indiscriminate use and resulted in sudden outbreak. In view of these, pest management using non-chemical methods gained importance including biological control.

One of the most important naturally occurring pathogens of *D. Pulverulentalis* larvae is Nuclear Polyhedrosis Virus (NPV), a baculovirus capable of causing epizootics in the larval population that result in declines or elimination of populations (Priyadharshini and Narayanaswamy, 2012). One of the major drawbacks of using entomopathogens as biopesticides is their lack of persistence in the environment. 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). Hence the present investigation was made on the larvicidal effect of *Dp*NPV formulation against *D. Pulverulentalis*.

MATERIALS AND METHODS

A laboratory assay was conducted to evaluate the virulence of *Dp*NPV formulation with different combinations against early third instars of *D. pulverulentalis* using leaf disc method as described by Sajap *et al.* (2007). Fifty ml of virus suspension was spread evenly over fresh mulberry leaf discs (15 mm in diameter) and dried at room temperature for 15 min. The leaf discs were then placed in 9 cm petri dishes lined with two layers of filter paper. Third instars were transferred individually to the petri dishes. After consumption of entire leaf disc, the larvae were fed with untreated leaves and placed in a room at 27 ± 2^0C temperature and

70±5% RH. The leaves were changed daily. Larval mortality was monitored daily until pupation. Larvae were fed with leaves treated with distilled water in control treatment. The experiment was conducted in three replicates with 30 larvae/treatment. Larval mortality caused by virus with adjuvants was also compared with mortality caused by virus alone (Karma choden *et al*., 2012).

RESULTS AND DISCUSSION

The control of pest by the massive use of chemical insecticides has resulted in development of resistance of insects, non-specificity of action of chemicals which destroys beneficial species and synthetic insecticides cause potential hazards to human, live stock and wild life and increased the emission of green house gases which will alter the climate and natural environment. Hence, the recent developments in pest management techniques are inclined towards the utilization of non-conventional methods of pest control, *i.e.* Microbial control. Nuclear polyhedrosis cause natural epizootic disease in pest population of mulberry eco-system has an excellent potential as biological pesticides.

Bioassays conducted to determine the possible effects of different formulations of *Dp*NPV on leaf webber resulted in maximum larval mortality. The mortality of leaf webber was higher in *Dp*NPV formulations when compared to *Dp*NPV suspension. Among the various formulations tested, the phagostimulants *i.e.,* starch, sucrose and glycerol at 10 per cent concentration with the combination of UV protectant tinopal 0.2 per cent and virus *Dp*NPV@ $1x10^9$ POB showed maximum mortality of 82.33, 81.44 and 80.00 per cent respectively at 12 DAS, which was followed by T_4 (76.55), T_8 (75.44), T_{12} (76.99). (Fig.1). The above combination of phagostimulants at 10 per cent concentration with UV protectant congored one per cent caused mortality which were on par. With the combination of phagostimulants at 5 per cent concentration and tinopal 0.2 per cent showed 70.03, 69.99, 69.66 per cent mortality which was followed by T_3 (62.22%), T_7 (62.88%) and T_{11} (61.11%) which were on par with each other. When *Dp*NPV@ $1x10^9$ POB/ml alone were used minimum mortality of 55.01 per cent mortality was recorded at 12 DAS which was followed by untreated control (4.88%) (Table 1). Viral concentration of 2.2 x 10^6 PIBs/ml of *Sl*NPV inflicting approximately 50 per cent mortality to the five day old larvae was demonstrated by Trang and Chaudri (2002).

Masetti *et al*. (2008) reported that four days after treatment of the *Sl*NPV to third instar larvae of *S. littoralis* caused mortality of 28 per cent. Hackett *et al*. (2000) reported that the overall mortality rate was lower in cases of *Ha*GV, *Ha*NPV and *Hz*NPV than in cases where separate viruses were used in *H. armigera*. This could be attributed to an interfering factor present in one or both of the viruses. Interference between NPV and GV in *C. fumiferana* was also illustrated by Bird (1959). It was suggested that the faster acting NPV could prevent infection by GV.

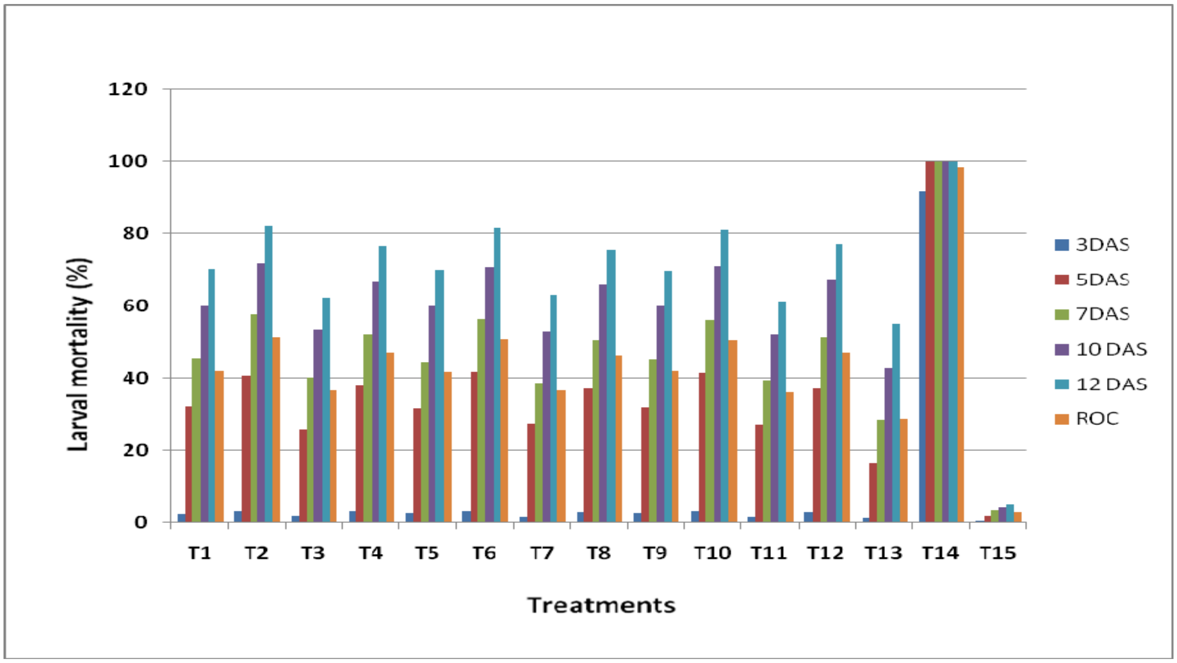
From the present study, it is concluded that *Dp*NPV formulations possess larvicidal activity and used for the management of *D. pulverulentalis.* Also it could be an attractive candidate, eco-friendly and safe to silkworm to use as a biopesticide in mulberry ecosystem and does not produce residue problem, resistance development and environmental hazards.

Table 1. Bioefficacy of *Dp*NPV formulations against larvae of *D. pulverulentalis* (H.) under *in-vitro* condition

T. No.	Treatments	Larval mortality (%)					Mean
		3DAS	5DAS	7DAS	10 DAS	12 DAS	
T_1	Starch 5% + Tinopal 0.2% + Sorbitol 1% +*Dp*NPV*	2.22 (8.57)^{d}	32.22 (34.58)^{cde}	45.11 (42.19)^{efgh}	59.88 (50.70)^{efg}	70.03 (56.81)^{efg}	41.89 (40.33)^{def}
T_2	Starch 10% + Tinopal 0.2%+ Tween 80 1% + *Dp*NPV*	3.11 (10.16)^{b}	40.66 (39.62)^{bc}	57.44 (49.28)^{b}	71.66 (57.84)^{b}	82.33 (65.14)^{b}	51.04 (45.60)^{b}
T_3	Starch 5% + Congored 1%+ Sorbitol 1% + *Dp*NPV*	1.44 (6.89)^{e}	25.66 (30.43)^{e}	39.98 (39.22)^{fgh}	53.48 (47.00)^{gh}	62.22 (52.07)^{ghi}	36.56 (37.20)^{de}
T_4	Starch 10% + Congored 1% + Tween80 1% + *Dp*NPV*	2.99 (9.96)^{bc}	37.88 (37.99)^{bcd}	51.88 (46.08)^{bc}	66.44 (54.60)^{cd}	76.55 (61.04)^{cd}	47.15 (43.37)^{bc}
T_5	Sucrose 5% + Tinopal 0.2% + Sorbitol 1% + *Dp*NPV*	2.67 (9.40)^{bcd}	31.59 (34.20)^{de}	44.21 (41.68)^{efgh}	60.04 (50.79)^{ef}	69.99 (56.78)^{ef}	41.70 (40.22)^{ef}
T_6	Sucrose 10% + Tinopal 0.2% + Tween80 1% + *Dp*NPV*	3.21 (10.32)^{b}	41.66 (40.20)^{b}	56.33 (48.64)^{cd}	70.54 (57.13)^{bc}	81.44 (64.48)^{c}	50.64 (45.36)^{bc}
T_7	Sucrose 5% + Congored 1% + Sorbitol 1% + *Dp*NPV*	1.41 (6.82)^{e}	27.22 (31.45)^{e}	38.57 (38.39)^{hi}	52.66 (46.52)^{h}	62.88 (52.46)^{ghi}	36.55 (37.20)^{fg}
T_8	Sucrose 10% + Congored 1% + Tween80 1% + *Dp*NPV*	2.87 (9.75)^{bc}	36.99 (37.46)^{bcd}	50.44 (45.25)^{defg}	65.75 (54.18)^{def}	75.44 (60.29)^{ef}	46.30 (42.88)^{cd}
T_9	Glycerol 5% + Tinopal 0.2% + Sorbitol 1% + *Dp*NPV*	2.51 (9.12)^{cd}	31.89 (34.38)^{cde}	44.89 (42.07)^{efgh}	59.88 (50.70)^{fgh}	69.66 (56.58)^{fgh}	41.77 (40.26)^{ef}
T_{10}	Glycerol 10% + Tinopal 0.2% + Tween80 1% + *Dp*NPV*	3.01 (9.99)^{bc}	41.33 (40.01)^{b}	55.89 (48.38)^{de}	71.04 (57.44)^{cde}	80.88 (64.07)^{cde}	50.43 (45.25)^{c}
T_{11}	Glycerol 5% + Congored 1% + Sorbitol 1% + *Dp*NPV*	1.33 (6.62)^{ef}	26.87 (31.22)^{e}	39.32 (38.83)^{gh}	51.88 (46.08)^{hi}	61.11 (51.42)^{hi}	36.10 (36.93)^{de}
T_{12}	Glycerol 10% + Congored 1% + Tween80 1% + *Dp*NPV*	2.93 (9.86)^{bc}	37.00 (37.46)^{bcd}	51.22 (45.70)^{def}	67.22 (55.07)^{def}	76.99 (61.34)^{def}	47.07 (43.32)^{de}
T_{13}	*Dp*NPV @ 1x10^{9} POB/ml	1.00 (5.74)^{f}	16.20 (23.73)^{f}	28.33 (32.16)^{i}	42.66 (40.78)^{i}	55.01 (47.88)^{i}	28.64 (32.36)^{g}
T_{14}	Dichlorvos 76EC 0.2%	91.66 (73.21)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	98.33 (82.58)^{a}
T_{15}	Untreated Control	0.33 (3.29)^{g}	1.66 (7.40)^{g}	3.33 (10.51)^{g}	4.22 (11.85)^{j}	4.88 (12.76)^{j}	2.88 (9.78)^{h}
SEd		0.47	2.45	2.99	2.49	3.09	
CD (0.05)		0.97	5.03	6.14	5.1	6.13	

**Dp*NPV @ 1x10^{9} POB ml^{-1} ; DAS- Days after spraying; ROC – Reduction over control
Figures in the parentheses are arc sine transformed values
In a column, means followed by a common letter(s) are not significantly different by LSD (P=0.05)

Fig.1 Bioefficacy of *Dp*NPV formulations against larvae of *D. pulverulentalis* (H.) under *in-vitro* condition



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