

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.603.215>

Effect of Sunlight and UV Light against DpNPV (Nuclear Polyhedrosis Virus) Formulation on Larval Mortality of Mulberry Leaf Webber, *Diaphania pulverulentalis* Hampson

S. Prabhu^{1*} and C.A. Mahalingam²

¹Department of Agricultural Entomology, RVS Padmavathy College of Horticulture
(Affiliated to Tamil Nadu Agricultural University, Coimbatore), Sempatti- 624 707, India

²Department of Agricultural Entomology, Tamil Nadu Agricultural University,
Coimbatore- 641 003, India

*Corresponding author

ABSTRACT

Keywords

Mulberry,
*Diaphania
pulverulentalis*,
NPV, Sunlight,
UV light.

Article Info

Accepted:
24 February 2017
Available Online:
10 March 2017

Effect of sunlight and UV light on the inactivation of nuclear polyhedrosis virus to *Diaphania pulverulentalis* (Hampson). Larvae were studied under laboratory conditions. The larval mortality was significantly maximum (76.66%) and (86.65%) when DpNPV suspension and formulation was exposed to sunlight for 1 hour respectively. Minimum mortality of (48.33%) and (61.66%) of DpNPV suspension and formulation were observed when it was exposed to sunlight for 5 hours which indicating reverse relationship between the exposure duration to sunlight and larval mortality. Inactivation of DpNPV by UV light was demonstrated by the decreased mortality rate in larval groups inoculated with irradiated virus compared to those fed on non- radiated virus *i.e.*, control. Significant difference in larval mortality was observed when DpNPV was exposed to 5, 10, 15, 30 and 60 minutes to UV light, (88.33, 85.00, 80.00, 66.66 and 53.33 % mortality respectively). Whereas in formulated DpNPV, the rate of larval mortality recorded was 93.33, 90.00, 86.65, 71.66 and 66.66 per cent when exposed to 5, 10, 15, 30 and 60 minutes to UV light respectively. The inactivation of virus was directly related to the period of exposure to UV radiation. This finding indicated a loss in pathogenicity and the number of days to death was higher and the total mortality was lower from exposed virus than from unexposed virus.

Introduction

The leaf webber *Diaphania pulverulentalis* (Hampson) (Lepidoptera : Pyralidae), is a key pest of mulberry in India. As recently become a major problem for mulberry cultivators in south India causing a leaf yield loss of 12.8 per cent with an average incidence of 21.77 per cent. In recent years, *D. pulverulentalis*, a new invader from Karnataka to Tamil Nadu is posing a serious threat to farmers (Geetha Bai *et al.*, 1997).

Timely management of insect pests is essential to avoid economic loss to farmers. However, regular usage of toxic chemicals in mulberry garden to control the pests caused pollution and was detrimental to human beings and beneficial organisms including 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.

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).

One of the most important naturally occurring pathogens of *D. Pulverulentalis* larvae is Nuclearpolyhedrosis virus (NPV), a baculovirus capable of causing epizootics in the larval population that result in declines or elimination of populations (Narayanaswamy *et al.*, 2008).

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).

Priyadharshini, 2009 reported that, Baculoviruses, particularly, nucleopolyhedroviruses (NPV), are deleteriously affected by sunlight radiation. Shapiro *et al.*, (2002) conducted bioassay to compare the biological activities of *Hz*SNPV against *Heliothis zea* larvae and *Se*MNPV against *Spodoptera exigua* larvae with the virus concentrations ranging from 10^1 ($=0.0074$ PIB/mm²) to 10^6 PIBs/cup ($=744.4$ PIBs/mm²) before and after irradiation. The UV-B/UV-A combination tubes were effective in inactivating both viruses and the effects were dependent upon both the length of UV exposure and the virus concentrations. Temperature also interacted with sunlight to alter its effect on viruses. McLeod *et al.*, (1977) showed that *Hz*NPV

inactivation by UV lights increased significantly as the temperature increased from 15 to 45°C.

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, Tinopal, sugars such as sucrose, fructose and sorbitol have been proved to increase the efficacy of NPV formulation (Sajap *et al.*, 2009). Hence, the present study was to determine the effect of sunlight and UV light on the infectivity of both *Dp*NPV and its formulation to *D. Pulverulentalis* larvae.

Materials and Methods

Effect of sunlight exposed *Dp*NPV and their formulation

The persistence of *Dp*NPV was assessed by exposing the virus to sunlight for different periods. The viral suspension and *Dp*NPV formulation (Starch 10%+ Tinopal 0.2%+ Tween 80 1%+*Dp*NPV) containing 1×10^9 POB/ml was poured into petri plates and the polyhedra were exposed outdoors to direct sunlight in open dishes for 1, 2, 3, 4 and 5 hours. After exposure, the samples were resuspended in distilled water at the original concentration. Mulberry leaf discs were cut and treated with polyhedral bodies at the centre of the discs and spread uniformly using the polished blunt end of a sterile glass rod. After the suspension had dried off, the disc was turned over and the other surface was treated similarly. Larvae fed on leaf discs without virus treatment were also maintained as control (0 h). Twenty numbers of third instar larvae per replication were used in the assay and larvae were released individually. After consuming of whole discs, the larvae were transferred to fresh discs. Each treatment was replicated three times. The leaf discs

were placed in containers lined with moist filter paper discs to maintain the freshness and moisture content of the discs, the larval mortality was recorded 4-10 days post inoculation (Sajap *et al.*, 2007).

Effect of UV light exposed *Dp*NPV and their formulation

Laboratory assays were conducted to find out the UV stability of an *in-vivo* produced virus. Both virus suspension (1×10^9 POBs) and formulation (Starch 10%+ Tinopal 0.2%+ Tween80 1%+*Dp*NPV) containing 1×10^9 POB/ml were taken in open petri dishes (60 by 15 mm glass) placed 19.5 cm below the UV lamp. Wet virus was exposed for periods of 5, 10, 15, 30 and 60 minutes, respectively. After the exposure periods, the remaining volumes were determined and distilled water was added to each dish to replace water lost by evaporation. Lids were then placed on all dishes and were stored at 4°C until usage.

Leaf discs treated with *Dp*NPV inoculum were spread uniformly on both sides of mulberry leaf discs by using blunt ended sterile glass rod. Twenty number of third instar larvae of leaf webber were used per replication. The control was also maintained by treating the leaf disc with unexposed *Dp*NPV. Each treatment was replicated five times and the mortality was recorded at 4th, 7th and 10th day (Bullock *et al.*, 1970).

Results and Discussion

Effect of exposure duration of sunlight on *Dp*NPV and their formulation on the larval mortality of leaf webber, *D. pulverulentalis*

The present study is in conformity with Morris (1971) who reported that one hour and five hours exposure of NPV of western

hemlock looper (*Lambdina fuscicularlugubrosa*) to sunlight caused mortality of 72.0 and 50.0 per cent respectively. The rate of larval mortality in *D. pulverulentalis* due to inoculation of *Dp*NPV and their formulation differed significantly when exposed to different durations of sunlight. Among the exposure lots of *Dp*NPV suspension (Table 1) the larval mortality was significantly maximum (76.66 %) when *Dp*NPV was exposed to sunlight for 1 hr, which was followed by mortality of 68.33, 68.33, 53.33 and 48.33 per cent for 2, 3, 4 and 5 hr respectively.

The mortality caused by *Malacosoma disstria* NPV exposed to sunlight on the sweet gum foliage was 87.00 per cent at 0 h exposure (Broome *et al.*, 1974), which was slightly high in the similar study with (91.25 %) at the 0 h exposure (Priyadharshini, 2009). Ignoffo *et al.*, (1989) reported the effect on the inactivation of occluded baculoviruses. Whereas, with formulated *Dp*NPV (Starch 10%+ Tinopal 0.2%+ Tween 80 (1%)+*Dp*NPV @ 1×10^9 POBs ml⁻¹) the larval mortality was significantly maximum (86.65 %) when exposed to sunlight for 1 hr, which was followed by mortality of 78.33, 71.66, 65.00 and 61.66 per cent for 2, 3, 4 and 5 hr respectively, indicating inverse relationship between the exposure duration to sunlight and larval mortality. However, the mortality rate of 90.00 and 93.33 per cent were recorded in unexposed *Dp*NPV suspension and formulated *Dp*NPV (*i.e.*, control) respectively. (Fig. 1) Hence, the larval mortality decreased with increase in exposure duration of sunlight. This indicated a loss in pathogenicity and the number of days to death was higher and the total mortality was lower from exposed virus than from unexposed virus.

Table.1 Effect of sunlight exposed DpNPV suspension and its formulation against larvae of *D. pulverulentalis*

S.No	Sunlight Exposure duration (h.)	Larval Mortality(%)	
		<i>DpNPV</i> * Suspension	<i>DpNPV</i> * Formulation
1.	1	76.66 (61.11) ^b	86.65 (68.56) ^b
2.	2	68.33 (55.75) ^c	78.33 (62.25) ^c
3.	3	68.33 (55.75) ^c	71.66 (57.83) ^d
4.	4	53.33 (46.90) ^d	65.00 (53.72) ^e
5.	5	48.33 (44.04) ^e	61.66 (51.74) ^f
6.	Control	90.00 (71.56) ^a	93.33 (75.03) ^a
	SEd	0.38	0.61
	CD at (0.05%)	0.82	1.33

**DpNPV*@ 1x10⁹ POB/ml

Values are mean of three replications

Values in parentheses are arc sine transformed values

Means followed by similar letter (s) are not significantly different by LSD

Table.2 Effect of UV light exposed DpNPV suspension and its formulation against larvae of *D. pulverulentalis*

S.No	UV Exposure duration (min.)	Larval Mortality (%)	
		<i>DpNPV</i> * Suspension	<i>DpNPV</i> * Formulation
1.	5	88.33 (70.02) ^b	93.33 (75.03) ^b
2.	10	85.00 (67.21) ^c	90.00 (71.56) ^c
3.	15	80.00 (63.43) ^d	86.65 (68.56) ^d
4.	30	66.66 (54.73) ^e	71.66 (57.83) ^e
5.	60	53.33 (46.90) ^f	66.66 (54.73) ^f
6.	Control	90.00 (71.56) ^a	95.00 (77.07) ^a
	SEd	0.45	0.39
	CD at (0.05%)	0.99	0.85

**DpNPV*@ 1x10⁹ POB/ml

Values are mean of three replications

Values in parentheses are arc sine transformed values

Means followed by similar letter (s) are not significantly different by LSD.

Fig.1 Effect of sunlight exposed *Dp*NPV suspension and formulation against *D. pulverulentalis*

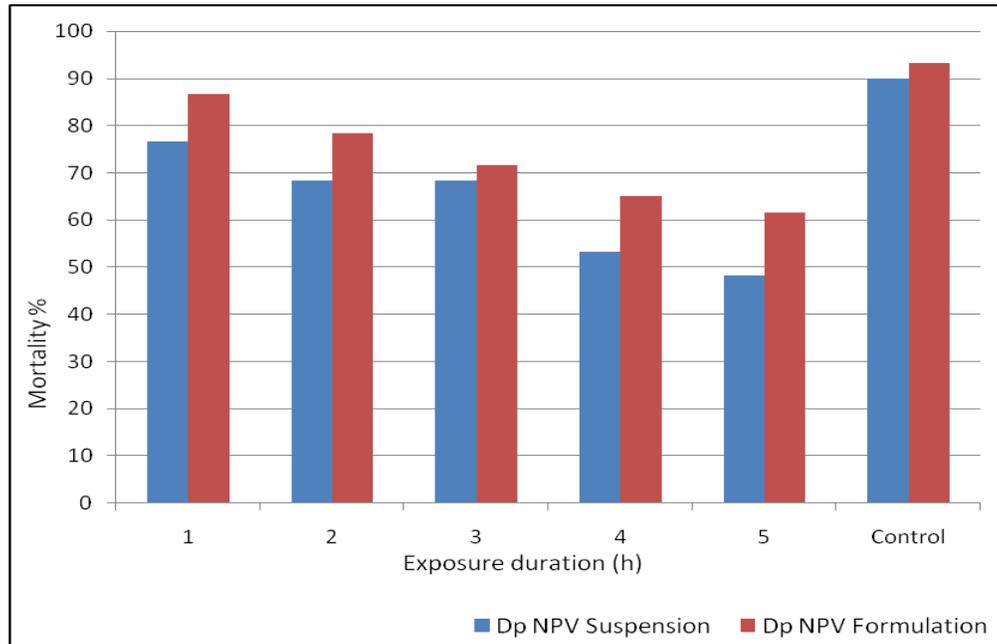
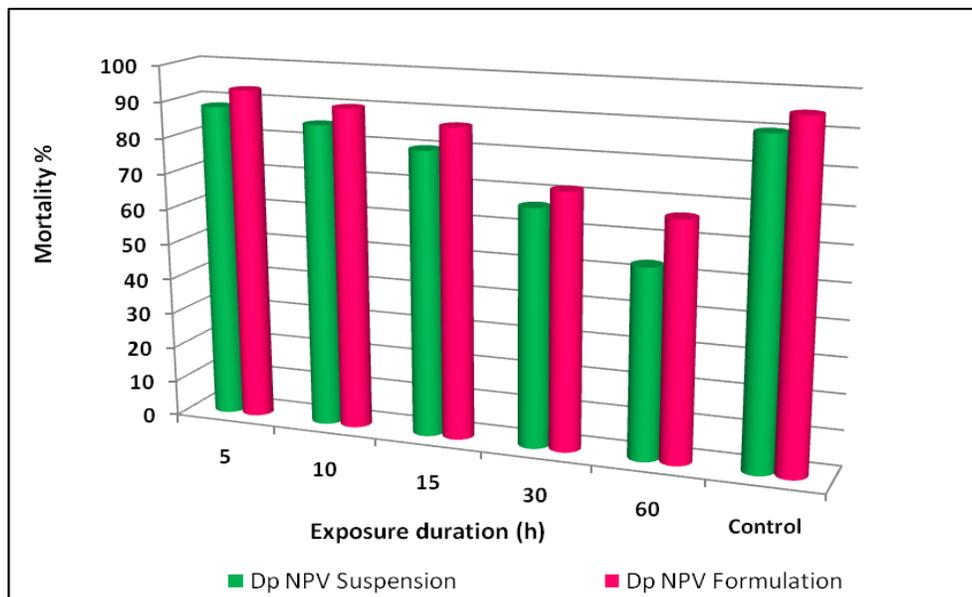


Fig.2 Effect of UVlight exposed *Dp*NPV suspension and formulation against *D. pulverulentalis*



Effect of sunlight on the infectivity of NPV *Hyphantria cunea* determined in the laboratory and exposure of suspension of the virus to direct sunlight for 3 h resulted in 50 per cent reduction in the infectivity (Nordin,

1976). Also the effect of sunlight on the infectivity of NPV *Mythimna seprata* (Manjunath and Mathad, 1981; Parameshwar-Hugar *et al.*, 1996); *Trichoplusia ni* (Biever and Hostetter, 1985) and *Spodoptera litura*

(Kulkarni *et al.*, 1999) has been were documented.

Effect of UVlight exposed *Dp*NPV and their formulations on the larval mortality of leaf webber, *D. pulverulentalis*

Survival might be achieved by persistence of polyhedra in soil or decaying leaf matter, particularly during periods when the insect host is not available (Hughes *et al.*, 1997). Following application to plant surfaces, polyhedra are rapidly inactivated by solar ultraviolet (UV) radiation, particularly in the UV-B range of 280~320 nm (Killick, 1990; Morris, 1971).

Insect viruses are known to be inactivated by artificial radiation (Watanabe, 1951; David, 1969; Bullock *et al.*, 1970; Jaques, 1968). The purpose of the present study was directed towards obtaining basic information on the response of the nuclear polyhedrosis virus to UV radiation.

Significantly difference in larval mortality was observed when *Dp*NPV was exposed to 5, 10, 15, 30 and 60 minutes to UV light, (88.33, 85.00, 80.00, 66.66 and 53.33 per cent, respectively) (Fig. 2) However, virus without UV exposure caused mortality of 91.25 per cent which showed no significant loss in activity of *Dp*NPV (Priyadharshini, 2009). Shapiro *et al.*, (2002) reported the effects of UV light on the activity of corn earworm, beet army worm and leaf webber nuclear polyhedrosis virus. Jones and Mckinnely (1986) demonstrated that > 90 per cent inactivation of *Spodoptera littoralis* nuclear polyhedrosis (*Sl*MNPV) occurred within 4 hours and more than >99 per cent inactivation occurred within 8 hours of exposure to UV light.

Several workers tested different adjuvants as UV protectants *viz.*, carbon, aluminium powder, aluminium oxide and cellulose

(Ignoffo and Batzer, 1971), boric acid 0.1 to 1.0 per cent (Bijur *et al.*, 1993), Ranipal and Robin blue @ 0.5 per cent (Rabindra *et al.*, 1989), Tinopal LPW (Ignoffo and Garcia, 1995), Ranipal BVN and Ranipal 2B (Murali baskaran *et al.*, 1997). Whereas, in UV treated *Dp*NPV formulation showed the higher rate of larval mortality (93.33, 90.00, 86.65, 71.66 and 66.66 per cent mortality in 5, 10, 15, 30 and 60 hour duration respectively). However, virus with no UV exposure caused mortality of 90.00 per cent in *Dp*NPV suspension and 95.00 per cent in *Dp*NPV formulation, which showed no significant loss in activity. Hence, the inactivation of virus was directly related to the period of exposure to UV radiation. The viral activity of the irradiated suspensions decreased with increased exposure duration to UV light. The inactivation of virus was directly related to the period of exposure to UV radiation (Table 2).

Rabindra *et al.*, (1989) reported that Robin blue and Tinopal when used as adjuvants increased the persistence of the virus on foliage and suggested that this could be attributed to the UV protection. Shapiro *et al.*, (1983) evaluated different adjuvants and concluded that molasses, shade and coax served as UV protectants at 5 per cent concentration. Also, Stilbene derived optical brighteners could improve the insecticidal properties of baculoviruses (Lasa *et al.*, 2007).

In conclusion, investigations were carried out on the Development and evaluation of formulation of *Dp*NPV for the management of leaf webber *Diaphania pulverulentalis* (Hampson) in mulberry ecosystem. The results obtained from various laboratory experiments are concluded that, the larval mortality was significantly maximum (76.66 %) when *Dp*NPV suspension was exposed to sunlight for 1 hr, which was followed by mortality of 68.33, 68.33, 53.33 and 48.33 per cent for 2, 3, 4 and 5 hr respectively. Whereas,

comparatively formulated *DpNPV* (Starch 10%+ Tinopal 0.2%+ Tween 80 (1%)+*DpNPV* @ 1×10^9 POBs ml^{-1}) showed significantly maximum larval mortality (86.65 %) when exposed to sunlight for 1 hr, than *DpNPV* alone, which was followed by mortality of 78.33, 71.66, 65.00 and 61.66 per cent for 2, 3, 4 and 5 hr respectively, indicating inverse relationship between the exposure duration to sunlight and larval mortality.

Significant difference in larval mortality was observed when *DpNPV* was exposed to 5, 10, 15, 30 and 60 minutes to UV light, (88.33, 85.00, 80.00, 66.66 and 53.33 % mortality, respectively. Whereas, in formulated *DpNPV*, the rate of larval mortality recorded was higher (93.33, 90.00, 86.65, 71.66 and 66.66 % respectively). The inactivation of virus was directly related to the period of exposure to UV radiation.

Acknowledgement

The senior author sincerely acknowledges the Rajiv Gandhi National Fellowship (RGNF) for the financial support in the form of Senior research fellowship for his Ph.D. research. Thanks are also due to the Head and Chairman for providing field and laboratory facilities at the Department of Sericulture, Tamil Nadu Agricultural University, during the course of this investigation.

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How to cite this article:

Prabhu, S. and Mahalingam, C.A. 2017. Effect of Sunlight and UV Light against DpNPV (Nuclear Polyhedrosis Virus) Formulation on Larval Mortality of Mulberry Leaf Webber, *Diaphania pulverulentalis* Hampson. *Int.J.Curr.Microbiol.App.Sci*. 6(3): 1897-1905. doi: <https://doi.org/10.20546/ijcmas.2017.603.215>