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SCREENING OF ADJUVANTS AS SUNLIGHT PROTECTANT FOR NPV AND EVALUATION OF STORAGE CONDITIONS AGAINST *SPODOPTERA LITURA*

Navdisha[1], Neelam Joshi[2]* and Sudhendu Sharma[2]

[1]Department of Microbiology; [2]Department of Entomology,
Punjab Agricultural University, Ludhiana 141004, Punjab, India
*Email: neelamjoshi_01@pau.edu (corresponding author): ORCID ID 0000-0002-4159-5691

ABSTRACT

This experimental study evaluated the effect of adjuvant supplementation on larval mortality of nuclear polyhedrosis virus under sunlight. Study revealed that Tinopal @1% provided best protection from sunlight and retained viral efficacy up to 68.75 and 66.75% in *Splt*NPV (native) and *Splt*NPV (NIPHM) respectively, against third instar larvae of *Spodoptera litura* (F). However, all adjutants resulted in significantly higher larval mortality as compared to control (virus alone) under sunlight. *Splt*NPV viral formulations stored at different storage conditions recorded minimum reduction in larval mortality, when stored in amber coloured glass bottle at refrigeration temperature (4± 2^{0}C).

Key words: *Spodoptera litura*, nuclear polyhedrosis virus, mortality, efficacy, refrigeration temperature, *Splt*NPV, Tinopal, adjuvants, storage, amber glass bottles

Spodoptera litura (F.) is a serious, cosmopolitan, polyphagous and nocturnal moth also known as 'Tobacco caterpillar' found in temperate and tropical region in many South Asian countries (Tenywa et al., 2018). It is a serious pest of various agricultural and horticultural crops. It feeds by skeletonizing various cash crops by decreasing their photosynthetic activity (Yinghua et al., 2017). The application of chemical and synthetic insecticides for instant pest management causes resistance and resurgence in pest population with many negative effects on environmental conditions (Rehan and Freed, 2014). Entomopathogens are ecofriendly alternative against serious crop pests and gaining popularity day by day. Nucleopolyhedrosis virus (NPV) belongs to the family Baculoviridae and is one of the potential microbial agents for crop pest management. These enveloped viruses have a typical feature of occlusion bodies that cause viral infection (Kaur et al., 2021). NPV exhibit non-toxic, non-persistent, highly epizootic, pathogenic, environmentally safe with narrow host spectrum (El Sayed et al., 2022). Viral efficacy is influenced by temperature, pH, sunlight, moisture, secondary metabolites and storage conditions (Raypuria et al., 2019). Sunlight is the primary factor limiting viral persistence in the environment. Virus inactivation by sunlight is caused primarily by ultraviolet light (UV) (Young 1998). The medium wave UV band (UV-B of 280-320 nm) is the most important photo activator of baculovirus, with considerable effect in the near UV region (320-400 nm). Direct exposure to solar radiation impairs the viability of virus. Use of various adjuvants stickers, sun screener, phagostimulants affect the efficacy of the virus this is attributed to the improved field persistence of the virus or due to increased consumption of the virus by pest. The addition of various adjuvants also enhances the shelf life and performance by stabilizing the microbial properties (Patil and Jadhav, 2016). The storage conditions of NPV also plays important role in shelf life and pathogenicity. Hence the present study was done to evaluate various adjuvants as sunlight protectant and to study storage conditions for their significant role in maintaining pathogenicity against *S. litura* larvae.

MATERIALS AND METHODS

Spodoptera litura larvae were collected from cabbage fields and reared under laboratory on cabbage leaves placed in glass jars covered with muslin cloth at ambient environment (25^{0}C). Two viral isolates viz., *Splt*NPV (native) and *Splt*NPV (NIPHM) procured from the National Institute of Plant Health Management, Hyderabad were used. These viral isolates were multiplied in the laboratory and @1×10^{10} OBs/ ml concentration was prepared. Various adjuvants viz., Tinopal @1%, Tween 80 @0.5% and crude sugar @10% were sterilized in hot air oven at 160^{0}C for 1 hr and individually added to optimum concentration of @1×10^{10} OBs/ml of *Splt*NPV. Twenty ml of each treatment was pipetted out in petri plate and exposed to direct sunlight for three hr from (12:00 - 3:00 pm)

on cloudless days along with untreated control and was evaluated for its pathogenicity against third instars larvae under laboratory conditions according to methodology of Sajap et al. (2009) with little modification. Storage conditions also play an important role in pathogenicity of nucleopolyhedrosis virus. *Splt*NPV (native) and *Splt*NPV (NIPHM) were stored individually in three different types of containers viz: glass bottles (ambered, colorless) and polyethylene bottles at two different temperatures i.e., refrigeration (4± 2°C) and room temperatures (25± 2^{0}C) for six months. Bioassay test was conducted after first, third and six months of storage against third instar larvae of *S. litura* by diet incorporation method to check the effect of storage on viral formulations. For bioassay studies, the larvae were allowed to feed on viral inoculated artificial diet in vials. There were four replications and 20 larvae per replication. The data of % larval mortality and reduction of larval mortality was recorded daily up to ten days. One-way (ANOVA) in SPSS 16.0 statistical software was used to compare the means at 5% level of significance by Tukey's post-hoc test. Means with $p < 0.05$ were considered to be significantly different from each other. The transformed mean values were obtained by applying one-way ANOVA under CRD in CPCS1 software.

RESULT AND DISCUSSION

Cumulative % larval mortality against 3rd instar larvae of *S. litura* after 6 days of treatment (DAT) recorded maximum % mortality with *Splt*NPV (native) supplemented with Tinopal @1% (Table 1). After 8 DAT, maximum % mortality was recorded in *Splt*NPV (native) and *Splt*NPV (NIPHM) respectively, both were supplemented with Tinopal @1% individually and were at par with each other. At 10 DAT; statistically significant increase in mortality over control was observed with different supplementations, maximum cumulative % mortality was recorded with both *Splt*NPV (native) and *Splt*NPV (NIPHM) supplemented with Tinopal @1% individually. However, minimum % larval mortality was recorded in *Splt*NPV (NIPHM) alone. Tinopal @1% gave best protection to viral efficacy under sunlight and this might be due to its optical brightener properties of Tinopal. However, all adjuvants tested gave some

Table 1. Evaluation of *Splt*NPV (native) and *Splt*NPV (NIPHM) with different adjuvants exposed to sunlight against *S. litura* larvae under laboratory conditions

Treatment	% larval mortality			
	Days after treatment			
	4DAT	6DAT	8DAT	10DAT
*Splt*NPV (native)+ Tinopal (@1%)+ Exposed*	25.00^{a} (29.98)	37.50^{a} (37.73)	53.75^{a} (47.13)	68.75^{a} (56.00)
*Splt*NPV (native)+ Crudesugar (@10%) +Exposed	23.75^{ab} (29.13)	35.00^{ab} (36.25)	46.25^{bc} (42.83)	58.75^{b} (50.02)
*Splt*NPV (native)+ Tween80 (@0.5%) +Exposed	17.50^{c} (24.66)	31.25^{bcd} (33.96)	43.75^{cd} (41.38)	51.25^{cd} (45.69)
*Splt*NPV (native) + (without adjuvant) +Exposed	10.00^{d} (18.42)	28.25^{de} (32.39)	35.00^{ef} (36.25)	46.25^{de} (45.69)
*Splt*NPV (NIPHM)+ Tinopal (@1%)+ Exposed	21.25^{abc} (27.41)	33.75^{abc} (35.73)	50.00^{ab} (44.98)	66.75^{a} (54.47)
*Splt*NPV (NIPHM)+ Crudesugar (@10%) +Exposed	18.75^{bc} (25.61)	33.00^{abc} (35.75)	43.75^{cd} (41.38)	56.25^{c} (48.57)
*Splt*NPV(NIPHM)+ Tween80 (@0.5%) +Exposed	8.75^{d} (17.05)	30.00^{cd} (33.19)	38.75^{de} (38.47)	50.00^{de} (44.98)
*Splt*NPV (NIPHM) + (without adjuvant) +Exposed	6.25^{d} (14.29)	25.25^{e} (29.98)	31.25^{f} (33.96)	45.00^{e} (42.11)
CD (p = 0.05)	2.71	1.78	1.92	1.87
F value	41.16	15.00	48.28	66.57

*****SpltNPV with and without adjuvant; DAT= Days after treatment; (POB's/ ml) = (1×10^{10} POB's/ ml), Mean value followed by same letter (a, b, c, d, e) in vertical column not significantly different (Tukey's post hoc test, p=0.05)

degree of protection against inactivation in direct sunlight exposure. Present results are in corroboration with Sajap et al. (2009) who evaluated various adjuvants for protecting *Splt*NPV from photoinactivation and reported most of the adjuvants gave some protection to the viral inactivation and further reported adjuvant Tinopal lowered LT_{50} value of virus 1.2 times than the unexposed virus and thus enhanced the viral efficacy.

The effect of direct sunlight and UV exposure was studied on *Dp*NPV (*Diaphania pulverilentalis* nucleopolyhedrosis virus). Various additives were supplemented under exposed conditions and effectivity on larvicidal activity was tested. They revealed progressive decrement in viral efficiency with prolong sunlight exposure and concluded that Tinopal supplemented was acted as optical brightener that provided excellent protection from sunlight inactivation by enhancing insecticidal properties. However, supplementation of Tween 80 (Polysorbate) provides least protection to virus it might be due to its surfactant nature with fatty acid chain moiety of 15 carbons and negative charge that stimulates the antiviral activity (Prabhu and Mahalingam, 2017). Effect of natural and synthetic additives against UV inactivation of baculoviruses was investigated by Gifani et al. (2021). They reported that the sodium alginate and titanium dioxide were the best additives as UV protectant compared other. However, Tween 80 gave least protection to the viral formulation. Chen et al. (2020) reported Tween 80 gave least protection to sunlight, as it attributed to amphiphilic nature of surfactant that allow its hydrophilic domains to interact with lipid membranes. This stimulates its interaction with other hydrophilic substances resulting in disruption and deactivations. (Wang and Granados, 2000; Mukawa et al., 2003; Okuno et al., 2003) revealed the ingestion of fluorescent brightener (Tinopal) by NPV can inhibits the formation of the peritrophic membrane, which acted as barrier to micro-organisms to host midgut. This mechanism involves alteration of the permeability in peritrophic membrane, allowing large number of ocular bodies to enter in midgut epithelium cells and cause infection. In addition, Washburn et al. (1998) revealed that fluorescent brighteners also have potential to block the sloughing of infected midgut epithelial cells that cause decrement in larval midgut resistance and increment in larval mortality. According to a recent study, a unique formulation technique that contained the baculovirus in an waxy shell efficiently protected the delicate viral DNA from solar degradation while remaining non-phytotoxic (Wilson et al., 2020). Adjuvants were evaluated by Muthuswami et al. (1994) for their UV protectant and phagostimulant properties against HaNPV under sunlight exposure. They reported that crude sugar is phagostimulant that enhanced the viral ingestion by larvae and also act as UV protectant by reducing LT50 value. Similarly, Tinopal and egg white acted as UV protectant and glycerol showed its evaporation retardation properties. Different adjuvants were added and tested by (Ithnin, 2014) and results were revealed on the basis of screening test, the formulation prepared by mixing adjuvants gave maximum per cent larval mortality. Thus, Tinopal acted as the best UV protectant among all for protection of *Splt*NPV due to its UV protectant and optical brightener properties. Some researchers have used natural compounds derived from leaves and fruits (referred to as herbal cosmetics) to protect entomopathogenic viruses from UV radiation. Flavonoids are the most studied sunlight protectant which includes quercetin (3, 40- dihydroxy flavonol). This might be due to the presence of aromatic rings in their molecular structure, flavonoids absorb UV rays between 200 and 400 nm, making them appropriate for use as a sunscreen agent (Cefali et al., 2016). Ibrahim et al (2019) analysed the relationship between viability of bioformulation and period of sunlight exposure against larvae of *H. armigera*, and the effect of ultraviolet light (UV) protectants on persistence of the entomopathogenic virus (*Ha*NPV). They reported that exposure time is directly proportional to the viral sensitivity, and some plant extracts may be useful for the *Ha*NPV under sunlight exposure.

Reduction in larval mortality of *Splt*NPV formulation after six months of storage recorded minimum reduction in larval mortality over control (3.17%) and (1.63%) in *Splt*NPV (native) and *Splt*NPV (NIPHM) respectively, when stored in amber coloured glass bottles followed by storage in colourless glass bottles with (3.22%) and (3.27%) reduction under refrigeration temperature (4± 2^0C) (Table 2). However, maximum reduction (30.15%) and (29.00%) was recorded, respectively, when stored in polyethylene bottles (25± 2^0C). This may be due to fact that at room temperature, there is significant pH fluctuation in viral suspension due to microbial disturbance. However, at refrigeration conditions (4± 2^0C) very slow conversion was recorded in pH value from acidic to normal, thus viral efficacy was maintained up to longer period of time. These findings are in accordance with earlier work Gopali and Lingappa (2001) and (Lasa et al., 2008) who studied the effect of storage conditions on efficacy of nucleopolyhedrosis and recorded maximum loss in efficacy, when stored

Table 2. Larval mortality in *S. litura*

Treatments	% Larval mortality over control (six months after storage)					
	*Splt*NPV (native)			*Splt*NPV (NIPHM)		
	After 0 month	After 6 months	% reduction of larval mortality over control	After 0 month	After 6 months	% reduction of larval mortality over control
Splt NPV stored in glass amber bottle under (4± 2°C)	78.75	76.25[a] (60.89)	3.17	76.25	75.00[a] (59.97)	1.63
Splt NPV stored in colourless glass bottle under (4± 2°C)	77.50	75.00[a] (59.97)	3.22	76.25	73.75[ba] (59.17)	3.27
Splt NPV stored in colourless polyethylene bottle under (4± 2°C)	78.75	72.50 (58.37)	7.93	77.50	70.00[b] (56.76)	9.67
Splt NPV stored in glass amber bottle (25± 2°C)	78.75	63.75[b] (52.96)	19.04	76.25	62.75[c] (52.22)	17.70
Splt NPV stored in colourless glass bottle (25±2°C)	76.25	62.50[b] (52.22)	18.03	75.00	58.75[c] (50.02)	21.66
Splt NPV stored in colourless polyethylene bottle (25± 2°C)	78.75	55.00[c] (47.85)	30.15	76.25	53.75[d] (47.13)	29.5
CD (p= 0.05%)	NS	(2.64)		NS	(1.89)	

Mean value followed by same letter (a, b, c, d, e) in column not significantly different Tukey's post hoc test (p=0.05). *Splt*NPV count: 1×10^{10}POB's/ ml; Values in parentheses arc sine transformation

under room temperature (25± 2^0C). Lasa et al. (2008) reported this might be due to deterioration caused by hydrolysis and autoxidation as a result of exposure to oxygen derived from lipids present in insect cadavers, which leads to proteolysis or production of free radicles and super oxides during autooxidation that are capable of distorting the structure of viral nucleic acid and thus loss of pathogenicity. They further reported that DNA of occulated virions significantly degraded at room temperature as compared at refrigerated conditions. Sireesha et al. (2010) conducted similar studies to record the impact of different storage containers and temperatures (refrigerator and room temperature) on *Ha*NPV formulations and reported, under room temperature viral efficacy decreased at faster rate in both amber and glass bottles, this gradual decrement in efficacy under room temperature was due to presence of microbial hinderance as the bacterial count in viral suspension was counted 3.47 times more when stored in room temperature**.** Mehrvar et al. (2016) tested the stability of *Ha*NPV formulated with talc stored at different storage temperature for six months. Bioassay studies revealed that LT_{50} value was higher, when stored at room temperature and less at refrigeration temperature. Ghosh et al. (2018) also evaluated the shelf life and cross infectivity of three formulation of *Sl*NPV (*Sl*NPV + antimicrobials, *Sl*NPV + UV protectants and *Sl*NPV without any supplementation) at different storage conditions and reported refrigeration was the best option for long term storage, as the bacterial contamination was found to be 3.7 times more in formulation stored at room temperature which affected its viral efficacy.

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AUTHOR CONTRIBUTION STATEMENT

Navdisha carried out experiment recorded data and wrote manuscript. Neelam Joshi designed experiment provided technical guidance and edited the manuscript. Sudhendu Sharma provided technical guidance.

CONFLICT OF INTEREST

No conflict of interest.

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