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RESEARCH ARTICLE :

Shelf-life study of *Dp* NPV (Nuclear Polyhedrosis Virus) suspension and formulation against larval mortality of *Diaphania pulverulentalis* Hampson

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SUMMARY : Bioassay was conducted at six regular intervals (monthly once for six months) against *D. pulverulentalis*, viral suspension stored at three different temperature levels *viz.*, refrigerated condition (0±1^{0}C), room temperature (25±1^{0}C) and high temperature (35±1^{0}C)revealed that the virus stored at high temperature (35^{0}C) readily lost its virulence with decreasing mortality from 77.92 per cent to 60.36 per cent on 5th day, Larval mortality decreased from 78.44 per cent to 55.18 per cent on 7th day and larval mortality decreases from 93.80 per cent to 57.92 per cent on 10th day. The larval mortality slightly decreased at 0^{0}C from 78.42 per cent to 76.95 per cent on 5th day, 80.21 per cent to 78.45 per cent on 7th day and 93.80 per cent to 92.23 per cent on 10th day. The suspension which is formulated with Starch 10% + Tinopal 0.2% + Tween 80 1% + *Dp*NPV@1x10^9 POB/ml stored at three different temperature like above mentioned levels, revealed that the virus stored at high temperature (35^{0}C) readily lost its virulence with decreasing mortality from 78.44 per cent to 55.18 per cent at 5th day. Larval mortality decreased from 89.76 per cent to 61.67 per cent at 7th day and 93.60 per cent to 62.65 per cent on 10th day. The larval mortality slightly decreased at 0^{0}C from 80.21 per cent to 78.45 per cent on 5th day, 89.81 per cent to 88.21 per cent on 7th day and 97.80 per cent to 89.88 per cent on 10th day.

KEY WORDS :
*Dp*NPV, Nuclear Polyhedrosis virus, Shelf life

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BACKGROUND AND OBJECTIVES

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 nucleo polyhedro viruses (NPVs) and the granulosis viruses (GV), infect many important insect pests (Blissard *et al*., 2000 and 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).

Several authors reported that virus can be preserved for more than ten years at 4^0C without loss in its virulence (Gopali and Lingappa, 2001). Gudauskas and Canerday (1968) found the thermal inactivation point of *Ha* NPV to be 75 to 80^0C for 10 minutes. It was concluded that virulence of virus depends on quality of virus, storage conditions, duration of storage, temperature and pH of the product.

Hence, for future field and laboratory use the polyhedra associated with many insect virus diseases are usually stored under refrigerated conditions as air-dried, purified polyhedra or as polyhedra with in dried larval cadavers. Under these conditions polyhedra were noted to resist deterioration for considerable periods of time (Sireesha *et al*., 2010). Some polyhedral viruses have been shown to retain their virulence for periods ranging from 2 to 25 years (Steinhaus, 1949 and Bergold, 1953).

The present investigation on the pathogenicity of *Dp* NPVsuspension and its formulation were kept in three different storage condition to carried out their stability loss during storage.

RESOURCES AND METHODS

Shelf life of *Dp* NPV :

The stability of the *Dp* NPV under storage was studied in the laboratory to determine the effects of storage on the activity of the virus. Three different temperatures were selected *viz*., refrigerated condition (0 ± 1^0C), room temperature (25 ± 1^0C) and high temperature (35 ± 1^0C). One hundred ml of *Dp* NPV liquid suspension was stored in high density polyethylene bottle containing 1×10^9 POB's/ml under room, refrigerated condition and high temperature (35^0C) conditions. One ml of *Dp* NPV samples were drawn from the respective bottles at 1, 3 and 6 months storage and the virulence was assessed adopting bioassay method described by Finney (1952) against third instar larvae of *D. pulverulentalis*. The experiment was continued upto one year from (2013-2014). Observation on larval mortality was recorded at 24 h intervals (Karma Choden *et al*., 2012).

OBSERVATIONS AND ANALYSIS

The results of investigation on the shelf-life of DpNPV suspension and formulationin order to identify and characterize with increased virulence for the management of leaf webber *Diaphania pulverulentalis* (Hampson) in mulberry ecosystemare discussed.

The viral suspension stored at three different temperature levels *viz*., refrigerated condition (0 ± 1^0C), room temperature (25 ± 1^0C) and high temperature (35 ± 1^0C)at six regular intervals (monthly once for six months). Results revealed that the virus stored at high temperature (35^0C) readily lost its virulence with decreasing mortality from 77.92 per cent to 60.36 per cent at 5th day, larval mortality decreased from 78.44 per cent to 55.18 per cent at 7th day and larval mortality decreased from 93.80 per cent to 57.92 per cent at 10th day. The larval mortality slightly decreased at 0^0C from 78.42 per cent to 76.95 per cent at 5th day, the mortality was increased to 80.21 per cent to 78.45 per cent at 7th day and 93.80 per cent to 92.23 per cent at 10th day (Table 1).

Though it is an effective biopesticide it is under exploited due to problems in storage. Unlike chemical pesticides, viral pesticides often have a shorter shelf-life of infectivity (Shieh, 1978) that requires special attention for commercial operations. Viral insecticides cannot be developed commercially until their formulations are physically, chemically and environmentally stable in storage and distribution. For industrialization of product, formulation of pathogen product should have a shelf-life of more than 18 months (Couch and Ignoffo, 1981).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 and Rabindra and Jayaraj, 1995).

The virulence of virus depends on quality, storage conditions and duration of storage, storage temperature and pH of the product (Sireesha *et al*., 2010). The formulated *Dp*NPV bioassay were conducted at six regular intervals (monthly once for six months) against *D. pulverulentalis*. A viral suspension was formulated with (starch 10% + Tinopal 0.2% + Tween 80 1% + *Dp*NPV@$1x10^9$ POB/ml) and were stored at three different temperature levels *viz*., refrigerated condition (0 ± 1^0C), room temperature (25 ± 1^0C) and high temperature (35 ± 1^0C). Results of bioassay at regular interval against *D. pulverulentalis* revealed that the virus

stored at high temperature (35^{0}C) readily lost its virulence with decreasing mortality from 78.44 per cent to 55.18 per cent at 5th day, larval mortality decreased from 89.76 per cent to 61.67 per cent at 7th day and larval mortality decreased from 93.60 per cent to 62.65 per cent at 10th day. The larval mortality is slightly decreased at 0^{0}C from 80.21 per cent to 78.45 per cent at 5th day, the mortality was increased to 89.81 per cent to 88.21 per cent at 7th day and 97.80 per cent to 89.88 per cent at 10th day (Table 2).

According to Gopali and Lingappa (2001), reported that virus can be preserved for more than ten years at 4^{0}C without loss in its virulence. Gudauskas and Canerday (1968) found the thermal inactivation point of *Ha*NPV to be 75 to 80^{0}C for 10 minutes. It was concluded that virulence of virus depends on quality of virus, storage conditions, duration of storage, temperature and pH of the product.

Unlike chemical pesticides, these NPVs are multiplied on living insects presence of insect debris and putrefaction due to bacterial contamination is inevitable. Hence, for future field and laboratory use the polyhedra associated with many insect virus diseases are usually stored under refrigerated conditions as air dried, purified

Table 1 : Per cent larval mortality of *D. pulverulentalis* due to *Dp* NPV Suspension after different storage methodsand intervals

Storage period (Months)	Day after treatment under different storage conditions											
	5th day				7th day				10th day			
	0°C	25°C	35°C	Mean	0°C	25°C	35°C	Mean	0°C	25°C	35°C	Mean
1	78.42	78.23	77.92	78.19	80.21	79.21	78.44	79.28	93.80	93.44	93.70	93.64
	(62.31)	(62.18)	(61.97)	(62.15)^a	(63.58)	(62.87)	(62.33)	(62.92)^a	(75.58)	(75.15)	(75.46)	(75.39)^a
2	77.86	77.06	75.94	76.95	79.95	78.11	77.65	78.57	93.66	92.37	91.37	92.47
	(61.93)	(61.38)	(60.62)	(61.30)^a	(63.39)	(62.10)	(61.78)	(62.42)^a	(75.41)	(73.96)	(72.91)	(74.07)^a
3	77.65	75.97	74.85	76.15	79.61	76.51	74.47	76.86	93.54	92.14	90.31	91.99
	(61.78)	(60.64)	(59.90)	(60.76)^a	(63.15)	(61.00)	(59.65)	(61.24)^a	(75.27)	(73.71)	(71.86)	(73.55)^a
4	77.34	62.02	56.81	65.39	79.26	71.86	69.86	73.66	92.72	75.91	61.13	76.58
	(61.57)	(51.95)	(48.91)	(53.96)^b	(62.90)	(57.96)	(56.70)	(59.12)^b	(74.34)	(60.60)	(51.43)	(61.05)^b
5	77.13	58.40	51.28	62.27	79.02	68.22	61.78	69.67	92.48	70.92	60.97	74.79
	(61.43)	(49.83)	(45.73)	(52.10)^b	(62.73)	(55.68)	(51.81)	(56.58)^b	(74.08)	(57.36)	(51.33)	(59.86)^b
6	76.95	55.18	48.95	60.36	78.45	62.54	55.18	65.39	92.23	65.95	57.92	72.03
	(61.30)	(47.97)	(44.39	(50.97)^b	(62.34)	(52.26)	(47.97)	(53.96)^b	(73.81)	(54.30)	(49.55)	(58.07)^b
S.E.$\pm$				1.673				2.621				2.672
C.D. (P=0.05)				3.646				5.723				5.822

Numbers followed by the same letter are not statistically significant (p<0.001) by LSD
1.Refrigerated condition – (0^{0}C); 2.Room temperature – (25^{0}C); 3.High temperature – (35^{0}C)

Table 2 : Per cent larval mortality of *D. pulverulentalis* due to *Dp* NPVformulation after different storage methodsand intervals

Storage period (Months)	Day after treatment under different storage conditions											
	5th day				7th day				10th day			
	0°C	25°C	35°C	Mean	0°C	25°C	35°C	Mean	0°C	25°C	35°C	Mean
1	80.21	79.21	78.44	79.28	89.81	89.23	89.76	89.60	97.80	96.44	93.60	95.94
	(63.58)	(62.87)	(62.33)	(62.92)^a	(71.38)	(70.84)	(71.33)	(71.18)^a	(81.47)	(79.12)	(75.34)	(78.37)^a
2	79.95	78.11	77.65	78.57	89.67	88.45	85.81	87.97	97.10	92.66	90.43	93.39
	(63.39)	(62.10)	(61.78)	(62.42)^a	(71.25)	(70.13)	(67.87)	(69.70)^a	(80.19)	(74.28)	(71.97)	(75.10)^a
3	79.61	76.51	74.47	76.86	89.32	84.21	81.67	85.06	96.34	89.88	84.88	90.36
	(63.15)	(61.00)	(59.65)	(61.24)^a	(70.92)	(66.58)	(64.65)	(67.26)^a	(78.97)	(71.45)	(67.11)	(71.91)^b
4	79.26	71.86	69.86	73.66	89.11	81.01	75.98	82.03	93.76	85.77	78.88	86.13
	(62.90)	(57.96)	(56.70)	(59.12)^b	(70.73)	(64.16)	(60.65)	(64.91)^b	(75.53)	(67.83)	(62.64)	(68.13)^b
5	79.02	68.22	61.78	69.67	88.65	78.12	67.54	78.10	91.44	79.99	69.98	80.47
	(62.73)	(55.68)	(51.81)	(56.58)^b	(70.31)	(62.11)	(55.26)	(62.09)^b	(72.98)	(63.42)	(56.77)	(63.77)^c
6	78.45	62.54	55.18	65.39	88.21	65.43	61.67	71.77	89.88	67.65	62.65	73.39
	(62.34)	(52.26)	(47.97)	(53.96)^b	(69.91)	(53.98)	(51.74)	(57.90)^c	(71.45)	(55.33)	(52.32)	(58.94)^d
S.E.$\pm$				2.7271				2.621				2.965
C.D. (P=0.05)				5.9418				5.723				6.374

Numbers followed by the same letter are not statistically significant (p<0.001) by LSD
1. Refrigerated condition – (0^{0}C); 2. Room temperature – (25^{0}C); 3. High temperature – (35^{0}C)

polyhedra or as polyhedra with in dried larval cadavers. Under these conditions polyhedra were noted to resist deterioration for considerable periods of time (Sireesha *et al*., 2010). Some polyhedral viruses have been shown to retain their virulence for periods ranging from 2 to 25 years (Steinhaus, 1949 and Bergold, 1953).

This difference on larval population indicated that the freshness of product could enhance significantly the reduction of larvae and fresh product would be more efficient as compared to stored products. However, previously commercial formulation was recommended for the management of *H. armigera* (Srinivasa *et al*., 2008 and Jeyarani and Karuppuchamy, 2010).

The present investigation revealed that, the virus suspension when stored at high temperature leads to decrease in larval mortality and storage at low temperature leads to high larval mortality. The formulated viral suspension showed high larval mortality when compared to the viral suspension alone.

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