



STUDIES ON THE MODE OF TRANSMISSION OF *BOMBYX MORI* NUCLEAR POLYHEDROSIS VIRUS IN SILKWORM, *BOMBYX MORI* L

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ABSTRACT

Disease free layings of silkworm breed, DUN₂₂ were procured, incubated, brushed and reared upto 3rd instar enmass following the standard rearing method. Just after 3rd moult, the experiment was laid down into four treatments (T₁-T₄) as per the experimental design. Five replications of 100 larvae in each treatment were maintained and data with regard to 5th instar and total larval duration, diseases incidence, yield and pupation rate were recorded and analysed statistically. Perusal of data revealed significant difference in afore mentioned economic traits and therefore concluded that *Bombyx mori* Nuclear Polyhedrosis Virus (*BmNPV*) spreads infection secondarily and also had negative impact on the silkworm, *B. mori*.

Keywords: *Bombyx mori*, Infection, Nuclear Polyhedrosis virus and Viral disease.

INTRODUCTION

The silk industry faces severe setbacks in the past due to frequent disease outbreaks since silkworm *Bombyx mori*, is highly susceptible to the diseases like pebrine, flacherie, muscardine and grasserie. The diseases contributing considerably to the cocoon crop which directly affect the farming community due to reduced returns and affecting the earning foreign exchange (Govindan and Devaiah, 1995). Approximately 40 percent crop losses are attributed to diseases (Sheebarajakumari *et al.*, 2007). The loss due to *BmNPV* has been reported to the extent of 30 - 40 percent (Illahia *et al.*, 2007) and is perhaps the most extensively studied disease among all the silkworm diseases. It has been observed that, after through disinfection in the rearing environment there is always sporadic incidence of grasserie disease which is one of the reasons of low productivity. It was felt necessary to assess the mode of transmission of *BmNPV* and its impact on silkworm.

MATERIALS AND METHODS

The present study was carried out in the laboratory at College of Temperate Sericulture (CoTS), Mirgund, SKUAST-Kashmir. The disease free layings of silkworm breed, DUN₂₂ were reared upto 3rd instar enmass following the standard rearing method (Anonymous, 2003). After 3rd moult the worms were counted as per the treatments with five replications of 100 larvae and the data with regard to larval duration, disease incidence, cocoon yield and pupation were recorded and analyzed statistically. The experiment was laid down into four batches as per the experimental design and the treatment details (T₁-T₄). In T₁ 1ml of 1x10⁶ POB/ml of *BmNPV* was inoculated to hundred larvae through mulberry leaf immediately after 3rd moult, in T₂ one ml of 1x10⁶ POB/ml of *BmNPV* inoculum was sprayed on to the 100 larvae and rearing seat paper, in T₃ *BmNPV* infected silkworms (carriers) were introduced in a healthy population of silkworms at the ratio of 8:92 thus formed a group of 100 larvae including carriers and T₄ was reared without any treatment for comparison and served as

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control.

Isolation and purification of *Bombyx mori* Nuclear Polyhedral bodies

The *BmNPV* were collected from infected larvae and the same was centrifuged at 5000 rpm for 5mins. The sample was collected and suspended in distilled water and then filtered through absorbent cotton to remove the debris. The final sample was diluted in distilled water and then suspended in physiological saline, stored as stock solution at 5°C.

RESULTS AND DISCUSSION

Perusal of data revealed that significant difference both in

5th as well as total larval duration (Table 1) however shortest 5th instar larval duration was recorded in T₄ (180.01 hrs) and the longest in T₁ (212.86 hrs). Similar trend was recorded for total larval duration being shortest in T₄ (660.01hrs) and longest in T₁ (692.86hrs). Disease incidence was recorded and presented (Table 2) and a significant difference was recorded with respect to larval mortality being highest in T₁. Percent change with regard to larval mortality was calculated and observed highest in T₁ (97.74%) followed by T₃ (96.75%) and T₂ (95.96%). Pupal mortality viz., 23.00, 16.00, 19.00 % were recorded in T₁, T₂ and T₃ respectively. Further, percent change with respect to pupal mortality was to an extent of 95.65%, 94.73% and 93.75% in T₁, T₃, and T₂ respectively over control.

Table 1. Effect of *BmNPV* infection on 5th instar and total larval duration.

Treatment	5 th Instar larval duration	Total larval duration	Per cent change over control	
	(hrs)	(hrs)	5 th Instar larval duration	Total larval duration
T ₁	212.86 ^d	692.86 ^d	- 18.24	- 4.97
T ₂	200.53 ^b	680.53 ^b	- 11.39	- 3.10
T ₃	209.86 ^c	685.86 ^c	- 16.58	- 3.91.
T ₄	180.01 ^a	660.01 ^a	-	-
C.D (P≤0.05)	5.73	4.00	-	-
SE(m)	1.73	1.21	-	-

- Means with different super script are significantly different from each other

Cocoon yield by number was recorded in different treatments and the lowest cocoon yield was recorded in T₁ (4100.00) followed by T₃ (5900.00) and T₂ (6700.00). A drastic decrease of 58.02%, 39.59% and 31.59% was recorded in T₁, T₃ and T₂ over the control (Table 3). Similarly lower cocoon yield by weight was recorded in T₁ (5.93kg) as compared to 17.79Kgs in T₄. Significant differences were observed in pupation rate viz., 43.90%, 76.11%, 67.79%, and 98.98% in T₁, T₂, T₃ and T₄ respectively. Further, percent change was calculated and 55.64%, 31.51% and 23.10% were recorded in T₁, T₃ and T₂ respectively over T₄ (Table 4).

Table 2. Disease incidence due to *BmNPV* infection.

Treatment	Larval mortality	Pupal mortality	Percent change over control	
	(%)	(%)	Larval mortality	Pupal mortality
T ₁	59.00 ^d (50.16)	23.00 ^d (28.64)	97.74	95.65
T ₂	33.00 ^b (35.03)	16.00 ^b (23.56)	95.96	93.75
T ₃	41.00 ^c (39.79)	19.00 ^c (25.82)	96.75	94.73
T ₄	1.33 ^a (6.53)	1.00 ^a (5.73)	-	-
C.D (P≤0.05)	2.17	1.21	-	-
SE(m)	0.88	0.36	-	-

- Values in parenthesis are Arc sine transformed values
- Means with different super script are significantly different from each other

Mulberry silkworm, *Bombyx mori* L. is affected by different diseases which inflict great losses to sericulture. Among these diseases, *BmNPV* is the major one which accounts for 30-40% average crop loss in silkworm rearing and occurs during all seasons (Illahi and Nataraju, 2007). The disease spreads quickly and takes heavy toll of silkworms and even results in total crop failure when infection is severe. Apart from causing larval mortality, grasserie is also responsible for the post cocoon mortality (Chandrashakera *et al.*, 2004). A study was carried out to investigate the spread and impact of *BmNPV* infection on silkworm. Perusal of data revealed that the infection had shown significant effect on the economic characters studied. The 5th as well as total larval duration prolonged in infected batches which could be due to cessation of feeding and is in agreement with the findings of Gururaj *et al.*, (1999) and Mikhailov *et al.*, (1992) who reported that silkworm infected with viral disease loose feeding ability due to the decreased digestive enzymes activity and variation in hormones titers. Vijayakumari *et al.*, (2001)

have also reported prolongation of 5th instar duration in *BmNPV* infected silkworms is due to the increased production of juvenile hormone with decrease in ecdysone titer. Disease incidence was recorded in all treated batches but higher mortality was recorded in T₁ as compared to other two treatments. This may be due to the ingestion of higher dose of viruses and its quick dissolution in the midgut and libration of virions that invaded the neighbouring susceptible cells of the host and lead to the mortality in one cycle of its multiplication. However in case of T₂ and T₃ batches it might have required more such multiplication of virus cycles to exhibit the mortality. The present finding corroborates the study of Chandrasekharan *et al.*, (2006) who reported that higher dose of grasserie virus can cause mortality to the larvae within one cycle of its multiplication, but low dose may require more such multiplication cycles to cause mortality. It is also established from the present investigation that the *BmNPV* spreads infection secondarily by different modes however the degree of infection varies.

Table 3. Effect of *BmNPV* infection on Cocoon yield.

Treatment	Cocoon yield/10000 larvae by number	Cocoon yield/10000 larvae by weight (kg)	Per cent change in cocoon yield over control by	
			No.	Wt.
T ₁	4100.00 ^d	5.93 ^d	58.02	66.66
T ₂	6700.00 ^b	10.72 ^c	31.39	39.74
T ₃	5900 .00 ^c	9.65 ^b	39.59	45.75
T ₄	9766.66 ^a	17.79 ^a	-	-
C.D (P≤0.05)	240.59	1.33	-	-
SE(m)	72.64	0.56	-	-

- Means with different super script are significantly different from each other

Table 4. Effect of *BmNPV* on Pupation rate.

Treatment	Pupation (%)	Percent change over control
T ₁	43.90 ^d (41.47)	55.64
T ₂	76.11 ^b (60.71)	23.10
T ₃	67.79 ^c (55.40)	31.51
T ₄	98.98 ^a (83.35)	-
C.D (P≤0.05)	1.70	-
SE(m)	0.51	

- Values in parenthesis are Arc sine transformed values
- Means with different super script are significantly different from each other

This is in agreement with the investigation carried out Nataraju (1998) who reported the introduction of *BmNPV* carriers into the healthy population of silkworms resulted in the infection but the infection range in the population was in proportionate with the number of carriers introduced. In the silkworm, *B. mori* common sources of pathogens for infection and spread of diseases are the contaminated

rearing trays and seat papers (Miyajima, 1979; Baig *et al.*, 1990) which are used for the rearing of mulberry silkworm.

CONCLUSION

The pathogens are extruded by infected silkworms through gut juice, faecal matter, dead worms contaminating rearing

trays and mulberry leaves becomes source of infection in healthy population. Results of the present study elucidated that infection have influenced pupation significantly too and only 43.90%, 76.11% and 67.79% pupation was recorded in T₁, T₂ and T₃ respectively as compared to (98.98%) in T₄. Lower pupation in T₁ could be attributed to per oral inoculation of *BmNPV*. It is therefore, concluded that *BmNPV* spread infection through all the three modes studied however the intensity of infection varies in different treatments however, utmost care should be given to exclude the pathogen from the rearing environment by following the recommended prophylactic measures in order to harvest the good cocoon crop.

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