International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 9, Issue 4, pp: 9-12, 2024
https://doi.org/10.55126/ijzab.2024.v09.i04.003 **http://www.ijzab.com**

Research Article

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

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Article History: Received 29th March 2024; Accepted 17th May 2024; Published 1st July 2024

ABSTRACT

Disease free layings of silkworm breed, DUN_{22} 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 (T_1-T_4) 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 (*Bm*NPV) 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 *Bm*NPV 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 *Bm*NPV 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, DUN_{22} 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 (T_1-T_4). In T_1 1ml of $1x10^6$ POB/ml of *Bm*NPV was inoculated to hundred larvae through mulberry leaf immediately after 3rd moult, in T_2 one ml of $1x10^6$ POB/ml of *Bm*NPV inoculum was sprayed on to the 100 larvae and rearing seat paper, in T_3 *Bm*NPV 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 T_4 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 *Bm*NPV 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^{o}C.

RESULTS AND DISCUSSION

Perusal of data revealed that significant difference both in 5^{th} as well as total larval duration (Table 1) however shortest 5^{th} instar larval duration was recorded in T_4 (180.01 hrs) and the longest in T_1 (212.86 hrs). Similar trend was recorded for total larval duration being shortest in T_4 (660.01hrs) and longest in T_1 (692.86hrs). Disease incidence was recorded and presented (Table 2) and a significant difference was recorded with respect to larval mortality being highest in T_1. Percent change with regard to larval mortality was calculated and observed highest in T_1 (97.74%) followed by T_3 (96.75%) and T_2 (95.96%). Pupal mortality *viz.,* 23.00, 16.00, 19.00 % were recorded in T_1, T_2 and T_3 respectively. Further, percent change with respect to pupal mortality was to an extent of 95.65%, 94.73% and 93.75% in T_1, T_3, and T_2 respectively over control.

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

Treatment	5^{th} Instar larval duration (hrs)	Total larval duration (hrs)	Per cent change over control	
			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 T_1 (4100.00) followed by T_3 (5900.00) and T_2 (6700.00). A drastic decrease of 58.02%, 39.59% and 31.59% was recorded in T_1, T_3 and T_2 over the control (Table 3).Similarly lower cocoon yield by weight was recorded in T_1 (5.93kg) as compared to 17.79Kgs in T_4. Significant differences were observed in pupation rate *viz.*, 43.90%, 76.11%, 67.79%, and 98.98% in T_1, T_2, T_3 and T_4 respectively. Further, percent change was calculated and 55.64%, 31.51% and 23.10% were recorded in T_1, T_3 and T_2 respectively over T_4 (Table 4).

Table 2. Disease incidence due to *Bm*NPV 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, *Bm*NPV 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 *Bm*NPV 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 *Bm*NPV 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 T_1 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 T_2 and T_3 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 *Bm*NPV 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 *Bm*NPV 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 T_1, T_2 and T_3 respectively as compared to (98.98%) in T_4. Lower pupation in T_1 could be attributed to per oral inoculation of *Bm*NPV. It is therefore, concluded that *Bm*NPV 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.

ACKNOWLEDGMENT

The authors express sincere thanks to the head of the Division of Sericulture Crop Improvement, Mirgund, College of Temperate Sericulture Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Post Box No. 674, GPO- Srinagar, Kashmir, India for the facilities provided to carry out this research work.

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