

Research Article

Effect of nucleopolyhedrosis virus on silkworm *bombyx mori* linn and its transmission from further generation

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Abstract:

Sericulture is one of the most agro-based industries in World. From time, sericulture practices have undergone changes to improve productivity. Disease development and mortality are ever present phenomena in the silkworms as in other living organism. Among silkworm disease, Grasseric, a viral disease of silkworm, *Bombyx mori* L. has been causing great economic loss to sericulturist. Perhaps a good understanding of this disease, its mode of transmission and recent developments are of great relevance for combating the disease. The present study review deals with a detailed account of the LC₅₀ for larval mortality during fifth instar was 0.096 X 10⁶. Nucleopolyherosis virus inclusion bodies ml⁻¹ in Pure Mysore and 0.218 X 10⁶ PIBs ml⁴ in NB₄D₂ silkworms. Besides mortality of silkworms, there was a significant reduction in the cocoon weight, shell weight and shell ratio in the surviving silkworms. The F₁ progeny obtained by selfing the moths emerged from the silkworms inoculated with BmNPV exhibited inferiority in fecundity, hatchability, larval weight, cocoon yield, cocoon weight shell weight, shell ratio, filament length, denier and effective rate of rearing when compared to control batches.

Key word: Grasseric, *Bombyx mori*, BmNPV, commercial character and Developmental character.

Introduction:

The silkworm *Bombyx mori* Linn has been utilized for the production of silk which is generally referred as “Queen of fabrics” due to

its luster, softness color, biodegradability, elegance, biocompatibility, strength and flexible properties and also used as powerful biological

model system. The disease caused by *Bombyx mori* nuclear polyhedrosis virus BmNPV causes drastic damage in the cocoon yield affecting the income of silkworm growers. Grasseric disease causes more than 15% loss in yield and accounts for 25-58% in total disease incidence (28). The NPV infects various tissues and multiplies in the nucleus forming inclusion bodies called polyhedral, which are occluded viral particles (13). The silkworm cocoon crops are highly unpredictable due to several factors including various disease of various pathogens the nucleopolyherosis virus BmNPV of *Bombyx mori* L is one of the most widely distributed viruses in the sericultural belt of Eastern Uttar Pradesh and causes considerable damage in silk production. In Eastern Uttar Pradesh alone the infection of BmNPV accounted 27.76% (27). A major disease problem next pebrine *Nosema bombycis* infection in mass rearing of this insect in tropical countries like India is Grasseric, a polyhedrosis disease caused by *Bombyx mori* nucleopolyherosis disease caused by *Bombyx mori* nucleopolyherosis virus BmNPV. The appearance of BmNPV occlusion bodies (OBS) in the blood cells of the infected silkworm was first described independently by (17). In spite of attempting disease prevention measures we observed the incidence of BmNPV infection larvae rearing at fifth instar stage quite frequently, although the extent of the problem varied from one batch to the other. Kukan., (15) reviewed evidence that virus could be transmitted from parent to progeny in lepidopteron and could be found in caterpillars reared from surface decontaminated eggs. To investigate the larval mortality increased larval and pupal duration, reduced larval and pupal weight, reduced moth emergence with deformed undersized adults, reduced fecundity in the diseased individuals. The objectives were to determine the transmissibility of BmNPV in *Bombyx mori*., via parent to progeny, which is one of the most important conditions for the

commercial character of silkworm and sericulture industry. Therefore, to investigate such possibility of transmission of BmNPV in *Bombyx mori* from parent to progeny was carried out.

Materials and Methods:

Two mulberry silkworm races namely Mysore (multivoltine) and NB₄D₂ biovoltine at the age of fifth instar first day a stock of BmNPV nuclear polyhedrosis virus were used for the study. The nuclear polyhedrosis inclusion bodies (PIBs), obtained from sericulture grainage and the silkworm larvae inoculated into the observation for multiplication. Isolation and purification of PIBs were carried out by following the method described by (3)

The silkworm rearing was conducted in the laboratory following the method described by (14). For determination of breed susceptibility, a total of seven batches, each batch containing 50 worms in triplicate were inoculated by oral injection with 40µL of different concentrations of PIBs viz., 6.25X10⁶, 3.125 X 10⁶, 1.562 X 10⁶, 0.781 X 10⁶, 0.391 X10⁶, 0.195 X10⁶ and 0.0976 X 10⁶ per ml nuclear polyhedrosis in 0.75 % NaCl solution. The control worms received the same amount of 0.75% NaCl solution only. Later the worms were allowed to complete larval stage, spinning, pupation and moth emergence. Number of cocoons harvested from each batches were considered for calculation for calculation of breed susceptibility. The moths emerged from BmNPV inoculated larvae were selfed and the F₁ progeny raised were used to study the effects of BmNPV on the economic traits of silkworm. Two doses of BmNPV viz., 1.562 X 10⁶ per ml (T₁) and 3.125 X 10⁶ per ml (T₂) were selected for inoculation after studies on breed susceptibility. The larvae were allowed to complete larval duration spinning, pupation and moth emergence. Cocoons were harvested race wise and treatment wise at room temperature 26 ± 1°C and relative humidity of 80 ± 5%RH. The

procedure followed for the preparation of layings and incubation silkworm rearing and assessment of economic traits are as described by (21, 14, 19) respectively. The data derived from the above experiments were statistically analyzed by on way ANOVA (10) and Duncan multiple range test (8). The LC₅₀ values were calculated for BmNPV infection by using probit analysis (9).

Results and Discussion:

The LC₅₀ for larval mortality during fifth instar was $0.096 \pm 0.0024 \times 10^6$ PIBs per ml in pure Mysore followed by NB₄D₂ ($0.28 \pm 0.00027 \times 10^6$ PIBs/ml). This indicates that pure Mysore was more sensitive to BmNPV when compared with NB₄D₂ race. In pure Mysore as well as NB₄D₂, the viability rate was higher in the control batches and gradual reduction was noticed as the dose of BmNPV increased. According to (4) the pure Mysore is sensitive to BmNPV than NB₄D₂ in early stages of larval development. However, contrary to general

trend, the multivoltine pure Mysore was found to be more sensitive to BmNPV as compared to a biovoltine NB₄D₂ race. Such a contradiction might have direct relationship to the body weight as well as larval duration that is lighter race with longer larval duration pure Mysore is more susceptible to BmNPV as against the heavier race with lesser larval duration NB₄D₂. Apart from the mortality of silkworm there was a significant reduction in the cocoon weight, shell weight and shell ratio in the surviving silkworms of both pure Mysore as well as NB₄D₂ race (Table-1). This might be due to reduced feeding accompanied by the reduced digestibility and absorption since the pathogen destroys the pathogen might be utilized for its reproduction and metabolism. In addition the energy derived from the digestive food must have distributed between the host as well as virus for their mutual defense purpose.

Table-1: Effect of Nuclear polyhedrosis on larval mortality and cocoon characters.

Dose of BmNPV	RACE							
	PURE MYSORE				NB ₄ D ₂			
	No. Cocoon harvested	Cocoon Weight (g)	Shell Weight (g)	Shell Ratio (%)	No. Cocoon harvested	Cocoon Weight (g)	Shell Weight (g)	Shell Ratio (%)
C ₀ (Control)	45	0.92	0.14	13.99	44	1.78	0.37	18.88
C ₁ (0.098X10 ⁶ ml ⁻¹)	20	0.78	0.11	12.33	28	1.80	0.31	16.77
C ₂ (0.195X10 ⁶ ml ⁻¹)	19	0.81	0.087	10.88	20	1.92	0.29	15.88
C ₃ (0.391 X10 ⁶ ml ⁻¹)	16	0.82	0.082	09.77	17	1.88	0.28	15.22
C ₄ (0.781 X10 ⁶ ml ⁻¹)	14	0.78	0.079	09.55	15	1.70	0.26	15.09
C ₅ (0.562 X10 ⁶ ml ⁻¹)	12	0.73	0.071	09.22	13	1.68	0.25	14.99
C ₆ (3.125 X10 ⁶ ml ⁻¹)	09	0.71	0.069	09.11	10	1.69	0.23	14.88
C ₇ (6.25 X10 ⁶ ml ⁻¹)	06	0.70	0.065	09.34	08	1.59	0.22	14.37
F-Ratio	172.56	132.11	11.99	5.44	12.88	211.77	399.18	10.12
Probability	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000

Table-2: Effect of BmNPV Nuclear polyhedrosis on commercial characters of F₁ Progeny Race: Pure Mysore.

Dose Of BmNPV	Survival Yield 1000 healthy larvae for observation Pure Mysore Race											
	Fecundity	Hatching (%)	Wt. of 10 V th Instar larvae	Larval Duration (Hrs)	Survive Larvae No.	Wt. Of Larvae (Kg)	Single Cocoon Wt. (gm)	Single Shell Wt. (gm)	Shell Ratio (%)	Filament Length (m)	Denier	ERR
Control (00)	490	93.44	20.88	670	940	0.76	0.82	0.12	13.23	380	1.92	94.0
T ₁	432	95.66	19.88	670	930	0.67	0.78	0.11	12.77	349	1.89	91.2

1.56X10⁶ ml⁻¹												
T₂ 3.12X10⁶ ml⁻¹	405	94.77	19.32	670	925	0.64	0.74	0.09	12.32	322	1.81	90.1
F-ratio	41.89	95.54	114.77	-----	35.78	23.56	26.57	9.15	3.11	55.61	2.31	7.11
Probability	0.00	0.00	0.00	-----	0.00	0.00	0.00	0.01	0.12	0.00	0.17	0.02

Table-3: Effect of BmNPV Nuclear polyhedrosis on commercial characters of F₁ Progeny Race: NB₄D₂.

Dose Of BmNPV	Survival Yield 1000 healthy larvae for observation NB ₄ D ₂ Race											
	Fecundity	Hatching (%)	Wt. of 10 V th Instar larvae	Larval Duration (Hrs)	Survive Larvae No.	Wt. Of Larvae (Kg)	Single Cocoon Wt. (gm)	Single Shell Wt. (gm)	Shell Ratio (%)	Filament Length (m)	Denier	ERR
Control (00)	570	97.55	34.06	503	940	1.75	1.891	0.312	18.99	1050	2.18	93.6
T₁ 1.56X10⁶ ml⁻¹	521	94.44	31.71	503	932	1.65	1.742	0.29	16.77	945	2.16	92.6
T₂ 3.12X10⁶ ml⁻¹	497	93.32	30.01	503	923	1.58	1.679	0.28	16.02	882	2.08	91.9
F-ratio	25.89	32.21	32.23	---	16.21	43.13	40.31	31.5	12.14	15.69	21.42	271.7
Probability	0.001	0.001	0.001	---	0.000	0.000	0.000	0.01	0.008	0.004	0.002	0.000

The F₁ progeny obtained by selfing the moths emerged from the silkworms inoculated with BmNPV exhibited inferiority as compared to control batches in almost all the economic characters analyzed. The size of the eggs in the silkworm batches treated with BmNPV (7) was in pure Mysore but NB₄D₂ did not show significant reduction in the egg size(20). Almost all commercial characters like fecundity, hatchability, larval weight, cocoon yield, cocoon weight shell weight and ratio filament length denier and effective rate of rearing showed reduction when compared to the control sets of both the races (Table-2 and Table-3) varietal differences for studied traits in *Bombyx mori* has been reported by (1, 16, 11). Similar studies on varietal diversity have also been sustained by the finding of (26, 32, 33, 24). The reduction in the size and fecundity might be due to the deviation of digested food from normal metabolic state to synthesis of viral proteins and or due to reduced rate of ingestion digestion and assimilation of food owing to the malfunction of the midgut of

the worms infect with BmNPV. The inferiority in the economic traits of progeny might be due to the following reasons. Firstly as the egg size was found to be smaller in the BmNPV treated batches, the quantity of yolk, which is reserved for embryonic development, was also reduced; ultimate weak larva may hatch out(18). Secondly, it is also possible the BmNPV can be transmitted from generation to generation in an occult state, which might render the larva of *Bombyx mori* weak and incapable to perform normal metabolism. Thus inferior economic characters are produced. The findings of (12, 2, 23, 24) (Hukuhara, (12) and Aruga and Nagashima, (2) and Nagaku et al., (23); Pal and Moorthy, (24)) support such reasoning. They reported that BmNPV is transmitted from progeny of *Bombyx mori*. In contrast (31, 22)(Sikorowski et al., 1973; Neilson, M. M., 1965) reported that NPV is transmitted in the tobacco budworm, *Heliothis virescens*, on the surface of the eggs rather than inside them. Bullock et al., (5) and Rao et al., (25) also concluded that the surface contamination of eggs

is an important means of transmitting the NPV of the pink bollworm. However, (30) observed that the particles are present in the haemolymph of *Heliothis virescens* larvae and in the adult (29). This result suggested that the developing eggs might be under the influence of haemolymph, which contains virus particle. Thus inferior commercial characters might be due to weak larvae hatched out from the undersized eggs produced under the influence of BmNPV.

Conclusion:

Thus it may be inferred that the progeny obtained by selfing the moths emerged from BmNPV inoculated silkworms, exhibited inferiority in almost all characters analyzed and this knowledge can be used in the sericulture industry during the selection of parent seed cocoons for the preparation of disease free laying either for commercial purposes, which have a vital role in improvement of sericulture rearers and silk industry.

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