



**EFFECTS OF INDUCED TOLERANCE AGAINST NUCLEAR POLYHEDROSIS ON
ECONOMIC CHARACTERS OF SILKWORM *BOMBYX MORI* L. AND THEIR
INHERITANCE**

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ABSTRACT

To study the effects of induced tolerance in silkworm *Bombyx mori* L. against nuclear polyhedrosis virus two mulberry silkworm breeds viz., Pure Mysore and NB₄D₂ were selected. The breed susceptibility was determined during fifth instar. The silkworms were fed with known amount of inactivated nuclear polyhedral inclusion bodies (NPIBs) thrice at various time intervals for induction of tolerance followed by the challenge inoculation with known amount of live NPIBs. The silkworm larvae of the control, induced, induced followed by live PIBs inoculated and live NPIBs inoculated batches were allowed to continue development. The breed susceptibility, effect of polyhedrosis as well as induced tolerance on economic traits and their inheritance were studied. The induced and induced followed by live NPIBs inoculated batches exhibited almost similar pattern as in case of control larvae. This result paves light towards development of a practical control measure to be applied during mass rearing against the dreaded nuclear polyhedrosis in silkworm *Bombyx mori* L.

Keywords: Silkworm, Induced Tolerance, Nuclear Polyhedrosis Virus, Economic Characters, Inheritance

INTRODUCTION

The silkworm, *Bombyx mori*, lost its natural domestication since time immemorial. resistance against varieties of disease These pathogens caused an annual crop loss causing germs because of continuous up to 40%, which runs to the loss of several

crores of rupees. Of all silkworm diseases, which cause damage, viral diseases are the most serious. In India, the silkworm cocoon crop loss due to nuclear polyhedrosis has been reported to an extent of 32.9-55.3 % among the total silkworm diseases [1] and most common in summer season [2]. Therefore, some attempts have been made to immunize insects with NPV. Carbone and Fortuna [3] and Gargiulo [4] vaccinated silkworms in large scale against jaundice virus. Aizawa [5] observed some degree of protection when silkworm pupae were injected with a vaccine prepared from formalinized infected insect blood. Nataraju *et al.* [6] made an effort to develop an oral vaccine against nuclear polyhedrosis virus in silkworms. Also Mahesha *et al.* [7] reported the induction of tolerance against nuclear polyhedrosis and their effects on some biomolecules. In contrast to pebrine (*Nosema bombycis* Nageli), there is no clear-cut evidence about the mode of transmission of viruses from generation to generation, or about its phenotypic effects on the commercial characters of the progeny. However, some scientists reported the effect of *BmCPV* on commercial characters and their transmission from generation to generation [8]. Therefore, the present investigation was undertaken in an attempt to acquire information on the effect of nuclear polyhedrosis and induced

tolerance against *BmNPV* on commercial characters and their inheritance from generation to generation.

MATERIALS AND METHODS

Two mulberry silkworm breeds namely Pure Mysore (multivoltine) and NB₄D₂ (tropical bivoltine) were selected for the present investigation. Disease free layings of pure breeds were obtained from the Germplasm, Department of Studies in Sericultural Sciences, University of Mysore, Mysuru, India. The silkworm rearing of both parents as well as from F₁ progeny was conducted in the laboratory following the method described by Krishnaswami [9]. The Nuclear Polyhedral Inclusion Bodies were collected, confirmed and *per orally* inoculated into the silkworm larvae immediately after fourth moult for the multiplication of virus. After the silkworms which exhibited milky white haemolymph were collected, the abdominal legs were punctured and the haemolymph was collected in a clean, pre cooled 1.5ml micro centrifuge tubes. Purification of NPIBs was carried out by following the method described by Balakrishnappa and Honnaiah [10]. Finally, the stock suspension was prepared which contained 7.125 X 10⁶ polyhedral inclusion bodies ml⁻¹. Enumeration of polyhedral inclusion bodies was done by following Neuber's haemocytometer. For the induction of the

tolerance, polyhedral bodies were treated in 2% formaldehyde solution for 24h at $30 \pm 1^\circ\text{C}$ and used [7].

For breed susceptibility, immediately after fourth moult, that is, after first two feedings during fifth instar, mulberry leaves of M₅ variety were washed with distilled water and surface sterilized with 70 per cent ethyl alcohol using sterile cotton wad. Then the leaves were cut to square shape (10 cm²) and 0.115 ml NPIBs suspension (from the stock of different concentrations of *BmNPV* viz., 5.44×10^7 , 4.76×10^7 , 4.08×10^7 , 3.4×10^7 , 2.72×10^7 , 2.04×10^7 and 1.36×10^7 polyhedral bodies ml⁻¹ NPIBs suspension in sterile distilled water) was uniformly smeared and fed to the silkworms. Such two leaves (40 cm²) smeared with NPIBs suspension were made use for 100 worms. The NPIBs smeared leaves were shade dried and chopped to the required size and fed to the silkworms. Suitable untreated batches were also maintained. All experimental batches were maintained in triplicate. Later, the worms were allowed to complete larval stage, spinning, pupation and moth emergence. Number of cocoons harvested from each batch was considered for calculation of the breed susceptibility.

For induction of tolerance, the mulberry leaves of M₅ variety were washed with sterile distilled water and surface sterilized

with 70 per cent ethyl alcohol using sterile cotton wad. Then the leaves were cut to square shape (10 cm²) and 0.1 ml inactivated NPIBs suspension (from the stock of $0.835 \times 10^7\text{ml}^{-1}$ NPIBs suspension in sterile distilled water) was uniformly smeared, shade dried and chopped to required size and fed to the silkworms. Such type of inoculation was carried out thrice i.e., at the age of third instar second day, again at the age of fourth instar second day and also, at the age of fifth instar first day. This was followed by treatment with 0.125 ml of live NPIBs (from the stock of $1.36 \times 10^7 \text{ml}^{-1}$) on second day of fifth instar [7]. Suitable untreated batches were also maintained. All experimental batches were maintained with 100 worms in triplicate. Treated worms were allowed to complete development. Of the cocoons harvested, only uniform and healthy cocoons were selected, and preserved. The procedure followed for the preparation of layings & incubation, silkworm rearing and assessment of economic traits are as described by Narasimhanna [11], Krishnaswamy [9] and Mahesha [12] respectively. The control batches, induced batches, induced followed by live virus treated and live virus treated batches are mentioned as C, I, IL and L respectively. The data obtained from the above experiments were statistically analyzed

through SPSS by one way ANOVA, [13], Scheffe's post hoc test [14] and probit analysis [15] wherever they were applicable.

RESULTS AND DISCUSSION

In Pure Mysore breed, the viability rate was higher in the control batches and gradual reduction was noticed as the dose of *BmNPV* increased. The control (N_0) worms recorded a mean viability of 100% followed by N_1 of 72%, N_2 of 64%, N_3 of 52%, N_4 of 41%, N_5 of 32%, N_6 of 22% and N_7 of 16% (Table 1). The N_1 , N_2 , N_3 , N_4 , N_5 , N_6 and N_7 are the silkworm batches inoculated with *BmNPV* doses of 5.44×10^7 , 4.76×10^7 , 4.08×10^7 , 3.4×10^7 , 2.72×10^7 , 2.04×10^7 and 1.36×10^7 NPIBs ml^{-1} respectively. In case of single cocoon weight also, same pattern was noticed. The gradual reduction in the cocoon weight from 1.062 g in the control to 0.755 g in N_7 (heavily inoculated batch) was observed. In case of shell weight, gradual reduction from 0.133 g in the control to 0.071 g in N_7 batch was noticed. There was sharp reduction in shell weight from N_0 to N_7 batches. In the shell ratio, the same pattern was noticed. A gradual reduction from 12.52% in N_0 batch was reduced to 9.41% in N_7 batches.

In the NB_4D_2 breed, again, the larval mortality was less in the control batches and gradual reduction was observed as the dose of *BmNPV* increased (Table 2). Silkworms of control (N_0) batch recorded a mean

viability of 96% (from treatment to cocoon spinning), followed by 68% in N_1 , 58% in N_2 , 48% in N_3 , 42% in N_4 , 34% in N_5 , 11% in N_6 and 12% in N_7 batches. In case of single cocoon weight, N_0 (control) batches revealed a highest mean weight of 1.895 g followed by 1.715, 1.695, 1.665, 1.573, 1.480, 1.448 and 1.380 g in N_1 , N_2 , N_3 , N_4 , N_5 , N_6 and N_7 batches respectively. In case of shell weight, again, the control (N_0) batch registered a highest weight of 0.366 g followed by 0.286, 0.267, 0.251, 0.236, 0.221, 0.214 and 0.198 g in N_1 , N_2 , N_3 , N_4 , N_5 , N_6 and N_7 sets respectively. The shell ratio also showed gradual reduction from 19.32% in the control to 14.39% in N_7 batch.

The F_1 progeny obtained by selfing the moths emerged from the silkworms inoculated with *BmNPV*, exhibited inferiority as compared to control as well as induced, induced followed by live PIBs inoculated batches in almost all the economic characters analyzed. The size of the eggs in the silkworm batches treated with NPIBs was small in both the strains. Almost all commercial characters like fecundity, hatchability, cocoon yield, cocoon weight, shell weight, shell ratio, filament length and effective rate of rearing showed reduction when compared to the control, induced and induced followed by

live NPIBs inoculated sets of both the strains (Tables 3 and 4).

In the present investigation, breed susceptibility was carried out by smearing the mulberry leaves with PIBs. Of the tested breeds, Pure Mysore exhibited more susceptibility when compared to NB₄D₂ breed. In both the breeds, the viability rate was higher in control sets; but, the treated with *BmNPV* PIBs showed gradual reduction as the dose of the pathogen increased. However, contrary to the general trend, the multivoltine, Pure Mysore was found to be more susceptible to *BmNPV* as compared to a bivoltine. Such a contradiction might have direct relationship to the body weight that is a lighter race is more susceptible than the heavier races. Similar observation was recorded by Mahesha [12] during cytoplasmic polyhedrosis. In addition to the mortality of silkworms there was a significant reduction in the cocoon weight, shell weight and shell ratio in the surviving silkworms of all the three silkworm varieties when compared to their respective control batches. The reduction in cocoon and shell weight might be due the reduced feeding since pathogen attack. Moreover, the food material absorbed by the pathogen might have been utilized for its multiplication. In addition, the energy derived from the ingested food, must have been distributed between the host

as well as the pathogen for their mutual defense mechanism.

The F₁ progeny obtained by selfing the moths emerged from the silkworms inoculated with *BmNPV*, exhibited inferiority as compared to their respective control batches in all the economic traits analyzed. Similar observations made by Vail *et al.* [16] and, Vail and Gouch, [17] in *Trichoplusia ni*; Bullock *et al.* [18] in *Pectinophora gossypiella*; Simmons and Sikorowski [19] and, Sikorowski and Thomson [20] in *Heliothis virescens*; Mahesha and Honnaiah [8] in *Bombyx mori*. However, Sikorowski *et al.* [21] observed that the virus particles are present in the haemolymph of *Heliothis virescens* larvae and in the adult. This result suggested that the developing eggs might be under the influence of haemolymph, which contains virus particles. In addition, there was variation protein content [22] and [23], reduced enzyme activities [24] and haemolymph glucose level [25] were also reported. Even in the case of nuclear polyhedrosis also, the eggs produced by the live *BmNPV* infected mother moth were less in number as well as size; altered enzyme activities [26] and [7] were reported. Thus, inferior commercial characters might be due to weak larvae hatched out from the undersized eggs produced under the influence of virus. On the other hand the

induced and induced followed by live virus treated exhibited almost similarity as in case of control silkworms in the expression of commercial characters. Hence, the present investigation clearly indicated that the progeny obtained by selfing the moths emerged from live *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 layings either for commercial or academic purposes, which have a vital role in the improvement of sericulture industry. The information gathered in this research work may be applied in grainage activities during the selection of seed cocoons for the preparation of DFLs. In addition, the progeny obtained by selfing the moths emerged from inactivated and inactivated followed by the live NPIBs inoculated silkworms, exhibited insignificant variation from the control sets of almost all characters analyzed. Hence, the immunization techniques used in this work may be used to develop a practical control measure to be applied at rearers' level to avoid crop loss during required season. In addition, the information obtained from this research work contributes to basic virology in general.

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Table 1: Effects of Nuclear Polyhedrosis on Larval Mortality and Cocoon Characters of Pure Mysore strain

Dose of <i>BmNPV</i> ↓	No. of cocoons harvested	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
N ₀	100	1.062	0.133	12.52
N ₁	72	0.955	0.116	12.14
N ₂	64	0.950	0.098	10.36
N ₃	52	0.909	0.089	9.87
N ₄	42	0.878	0.083	9.51
N ₅	32	0.851	0.079	9.33
N ₆	22	0.842	0.079	9.41
N ₇	16	0.755	0.071	9.41

The variations between control and experimental sets and among different doses are all significant at 5% level; Fifty worms in triplicate for each dose were used for inoculation.

Table 2: Effects of Nuclear Polyhedrosis on Larval Mortality and Cocoon Characters of NB₄D₂ strain

Dose of <i>BmNPV</i> ↓	No. of cocoons harvested	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
N ₀	96	1.895	0.366	19.32
N ₁	68	1.715	0.286	16.68
N ₂	58	1.695	0.267	15.79
N ₃	48	1.665	0.251	15.11
N ₄	42	1.573	0.236	15.04
N ₅	34	1.480	0.221	14.96
N ₆	22	1.448	0.214	14.82
N ₇	12	1.380	0.198	14.39

The variations between control and experimental sets and among different doses are all significant at 5% level; Fifty worms in triplicate for each dose were used for inoculation

Table 3: Effects of Nuclear Polyhedrosis on Commercial Characters of F₁ Progeny. Race: Pure Mysore

Dose of <i>BmNPV</i>	Fecundity	Hatching (%)	Yield per 10000 larvae brushed		Cocoon wt. (g)	Shell wt. (g)	Shell ratio (%)	Filament Length (mt)	ERR
			Number	Weight (kg)					
C	514	98.49	9460	10.141	1.072	0.144	13.43	455	94.60
I	504	98.21	9458	10.120	1.070	0.141	13.17	445	94.58
IL	489	98.11	9458	10.120	1.071	0.141	13.16	445	94.58
L	401	85.41	9309	9.039	0.971	0.120	12.36	379	93.09

The variation between control and experimental sets and among different treatments is all significant at 5% level.

Table 4: Effects of Nuclear Polyhedrosis on Commercial Characters of F₁ Progeny. Race: NB₄D₂

Dose of <i>BmNPV</i>	Fecundity	Hatching (%)	Yield per 10000 larvae brushed		Cocoon wt. (g)	Shell wt. (g)	Shell ratio (%)	Filament Length (mt)	ERR
			Number	Weight (kg)					
C	580	98.11	9449	18.425	1.950	0.376	19.28	985	94.49
I	575	98.16	9421	18.333	1.946	0.375	19.27	982	94.21
IL	576	98.02	9411	18.136	1.921	0.368	19.15	964	94.11
L	466	90.01	9311	15.955	1.774	0.284	16.00	743	93.11

The variations between control and experimental sets and among different treatments are all significant at 5% level.