

## RESEARCH PAPER

# Silkworm breeds and their hybrids of *Bombyx mori* L. to *bmnpv* stress

M.H. ASHA AND R.N. BHASKAR

Department of Sericulture, University of Agricultural Sciences, G.K.V.K., BENGALURU (KARNATAKA) INDIA

*BmNPV* (*Bombyx mori* nuclear polyhedrosis virus) causes nuclear polyhedrosis in silkworms. This paper reports on the relative susceptibility of silkworm pure breeds and their hybrids reared under *BmNPV* stress condition. Infection during fourth and fifth instar silkworm *Bombyx mori* L., with nuclear polyhedrosis virus caused reduction in larval weight and revealed significant results. However, maximum larval weight of 3.67 and 3.98g/10 was noticed in fourth instar inoculated batches ( $10^{-1}$  and  $10^{-3}$ ) of  $CSR_2$ . Among hybrids,  $CSR_4 \times CSR_{16}$  and  $CSR_2 \times CSR_4$  have recorded (5.34 and 5.35 g/10) and (4.77 and 5.47g/10) compared to other hybrids. On the other hand, fourth instar inoculated batches of fifth instar also recorded maximum larval weight in  $CSR_2$  (13.88 and 14.18g/10 and 11.68 and 11.74g/10). Further among hybrids of same instar inoculated,  $CSR_4 \times CSR_{16}$  recorded (19.06 and 19.90g/10 and 20.21 and 21.63g/10) which was found maximum than other hybrids. Effective rate of rearing (ERR) of fourth instar inoculated batches were realized differently due to the administration of *BmNPV*. However, the maximum ERR (59.33 and 64.00%) and (62.00 and 62.00%) was recorded in PM which exhibited more survival percentage followed by  $CSR_4$  (58.67 and 56.00%) and (58.67 and 59.33%) compare to other two breeds. Among hybrids,  $PM \times CSR_4$  was recorded highest ERR (60.67 and 58.00%) and (58.67 and 57.33%) when administered with  $10^{-1}$  and  $10^{-3}$ , respectively. The same trend has been noticed even in control lots. The results clearly indicated that, bivoltine breeds and their hybrids reflected low ERR percentage values inturn more sensitive to *BmNPV* stress.

**Key words :** Larval weight, ERR, *BmNPV*, Silkworm breeds

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## INTRODUCTION

Silkworm is susceptible to many diseases caused by viruses and this is one of the main problems in sericulture every year. Generally, 70 per cent of damages caused by diseases are due to viruses (Anonymous, 1976). Among viruses, nuclear polyhedrosis viruses (NPV) in recent years have caused the highest damage to silkworm (*Bombyx mori*) in tropical regions (Sivaprasad *et al.*, 2003 and Biabani *et al.*, 2005).

*BmNPV* affect midgut epithelial cells, trachea system, hemolymph cells, fat body, etc. The nuclear of middle and inner cells of silk gland are also sometimes invaded by this virus (Khurad *et al.*, 2004).

Domestication of the mulberry silkworm *Bombyx mori* L. has rendered it susceptible to a number of diseases caused by different pathogenic agents such as viruses, bacteria, fungus and microsporidia. *BmNPV*, which belongs to Baculoviridae,

causes nuclear polyhedrosis in silkworms. Nuclear polyhedrosis is the most common viral disease and is prevalent in almost all the sericultural areas in India. The incidence of nuclear polyhedrosis in India is reported to be 20-40 per cent (Chitra *et al.*, 1975) and was estimated at 33-55 per cent in different seasons in Karnataka, India (Nataraju *et al.*, 1998). This disease is caused by an occluded baculovirus, (*BmNPV*) and it is prevalent during all the rearing seasons. However, its severity was noticed during summer followed by rainy season (Selvakumar *et al.*, 2002). Prevention of this has become one of the most-important aspects in the success of commercial sericulture. In order to obtain high and stable cocoon yield it is necessary to reduce the incidence which inturn decrease pathogen quantity and pathogenicity and strengthen the larval health by increasing their disease resistance ability (Singh *et al.*, 2003). Larval susceptibility to viral disease greatly differed in various hybrids. Many researchers have reported that, inter-breed/strain differences

were noticed in susceptibility to *Bombyx mori* NPV, but very few studies have been carried out on the development of resistance of insects to pathogens (Furuta, 1995). The resistance in silkworm hybrids have physiological and genetic origins that are controlled by polygenes (Chen *et al.*, 2003). The persistence of *BmNPV* polyhedra, high temperature and humidity are the major factors that contribute to the crop losses due to nuclear polyhedrosis at farmer's level in India. The best approach to prevent an infectious disease such as nuclear polyhedrosis may be to use relatively tolerant silkworm breeds. This is due to the fact that the resistance to *BmNPV* is controlled by polygenes (Aratake, 1973). Before initiation of any breeding programme for disease resistance/tolerance, the tolerance level of the available breeding resource materials should be explored. The present study was undertaken to screen the available pure breeds for their relative tolerance to *BmNPV* and to identify and utilize the comparatively tolerant pure breeds for evolving certain crosses for use in future breeding programmes.

## RESEARCH METHODOLOGY

To know the susceptibility of silkworm breeds against *BmNPV*, the first day of fourth and fifth instar larvae of PM, CSR<sub>2</sub>, CSR<sub>4</sub>, CSR<sub>16</sub> and their hybrids *viz.*, PMxCSR<sub>2</sub>, PMxCSR<sub>4</sub>, PMxCSR<sub>16</sub>, CSR<sub>2</sub>xCSR<sub>4</sub>, CSR<sub>2</sub>xCSR<sub>16</sub> and CSR<sub>4</sub>xCSR<sub>16</sub> were used for *BmNPV* viral stress. After third moult, 50 larvae per each replication were inoculated per orally. The leaf bits (10x12 cm size) were prepared, washed in running water and shade dried and sterilized. These mulberry leaf bits were smeared evenly with the virus suspension @ 0.25ml of *BmNPV* PIB's by using non-absorbent cotton. After shade dried for five

minutes, the leaf fed to the silkworms. Control batches were fed with surface sterilized mulberry leaves for the first feed. Subsequent feeding was inoculum free leaves for both treated and untreated batches. These pure breeds and their hybrids were reared according to standard rearing practices (Dandin *et al.*, 2003). Data were collected and analysed by three factorial design on fourth instar larval weight (g/10), fifth instar larval weight (g/10), and ERR (%) for both treated and untreated batches.

## RESEARCH FINDINGS AND ANALYSIS

The larval weight of fourth and fifth instar silkworm revealed significant results. However, maximum in fourth instar inoculated larval weight (3.67 and 3.98g/10) was encountered for 10<sup>-1</sup> and 10<sup>-3</sup> fed batches of CSR<sub>2</sub>. Among hybrids, CSR<sub>4</sub>xCSR<sub>16</sub> and CSR<sub>2</sub>xCSR<sub>4</sub> has recorded (5.34 and 5.35 g/10) and (4.77 and 5.47g/10) compared to other hybrids. Fourth instar inoculated in fifth instar also significantly increased larval weight in CSR<sub>2</sub> of 13.88 and 14.18g/10 and 11.68 and 11.74g/10. Further among hybrids, CSR<sub>4</sub>xCSR<sub>16</sub> recorded maximum fifth instar larval weight of 19.06 and 19.90g/10 and 20.21 and 21.63g/10 was noticed in both the inoculated batches (Table 1 and 2). Present study indicated a wide significant variation in the fourth instar larval weight and fourth and fifth instar (fourth instar inoculated) larval weight of same instar administered with 10<sup>-1</sup> and 10<sup>-3</sup> and fifth instar inoculated larval weight. It is very much clear that, different races and their hybrids differ greatly in larval weight due to *BmNPV* infection. This experimental results are in conformity with Bhaskar *et al.* (1989) who reported that, the weight reduction of kenchu virus inoculated worms at 10<sup>th</sup> day ranged from 24.60 to 76.14

**Table 1 : Effect of *BmNPV* infection at fourth instar inoculated on different silkworm breeds and their hybrids on larval weight (g/10) and ERR**

Silkworm breeds/ hybrids	Fourth instar larval weight (g/10)			Fifth instar larval weight (g/10)			ERR (%)		
			Control			Control			Control
	10-1	10-3		10-1	10-3		10-1	10-3	
PM	1.40	1.58	1.74	3.88	4.42	4.90	59.33 (7.73)	64.00 (8.03)	86.00 (9.30)
CSR <sub>2</sub>	3.67	3.98	4.30	13.88	14.18	14.84	52.67 (7.29)	55.33 (7.47)	82.00 (9.08)
CSR <sub>4</sub>	3.22	3.25	3.75	10.82	11.68	12.03	58.67 (7.69)	56.00 (7.51)	82.67 (9.12)
CSR <sub>16</sub>	2.82	2.94	3.22	9.93	10.91	11.66	52.00 (7.24)	54.67 (7.43)	82.00 (9.08)
PMxCSR <sub>2</sub>	3.77	3.85	3.90	14.99	15.28	16.19	58.67 (7.69)	56.00 (7.51)	82.33 (9.10)
PMxCSR <sub>4</sub>	3.67	3.75	3.89	18.80	19.79	20.94	60.67 (7.81)	58.00 (7.65)	84.00 (9.19)
PMxCSR <sub>16</sub>	3.60	3.80	3.91	18.63	18.70	19.30	58.00 (7.65)	57.33 (7.60)	82.67 (9.12)
CSR <sub>2</sub> xCSR <sub>4</sub>	4.77	5.47	5.97	11.24	14.29	18.94	0.00 (0.71)	56.00 (7.51)	81.33 (9.04)
CSR <sub>2</sub> xCSR <sub>16</sub>	4.04	4.17	4.48	13.13	13.86	17.74	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
CSR <sub>4</sub> xCSR <sub>16</sub>	5.34	5.35	5.92	19.06	19.90	20.97	56.00 (7.51)	54.00 (7.38)	81.33 (9.04)
Mean	3.63	3.81	4.11	13.43	14.301	15.75	45.60 (6.20)	51.13 (6.88)	74.43 (8.279)
F test	*	*	*	*	*	*	*	*	*
S.E. ±	0.095	0.142	0.045	0.465	0.617	0.044	1.535 (0.100)	1.670 (0.111)	1.491 (0.081)
C.D. (P = 0.05)	0.282	0.419	0.132	1.373	1.820	0.131	4.528 (0.295)	4.940 (0.327)	4.398 (0.240)

\* indicate significance of value at P=0.05

NS = Non-significant

**Table 2 : Effect of *BmNPV* infection at fifth instar inoculated on different silkworm breeds and their hybrids on larval weight (g/10) and ERR (%)**

Silkworm breeds/ hybrids	Fifth instar larval weight (g/10)		Control	ERR (%)		Control
	10 <sup>-1</sup>	10 <sup>-3</sup>		10 <sup>-1</sup>	10 <sup>-3</sup>	
PM	5.21	5.38	4.96	62.00 (7.90)	62.00 (7.90)	85.34 (9.26)
CSR <sub>2</sub>	11.68	11.74	14.95	56.00 (7.51)	58.67 (7.69)	82.67 (9.12)
CSR <sub>4</sub>	11.32	12.24	12.08	58.67 (7.69)	59.33 (7.73)	84.33 (9.25)
CSR <sub>16</sub>	10.49	10.62	11.7	56.67 (7.56)	54.67 (7.42)	83.33 (9.15)
PMxCSR <sub>2</sub>	12.16	12.52	16.36	58.00 (7.65)	57.33 (7.60)	85.33 (9.26)
PMxCSR <sub>4</sub>	18.54	18.95	20.97	58.67 (7.69)	57.33 (7.60)	84.67 (9.23)
PMxCSR <sub>16</sub>	14.21	15.49	19.31	56.67 (7.56)	56.67 (7.56)	83.67 (9.17)
CSR <sub>2</sub> xCSR <sub>4</sub>	10.75	11.1	18.98	55.33 (7.47)	54.67 (7.42)	82.00 (9.08)
CSR <sub>2</sub> xCSR <sub>16</sub>	15.15	15.44	17.77	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
CSR <sub>4</sub> xCSR <sub>16</sub>	20.21	21.63	21.06	54.00 (7.38)	54.67 (7.43)	83.33 (9.15)
Mean	12.97	13.51	15.81	51.60 (6.91)	51.53 (6.91)	75.75 (8.34)
F test	*	*	*	*	*	*
S.E. ±	0.289	0.36	0.012	1.660 (0.110)	1.578 (0.104)	1.912 (0.104)
C.D.(P = 0.05)	0.851	1.071	0.035	4.897 (0.324)	4.654 (0.307)	5.640 (0.307)

\*indicates of significance of values at P=0.05, respectively

NS= Non-significant

per cent in PMxNB<sub>4</sub>D<sub>2</sub> and NB<sub>7</sub> breeds, respectively for the breeds PM and NB<sub>7</sub> as maternal parents. The same experimental data reflected in the present study. It is further confirmed by Narayanaswamy *et al.* (1985) that, the lethal time required for 50 per cent weight reduction in kenchu virus stock culture was 9 days in PM, 11 days 12 hrs in Hosa Mysore and 17 days 7 hrs in NB<sub>4</sub>D<sub>2</sub> while no weight reduction was noticed in NB<sub>7</sub> and NB<sub>18</sub> breeds.

The decrease in effective rate of rearing (%) was observed to be more at higher doses of *BmNPV* inoculated for fourth and fifth instar of pure breeds and their hybrids. The effective rate of rearing (ERR), which reflects the success of cocoon crop. ERR of fourth instar batches were realized differently due to the administration of *BmNPV*. However, the maximum ERR (59.33 and 64.00%) and (62.00 and 62.00%) was recorded in PM which exhibited more survival percentage followed by CSR<sub>4</sub> (58.67 and 56.00%) and (58.67 and 59.33%) compared to other two breeds. Among hybrids, PMxCSR<sub>4</sub> recorded highest ERR (60.67 and 58.00%) and (58.67 and 57.33%) when administered with 10<sup>-1</sup> and 10<sup>-3</sup>, respectively compared to other hybrids. The same trend has been noticed in control lots. It is very clearly indicated that, bivoltine breeds and their hybrids

reflected low ERR values inturn more sensitive to *BmNPV* infection. The above results are in parallel with those of Sharma *et al.* (2007) who studied that, inoculum dose at 5×10<sup>7</sup> has recorded 33 per cent survival rate (TN white) to 70.67 per cent (BL<sub>37</sub>). At 1×10<sup>8</sup> inoculum dose there was no survival in TN white while BL<sub>37</sub> showed highest survival (44%) followed by Mysore princes (39.67%). The survivability of the foundation crosses after inoculating with *BmNPV vis-à-vis* their parental breeds is showed a distinct superiority of the resultant crosses over their parental breeds in terms of their relative tolerance to *BmNPV*. It also confirmed with the findings of Sowmyashree and Nataraju (2007) and according to them, some of the breeds showing high survival per cent against high dose of *BmNPV* inoculums and also have high survival per cent with low concentration *BmNPV* inoculation and expose them to low temperature. Usha (1996) has also reported highest reduction in the rate of cocooning in NB<sub>7</sub>, lowest reduction in *C.Nichi* when infected with *BmNPV*. Jaiswal *et al.* (2004) have observed reduced ERR under higher NPV infections the ERR decreased considerably in NB<sub>18</sub> (79.27 with 11.06% mortality due to *BmNPV*) compared to Pure Mysore 92.48 per cent ERR with 2.18 per cent mortality due to NPV.

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