



## SENSITIVITY OF SILKWORM BREEDS AND THEIR HYBRIDS OF *Bombyx mori* L. TO *BmNPV* ON $ET_{50}$ FOR SYMPTOM EXPRESSION (DAYS) AND TOTAL LARVAL MORTALITY (%)

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### ABSTRACT

Sericulture is one of the oldest agro based industries in the world. Sericulture practices have undergone changes to improve productivity. Disease development and mortality are ever present phenomena in the silkworms as in other living organisms. The silkworm *Bombyx mori* due to its domestication, prone to many microbial infections they cause different diseases. Among silkworm diseases, grasserie, a viral disease of silkworm, *Bombyx mori* L. is causing great economic loss to sericulturists. Infection during fourth and fifth instar cause more  $ET_{50}$  value for symptom expression and less mortality in both the instars of Pure Mysore. Further, the same breed has taken more time (7.67 and 8.33 days) for first symptom expression. Among hybrids, PMxCSR<sub>16</sub> has recorded highest  $ET_{50}$  value (5.67 and 6.67 days) for both ( $10^{-1}$  and  $10^{-3}$ ) viral dilutions respectively. However, the minimum (36.00 and 39.33%) larval mortality was noticed in  $10^{-1}$  fed batches of pure Mysore and PMxCSR<sub>4</sub> hybrid respectively. Whereas, the fourth instar inoculated lots of CSR<sub>2</sub>xCSR<sub>4</sub> and CSR<sub>2</sub>xCSR<sub>16</sub> have reported 100 percent total larval mortality indicating their high sensitive to the disease while the hybrids involving Pure Mysore multivoltine breed as maternal parents exhibited more  $ET_{50}$  value. In fifth instar,  $10^{-1}$  fed batches caused 100% total larval mortality in CSR<sub>2</sub>xCSR<sub>16</sub> and minimum (38.00) was noticed in pure Mysore.

**KEYWORDS:** Silkworm, Pure breeds, Bivoltine, *BmNPV* and Susceptibility.

### INTRODUCTION

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. *Bombyx mori* nuclear polyhedrosis virus (*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% (Chitra *et al.*, 1975) and was estimated as 33-55% in different seasons in Karnataka, India (Nataraju *et al.*, 1998). The persistence of *BmNPV* polyhedral, 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.

### MATERIALS AND METHODS

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 crosses *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. All the parents and their hybrids were reared in three replications. After third moult 50 larvae per each replication were taken and 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 they are 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 was collected on two rearing traits such as  $ET_{50}$  for symptom Expression (days) and Total larval mortality (%) for both the treated and untreated batches. To know their sensitivity against *BmNPV*.

### RESULTS AND DISCUSSION

#### $ET_{50}$ for symptom expression (days)

Time taken for first symptom expression due to *BmNPV* found significantly different in both the doses ( $10^{-1}$  and  $10^{-3}$ ) administered to fourth instar larvae in all the breeds and hybrids. Among hybrids, PMxCSR<sub>16</sub> has recorded highest  $ET_{50}$  value (5.67 and 6.67 days) followed by CSR<sub>4</sub>xCSR<sub>16</sub> (5.67 and 6.33 days). The trend was found similar even in pure breeds. However, the Pure Mysore breed has taken more time (7.67 and 8.33 days) for first symptom expression due to *BmNPV* and found to be more tolerant than other three bivoltine breeds experimented *viz.*, CSR<sub>2</sub> (5.67 and 7.00 days), CSR<sub>4</sub> (6.67 and 6.67 days) and CSR<sub>16</sub> (5.33 and 5.67 days) (Table 1 & fig 1). At  $10^{-1}$  viral dilution to fifth instar larvae resulted non-significant

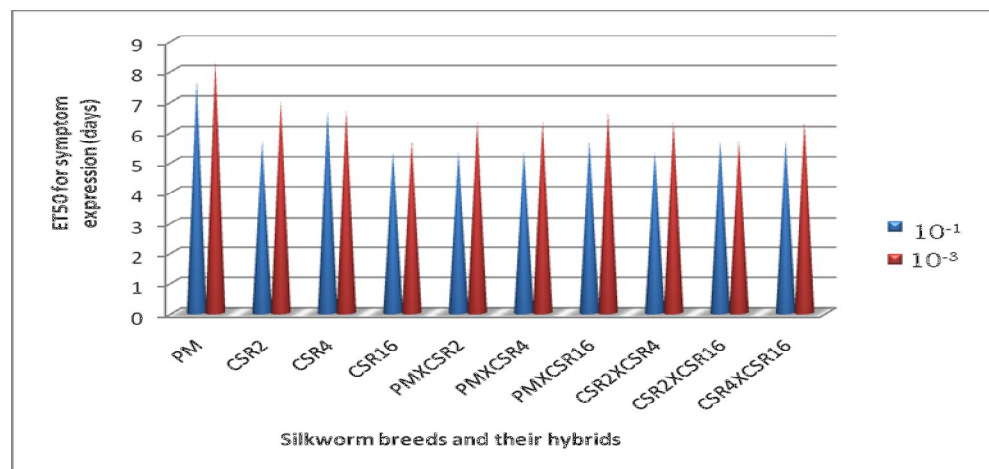
Sensitivity of *Bombyx mori* L. to BmNPV on ET<sub>50</sub> for symptom expression and total larval mortality results. Further, the 10<sup>-3</sup> fed batches taken different days to express the symptoms of NPV. In general bivoltine crosses have shown more ET<sub>50</sub> value viz., (7.67days) CSR<sub>2</sub>xCSR<sub>4</sub>, (7.67days) CSR<sub>2</sub>xCSR<sub>16</sub> and (8.33days) CSR<sub>4</sub>xCSR<sub>16</sub> compared to multivoltine crosses PMXCSR<sub>2</sub> (6.67days), PMxCSR<sub>4</sub> (7.33days) and PMxCSR<sub>16</sub> (7.33 days), respectively (Table 1 & fig 2).

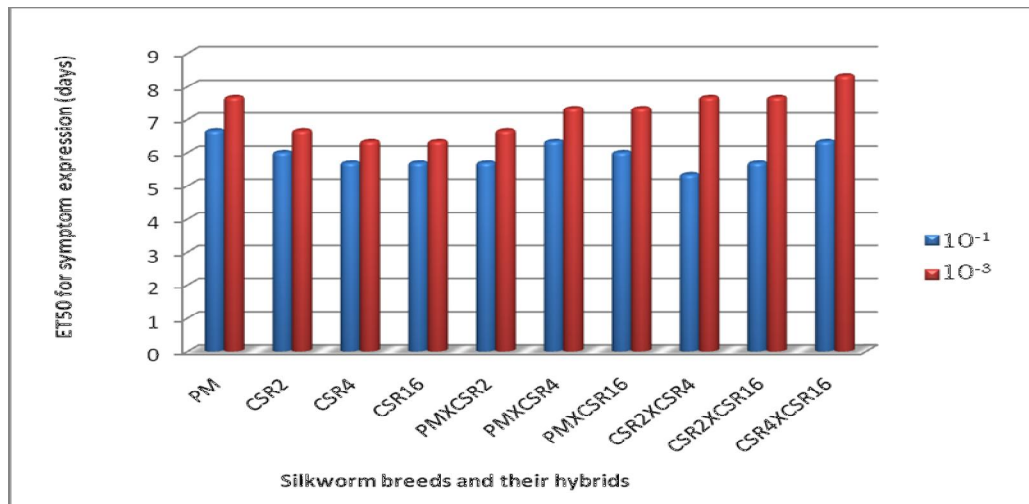
**TABLE- 1:** Influence of *BmNPV* infection at fourth and fifth instar inoculated batches of different silkworm breeds and their hybrids on ET<sub>50</sub> for symptom expression (days) and total larval mortality (%).

Silkworm Breeds/ Hybrids	ET <sub>50</sub> for symptom expression(days)		Total larval mortality (%)		ET <sub>50</sub> for symptom expression(days)		Total larval mortality (%)	
	IV <sup>th</sup> instar				V <sup>th</sup> instar			
	10 <sup>-1</sup>	10 <sup>-3</sup>	10 <sup>-1</sup>	10 <sup>-3</sup>	10 <sup>-1</sup>	10 <sup>-3</sup>	10 <sup>-1</sup>	10 <sup>-3</sup>
PM	7.67	8.33	47.33 (6.91)	36.00 (6.04)	6.67	7.67	38.00 (6.20)	38.00 (6.20)
CSR <sub>2</sub>	5.67	7.00	41.33 (6.47)	44.00 (6.67)	6.00	6.67	44.00 (6.67)	40.67 (6.41)
CSR <sub>4</sub>	6.67	6.67	40.67 (6.41)	44.67 (6.72)	5.67	6.33	41.33 (6.47)	41.33 (6.47)
CSR <sub>16</sub>	5.33	5.67	48.00 (6.96)	45.33 (6.77)	5.67	6.33	43.33 (6.62)	45.33 (6.77)
PMxCSR <sub>2</sub>	5.33	6.33	42.00 (6.51)	44.00 (6.67)	5.67	6.67	44.67 (6.72)	45.33 (6.76)
PMxCSR <sub>4</sub>	5.33	6.33	39.33 (6.30)	42.00 (6.52)	6.33	7.33	42.00 (6.52)	45.33 (6.77)
PMxCSR <sub>16</sub>	5.67	6.67	44.00 (6.67)	44.67 (6.72)	6.00	7.33	43.33 (6.62)	43.33 (6.62)
CSR <sub>2</sub> xCSR <sub>4</sub>	5.33	6.33	0.00 (0.71)	44.00 (6.67)	5.33	7.67	41.33 (6.47)	42.67 (6.57)
CSR <sub>2</sub> xCSR <sub>16</sub>	5.67	5.67	100 (10)	100 (10)	5.67	7.67	100 (10)	100 (10)
CSR <sub>4</sub> xCSR <sub>16</sub>	5.67	6.33	43.33 (6.62)	42.67 (6.56)	6.33	8.33	46.00 (6.82)	42.67 (6.57)
Mean	5.83	6.53	34.60 (5.43)	38.73 (6.00)	5.93	7.20	38.40 (5.97)	38.47 (5.98)
F test	*	*	*	*	NS	*	*	*
SEm ±	0.333	0.365	1.445 (0.112)	1.801 (0.136)	0.350	0.333	1.660 (0.126)	1.578 (0.120)
CD at 5%	0.983	1.077	4.263 (0.330)	5.313 (0.402)	1.031	0.983	4.897 (0.371)	4.654 (0.353)

Significant; NS: Non-Significant

**FIGURE-1:** Sensitivity of silkworm breeds and hybrids (fourth instar inoculated) on ET<sub>50</sub> for symptom expression (days) due to *BmNPV*.



**FIGURE-2:** Sensitivity of silkworm breeds and their hybrids (fifth instar inoculated) on ET<sub>50</sub> for symptom expression (days) due to *BmNPV*.**Total larval mortality (%)**

Laboratory experimental data on total mortality due to *BmNPV* administered to multivoltine and bivoltine breeds and their hybrids resulted decreased total mortality with increased viral dilutions. The 10<sup>-1</sup> fed batches caused 100 per cent total larval mortality in case of CSR<sub>2</sub>xCSR<sub>16</sub> followed by CSR<sub>2</sub>xCSR<sub>4</sub>. However, the minimum (36.00 and 39.33%) larval mortality was noticed in pure Mysore and PMxCSR<sub>4</sub> hybrid. The remaining bivoltine breeds *viz.*, CSR<sub>4</sub> (40.67 and 44.67 per cent), CSR<sub>2</sub> (41.33 and 44 per cent) and CSR<sub>16</sub> (48.00 and 45.33 per cent) recorded at 10<sup>-1</sup> and 10<sup>-3</sup> dilutions respectively. The Pure Mysore and their hybrids registered comparatively lesser total mortality (39.33 to 44.00 % and 42.00 to 44.67%) compare to bivoltine hybrids (16.27 to 19.36 and 34.60 to 44.00%). In fifth instar, the inoculated batches fed with 10<sup>-1</sup> dilution caused 100% total larval mortality in case of CSR<sub>2</sub>xCSR<sub>16</sub>. However, the minimum (38.00) and maximum (46.00%) larval mortality was noticed in pure Mysore and CSR<sub>4</sub> xCSR<sub>16</sub> hybrid. The remaining bivoltine breeds *viz.*, CSR<sub>2</sub> (44.00 and 40.67%), CSR<sub>4</sub> (41.33% and 41.33%) and CSR<sub>16</sub> (43.33 and 45.33 %) recorded same trend at 10<sup>-1</sup> and 10<sup>-3</sup> dilutions respectively.

These experimental results are in conformity with the findings of other researchers. However, administration of different dilutions of *BmNPV* to silkworm breeds and their hybrids exhibited significant results on both ET<sub>50</sub> for symptom expression and mortality. It is very clearly indicated that, increased viral dilution exhibited higher ET<sub>50</sub> value which was observed for PM 8.33 and 7.67 (10<sup>-3</sup>). In fifth instar inoculated batches recorded non significant at 10<sup>-1</sup>. In general PM and its components expressed higher ET<sub>50</sub> values compared to bivoltine components. Findings of Ravikumar *et al.* (2003) when different breeds and their hybrids administered with *BmNPV*, CSR<sub>4</sub>xCSR<sub>5</sub> expressed 12.74 and 12.49 days for ET<sub>50</sub> mortality than KSO-1 (12.30) and NP<sub>2</sub> (12.95) days respectively. Further, Baig *et al.* (1991) studied the relative susceptibility of different races of silkworm, according whom the bivoltine races were found to be comparatively more susceptible than multivoltine races. The hybrids of bivoltine (42 to 53%) and multivoltine

(24.50 to 32.33%) have recorded more disease due to grasserie. Bhaskar *et al.* (2002) have reported minimum ET<sub>50</sub> for mortality but maximum in KSO1 among bivoltine breeds. Further, the lowest ET<sub>50</sub> value (8 days) recorded for PMxNB<sub>4</sub>D<sub>2</sub> when fed with stock suspension of kenchu virus compared to 10<sup>-5</sup> dilution (33 days) they also confirmed that, PM and NB<sub>7</sub> and their components have recorded minimum and maximum ET<sub>50</sub> values Bhaskar *et al.* (1987) as reported in the present study. Results pertaining to total mortality were significant among fourth and fifth instar inoculated lots of silkworm breeds. In fourth instar, CSR<sub>16</sub> (48.00 to 45.33%) and (43.33 and 45.33%) was recorded maximum larval mortality in both the inoculated batches with *BmNPV* and 100 per cent mortality was witnessed by CSR<sub>2</sub>xCSR<sub>16</sub> in 10<sup>-1</sup> and 10<sup>-3</sup> viral dilutions. These results are in parity with findings of Sudhakara Rao *et al.* (2006) indicated that, the inheritance pattern of two selected donor parents *C. Nichi* and *A* were indicated that the mortality of *C. Nichi* (R) was only 21% and the mortality of *A* is found to be 27% whereas susceptible breed NB<sub>4</sub>D<sub>2</sub> (S) was recorded 100 per cent due to *BmDENV1*. Further they also confirmed, selected hybrids and their parents of *C. Nichi* and NB<sub>4</sub>D<sub>2</sub> changed their survival percentage *ie.*, *BmIFV* (71 to 87%) and *BmNPV* (60 to 65%) and indicated that, the evolved breeds and their hybrids acquired resistance characters against both the viral infections. Sowmyashree and Nataraju (2007) also inferred that, when (24)bivoltine and (23)multivoltine silkworm breeds administered with *BmNPV* (6x10<sup>-6</sup>/ml) the highest survival per cent of 58.80 noticed for CSR<sub>19</sub> compared to (CSR<sub>2</sub> 39.70%) and (CSR<sub>19</sub> 39.61%).

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Sensitivity of *Bombyx mori* L. to BmNPV on ET<sub>50</sub> for symptom expression and total larval mortality

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