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## Evaluation of bivoltine silkworm (*Bombyx mori* L.) breeds on survival against nuclear polyhedrosis virus (Grasserie) under temperate climatic conditions

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**Abstract**

The present studies on the evaluation of different bivoltine silkworm (*Bombyx mori* L.) breeds on survival against Nuclear Polyhedrosis Virus (Grasserie), under temperate climatic conditions was carried out at College of Temperate Sericulture, SKUAST-Kashmir, Mirgund. Preliminary evaluation of ten silkworm genotypes viz., APS4, APS8, CS6, CSR2, CSR4, DUN-6, DUN-22, NB4D2, SH6 and SANISH8 revealed that APS8 and NB4D2 breed possessed highest tolerance while, CSR2 and CSR4 were highly susceptible to BmNPV inoculation @ 20,000 PIB'S. The silkworm CSR4 showed drastic decrease in survival rate of (37.33%) in susceptible breed. However in tolerant breed APS8 recorded the survival rate of (72.67%). The maximum pupation rate of 91.36 per cent was recorded in case of NB4D2 and minimum pupation rate of 82.41 per cent was recorded in CSR2.

**Keywords:** Grasserie, Nuclear Polyhedrosis, PIB'S, *Bombyx mori* L.

**Introduction**

Sericulture is one of the oldest agro-based industries in the world. It is generally rural based on-farm and off-farm activity and has enormous employment generation potential. Sericulture has been recognized as one of the most appropriate avenue for socio-economic development particularly in a developing country like India especially for farmers with small land holdings as well as to other weaker section of the society. Sericulture in India has turned out to be a highly remunerative cash crop which provides attractive returns throughout the year. Sericulture occupies a unique position in Indian economy and assumes more importance in alleviating the problems of the rural people. Since it is practiced from time immemorial, it has now become the most important rural industry because of its low investment, maximum employment generation and quick turn over. Sericulture is deep-rooted in the culture and tradition of Indian society and has been considered as an economically viable, labour intensive agro industry in the rural area (Anonymous, 2013) [2]. The silk industry faces severe setbacks due to frequent disease outbreaks since most of the commercially reared silkworm species, including *Bombyx mori*, are highly susceptible to the diseases like pebrine, flacherie, grasserie and muscardine. In India approximately 40 percent crop losses are attributed to these diseases (Sheebarajakumari *et al.*, 2007) [10]. Among the diseases, viral diseases are most commonly occurring diseases (Samson *et al.*, 1990; Subbha Rao *et al.*, 1991) [8]. The virus formerly known as *Borrelina bombycis* (Bergold, 1947) [3] is presently termed as *Bombyx mori* L. nuclear polyhedrosis virus (BmNPV). The Nuclear polyhedrosis of silkworm is commonly known as Grasserie disease (French name), milky disease, fatty degeneration disease or Jaundice (American name), In Italy it is known as 'Giallume', in Japan as 'Nobyō' and in Germany as 'Gelbscht.' The names indicate either the yellowish colour of diseased insect or its swollen fat like appearance. In India, the disease is called by numerous local names such as 'Halu-Halu' or 'holuthonde roga' in Karnataka, 'Rosa' in West Bengal, 'Pal Puruga' in Andhra Pradesh, 'Pal Poochi' in Tamil Nadu, 'Baimair' in Kashmir, indicating the milky fluid

condition of the haemolymph in diseased worms. Grasserie infected worms show the symptoms of shiny body, aimless crawling, intersegmental swelling, white fluid coming out after skin rupture and dead worms hanging from the rearing stands and trays. It is also called "hanging disease." Grasserie disease usually affects fourth and fifth instars silkworms under natural conditions (Chitra *et al.*, 1975) [5]. If the infection is noticed during early instars, worms fail to spin cocoons and die, but worms can spin the cocoons and die inside producing melted cocoons, if infection occurs during later instars. The disease is caused by an occluded baculovirus. *Bombyx mori* nuclear polyhedrosis virus (BmNPV) and is prevalent during all the rearing seasons. However its severity was noticed during summer followed by rainy season (Selvakumar *et al.*, 2002) [9]. The silkworms infected by BmNPV show symptoms at the advanced stage of development and by that time the larvae have almost completed the feeding period, consuming the entire quantum of leaves and dies within a day or two. In India the loss due to BmNPV has been reported to the extent of 20-50 percent in commercial sericulture (Samson *et al.*, 1990) [8]. Rearing of bivoltine breeds and hybrids increase both quantity and quality of raw silk production however, these silkworm breeds are more susceptible to Grasserie as compared to polyvoltine breeds (Liushixian, 1984; Sivaprakasam and Rabindra, 1995) [6, 11]. Obviously this causes a big constraint for the successful adoption of bivoltine sericulture in a tropical country like India. Need was felt to identify silkworm breeds tolerant to BmNPV before taking up any breeding programme, specifically for evolving BmNPV tolerant breeds. Therefore, it would be essential to assess the susceptibility status of the available silkworm breeds against BmNPV. Hence, in the present study we evaluated the bivoltine silkworm (*Bombyx mori* L.) breeds against Nuclear Polyhedrosis Virus under temperate conditions.

### Material and Methods

The study on Evaluation of bivoltine silkworm (*Bombyx mori* L.) breeds on survival (Table-1) against Nuclear Polyhedrosis Virus (Grasserie) under temperate climatic conditions was carried out at College of Temperate Sericulture, SKUAST-Kashmir, Mirgund during 2017. The materials used and the techniques employed are presented below:

**Table 1:** Different genotypes of silkworm breeds for evaluation against nuclear polyhedrosis virus

S. No	Silkworm genotypes
1	APS-4
2	APS-8
3	CS-6
4	CSR-2
5	CSR-4
6	DUN-6
7	DUN-22
8	NB4D2
9	SH-6
10	SANISH-8

The silkworm breeds was carried out in a suitable rearing room which was thoroughly disinfected prior to incubation. The diseased free laying of breeds was surface sterilized and the eggs were incubated at  $25\pm 1^{\circ}\text{C}$  and  $75\pm 5\%$  relative humidity. The eggs were black boxed at pin head stage for 48 hours and exposed to bright light for uniform hatching on the expected day (Anonymus, 2003). Two hours after hatching,

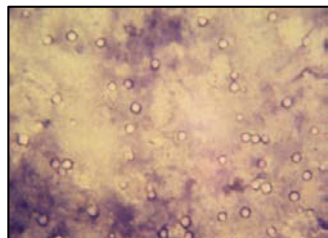
fresh tender mulberry leaves were cut to about  $0.5\text{ cm}^2$  and spread over the larvae on egg cards. The larvae of all breeds were reared up to third instar by following standard rearing practices and then separated into replications of 200 worms each for imposing the treatments.



**Fig 1:** Larvae of different genotypes of silkworm (*Bombyx mori* L.) breeds

### Preparation of BmNPV Inoculum

The concentration of polyhedral occlusion bodies was determined by using the Neubauer's haemocytometer. The haemocytometer is a glass slide generally used for counting the blood cells. It is also adopted to count the number of microbial cells. A drop of the stock suspension was first placed on the clean sterile haemocytometer. Then, a clean coverslip was placed over it and the number of polyhedral occlusion bodies was counted at the random fields and number of polyhedral occlusion bodies per ml estimated (Cantwell, 1974) [4]. The concentration was expressed as polyhedral occlusion bodies (PIB/ml). From the original stock suspension of the polyhedral concentration of 20,000 PIB'S/ml were prepared and used. The inoculation was carried out per orally by feeding silkworm with virus suspension on leaf. A normal control batch having 200 larvae for each race replications were smeared with distilled water and maintained for comparison and further use. The larvae were examined every day for BmNPV infection up to the day of mounting. The dead larvae and pupae were examined for microscopic symptoms. The total number of larvae survived and died due to nuclear polyhedrosis was recorded individually for each breed. Survived larvae were reared till cocooning and mounted for spinning. The cocoons were harvested on 6<sup>th</sup> day of spinning and assessed on 7<sup>th</sup> day for economic parameter.



**Fig 2:** Polyhedral bodies causing viral disease (Grasserie) of Silkworm (*Bombyx mori*)

### Observation Parameters

Survival rate (%) and pupation rate (%).

### Results

#### Survival rate (%)

The survival rate of different breeds under study ranged from 37.33 to 72.67 percent (Table-2). Among the inoculated silkworm breeds, APS<sub>8</sub> recorded the maximum survival rate of 72.67 percent, followed by NB<sub>4</sub>D<sub>2</sub> (67.67%), SH<sub>6</sub> (61.67%), CS<sub>6</sub> (58.67%), SANISH<sub>8</sub> (54.33%), DUN-6 (53.33%), DUN-22 (46.33%), APS<sub>4</sub> (45.00%) and CSR<sub>2</sub> (40.67%), whereas minimum survival rate of 37.33 percent in CSR<sub>4</sub>. The control batches maintained for each breed showed higher survival rate, in comparison to the inoculated breeds. The maximum percent decrease over control of 53.72 was observed in CSR<sub>4</sub>, whereas minimum percent decrease over control of 22.13 was observed in APS<sub>8</sub>.

**Table 2:** Effect of BmNPV infection on the survival rate of different breeds of silkworm, *Bombyx mori* L.

Genotype	Survival rate (%)		
	Inoculated	Control	Percent decrease over control
APS <sub>4</sub>	45.00 (42.11)	84.00 (66.47)	46.42
APS <sub>8</sub>	72.67 (58.50)	93.33 (75.07)	22.13
CS <sub>6</sub>	58.67 (49.97)	82.33 (65.15)	28.73
CSR <sub>2</sub>	40.67 (39.59)	82.07 (65.40)	50.44
CSR <sub>4</sub>	37.33 (37.64)	80.67 (63.92)	53.72
DUN-6	53.33 (46.89)	84.33 (66.71)	36.76
DUN-22	46.33 (42.87)	84.33 (66.71)	45.06
NB <sub>4</sub> D <sub>2</sub>	67.67 (55.33)	94.67 (76.86)	28.52
SH <sub>6</sub>	61.67 (51.73)	84.67 (66.96)	27.16
SANISH <sub>8</sub>	54.33 (47.47)	82.33 (65.15)	34.00
Mean	54	85.33	
SEm±	1.04	1.30	
CD at 5%	3.10	3.87	

Figures in parenthesis are arc-sine transformed values

#### Pupation rate (%)

The pupation rate of different breeds ranged from 80.38 to 91.36 per cent (Table-3). Among the inoculated silkworm breeds, NB<sub>4</sub>D<sub>2</sub> recorded the maximum pupation rate of 91.36 percent, followed by APS<sub>8</sub> (90.01%), DUN-22 (88.55%), SH<sub>6</sub> (88.33%), SANISH<sub>8</sub> (87.67%), DUN-6 (87.39%), CS<sub>6</sub> (83.67%), APS<sub>4</sub> (82.93%) and CSR<sub>2</sub> (82.41%), whereas minimum pupation rate of 80.38 per cent in (CSR<sub>4</sub>). The control batches maintained for each breed showed higher pupation rate in comparison to the inoculated breeds. The maximum per cent decrease over control of 11.68 was observed in CSR<sub>4</sub>, whereas minimum per cent decrease over control of 4.88 was observed in DUN-22.

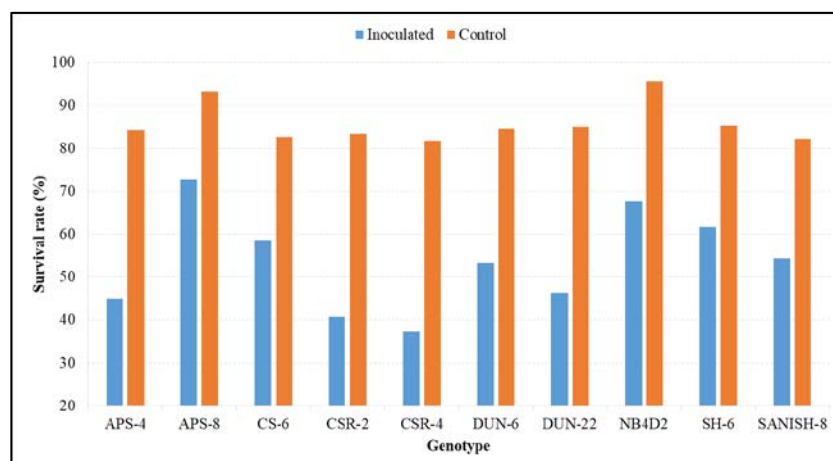
**Table 3:** Effect of BmNPV infection on the pupation rate of different breeds of silkworm, *Bombyx mori* L.

Genotype	Pupation rate (%)		
	Inoculated	Control	Percent decrease over control
APS <sub>4</sub>	82.93 (65.58)	89.24 (70.85)	7.07
APS <sub>8</sub>	90.01 (71.56)	96.59 (79.69)	6.81
CS <sub>6</sub>	83.67 (66.49)	92.90 (74.54)	9.93
CSR <sub>2</sub>	82.41 (65.23)	89.27 (70.91)	7.68
CSR <sub>4</sub>	80.38 (63.69)	91.01 (72.94)	11.68
DUN-6	87.39 (69.24)	93.64 (75.62)	6.67
DUN-22	88.55 (70.24)	93.10 (74.86)	4.88
NB <sub>4</sub> D <sub>2</sub>	91.36 (73.08)	97.17 (80.47)	5.97
SH <sub>6</sub>	88.33 (70.15)	93.48 (75.31)	5.50
SANISH <sub>8</sub>	87.67 (69.56)	94.52 (76.31)	7.24
Mean	86.27	93.10	
SEm±	1.73	1.58	
CD at 5%	5.16	4.70	

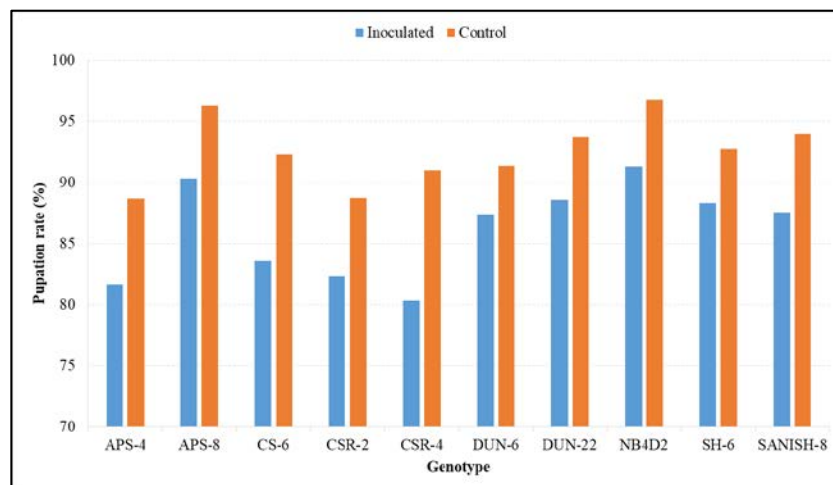
Figures in parenthesis are arc-sine transformed values

### Discussion

Screening of silkworm breeds against *Bombyx mori* Nuclear Polyhedrosis Virus showed significant difference among the breeds on larval parameters (Fig. 3 and 4). Though all the inoculated breeds recorded values lower than the normal rearing (Control rearing) however the degree of tolerance/susceptibility varied among the breeds. The maximum survival rate among the inoculated batches was observed in silkworm breed APS<sub>8</sub> (72.67%). Sivaprasad *et al.* (2003) [12] has also reported the highest survival rate of 75.67% in this breed when inoculated with BmNPV at 20,000 PIB'S which is the similar inoculation load used in the present study. The least survival rate was observed in CSR breeds with CSR<sub>4</sub> recording 37.33% followed by CSR<sub>2</sub> (40.67%). The low survival rate in CSR breeds due to BmNPV infection has also been reported by Mal Reddy *et al.* (2017) [17].



**Fig 3:** Effect of BmNPV infection on survival rate (%) of silkworm, *Bombyx mori* L.



**Fig 4:** Effect of BmNPV infection on pupation rate (%) of silkworm, *Bombyx mori* L.

### Conclusion

The present study of screening of silkworm breeds namely, APS4, APS8, CS6, CSR2, CSR4, DUN-6, DUN-22, NB4D2, SH6 and SANISH8 revealed that APS8 and NB4D2 expressed higher level of tolerance to BmNPV infection. On the contrary CSR2 and CSR4 breeds were highly susceptible for BmNPV infection at 20,000 PIB'S. The study can be used to evolve BmNPV tolerant hybrids for commercial use.

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