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Studies on mulberry silkworm cocoon traits due to *Bm* NPV infection

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Abstract
Silkworm diseases are caused by a number of pathogens that results in drastic reduction in the quantity and quality of cocoon crop. This ultimately has a direct effect on the farming community of the country due to reduced returns. Thus viral diseases of silkworm pose a serious problem in sericulture industry which need to be checked to the maximum possible level. Therefore, no serious attempts have been made systematically to screen different improved breeds and hybrids of mulberry silkworms for cocoon traits to *Bm*NPV infection. In this direction, to screened the tolerance of *Bm*NPV infection was under taken. The effective rate of rearing was significantly maximum in PM × CSR_2 (62.50%), which was on par with that of MH_1 × CSR_2 (61.11%)). Similarly, the effective rate of rearing varied from 88.00 per cent in CSR_2 × CSR_4 to 93.00 per cent in PM × CSR_2 of uninfected batches. Among the viral dilutions, the effective rate of rearing differed significantly in improved breeds and hybrids. The effective rate of rearing was significantly maximum in control batch (90.16%) followed by 10^{-6} PIBs (40.76%) and 10^{-3} PIBs concentration (37.04%).The cocoon, pupal and shell weights were found to be non-significant except shell ratio in improved breeds and hybrids of silkworm. The shell ratio was significantly maximum in CSR_2 (22.64%). It was significantly minimum in PM × CSR_2 (18.60%). Among the PIBs concentrations tested, the cocoon (1.30 g), shell (0.271g) and pupal weights (0.92 g) and shell ratio (20.64%) were significantly maximum at viral dilution of 10^{-6} PIBs followed by 10^{-3} PIBs (1.17g, 0.216g, 0.86g, 18.60 per cent, respectively) compared to the control (1.75g, 0.39g, 1.29g and 22.03 per cent, respectively).Among the interactions, the cocoon, shell and pupal weights and shell ratio were found to be non-significant.

Keywords: Mulberry silkworm, cocoon traits, *Bm*NPV, infection

Introduction
Silkworm is one of the most important domesticated insects where the growth and development is greatly influenced by environmental conditions. Success of silkworm breeds/hybrids largely depends on their adaptability to the environment in which it is destined to be reared. The biological as well as cocoon-related characters are influenced by ambient temperature, rearing seasons, quality mulberry leaf, and genetic constitution of silkworm strains. It is a well-established fact that under tropical conditions, unlike polyvoltines, bivoltines are more vulnerable to various stress like hot climatic conditions of tropics, poor leaf quality, and improper management of silkworm crop during summer that is not conducive for bivoltine rearing for technologically and economically poor farmers of India. Though the nature of silkworm healthiness is unclear, healthy silkworm varieties are important for the stabilization of silkworm crops. The use of commercial silkworm hybrids resistant to important silkworm diseases is economical and better option particularly in tropical areas. Due to fluctuating climatic conditions, inadequate silkworm disease management practices and poor quality mulberry leaf, frequent crop losses are witnessed especially due to grasserie disease with the farmers in tropical areas. The rate of disease induction is perhaps controlled by the host's developmental stage, particularly moulting period, indicating that physiological changes in silkworms may also play an important role in the induction of viral diseases.
Sericulture is economically very important for farming community especially for rural areas. Among the silkworm diseases, nuclear polyhedrosis virus (NPV) of *Bombyx mori* (*Bm*NPV) is known to occur in all larval instars and more commonly in 4th and 5th instars during all seasons and causing 20-50% cocoon crop losses. In viral diseases two common diseases are found these are nuclear polyhedrosis and cytoplasmic polyhedrosis.

Borellina virus cause nuclear polyhedrosis, which is principally the symptoms are skin become very thin, fragile and shiny and larvae become restless. Their skin rupture easily and a milky white fluid (haemolymph) oozes out though the holes. NPVs remain in a stable infectious state in the environment even after death of NPV infected host and a large number of progeny NPV particles are released due to rupture of its cuticle, which transfers to other susceptible individuals. The released NPV particles must remain viable to occur secondary transmission, which is accomplished in part by the polyhedrin protein matrix that surrounds the infective units, the virions and provides some degree of protection against environmental degradation. Keeping in view the investigation was carried out on mulberry silkworm traits due to *Bm*NPV infection.

Material and Methods

The four improved breeds (CSR_2, CSR_4, MH_1, BL_{43}) and five hybrids of mulberry silkworm ($CSR_2 \times CSR_4$, $CSR_4 \times CSR_2$, $MH_1 \times CSR_2$, $BL_{43} \times CSR_2$ and $PM \times CSR_2$) with two PIBs of 10^{-3} and 10^{-6} *Bm*NPV was tested under laboratory conditions. The standard rearing techniques were fallowed as recommended by Krishnaswami (1978) [1] and three replications by using 100 worms of each breed and their hybrid.

Grasserie diseased fifth instar larvae were collected and haemolymph was taken into sterilized glass tubes from infected worms shortly before death by puncturing the front pair of prolegs with the help of sterilized pin followed by a gentle pressing. The turbid milky haemolymph was stored and then subjected to refrigeration for several days till the polyhedra settled at the bottom. Later, the haemolymph was filtered through four layers of muslin cloth. The filtrate was subjected to centrifugation at 15,000 rpm for 15 minutes using Remi C-24 refrigerated centrifuge. The pellet was resuspended in sterile distilled water to half of the original volume and centrifuged at 5,000 rpm for 15 minutes. Another cycle of centrifugation at 15,000 rpm followed by 5,000 rpm was done for 15 minutes each. The process of the differential centrifugation was repeated till a milky whitish amorphous sediment of highly purified nuclear polyhedra was confirmed from microscopic examination. The polyhedra were suspended in distilled water and stored at 5°C in the refrigerator.

The polyhedral concentration was determined using the Neubauer's haemocytometer. From the original stock suspension of the polyhedra, two polyhedral concentrations *viz*., 10^{-3} (7.73×10^{-7}) and 10^{-6} (4.12×10^{-10}) were prepared and used for the study.

The infection was carried out orally by feeding the silkworms with virus suspension smeared mulberry leaves, soon after third moult. The leaf bits of 10 × 12 cm size were prepared using suitable aged leaf and washed in running water and sterilized by using 70 per cent alcohol (by cotton swab). The sterilized leaves were shade dried and such leaves were smeared evenly on both the sides with virus suspension (0.5 ml / replication) using non-absorbant cotton. The leaves were shade dried and fed to the silkworms. Control batches were fed with surface sterilized mulberry leaves for the first feed. For subsequent feedings, inoculum free leaves suitable for the age were provided for both treated and untreated batches. Two concentrations (10^{-3} and 10^{-6}) of polyhedral bodies were smeared uniformly to the surface of sterilized mulberry leaves and fed to improved breeds and hybrids of silkworm which were replicated thrice.

Results and Discussion

The effective rate of rearing ranged between 47.78 (CSR_2) to 62.50 per cent ($PM \times CSR_2$). It was significantly maximum in $PM \times CSR_2$ (62.50%), which was on par with that of $MH_1 \times CSR_2$ (61.11%) than the $CSR_4 \times CSR_2$ (56.56%), $BL_{43} \times CSR_2$ (56.45%) and $CSR_2 \times CSR_4$ (55.13%). Similarly, the effective rate of rearing varied from 88.00 per cent in $CSR_2 \times CSR_4$ to 93.00 per cent in $PM \times CSR_2$ of uninfected batches. Among the viral dilutions, the effective rate of rearing differed significantly in improved breeds and hybrids. The effective rate of rearing was significantly maximum in control batch (90.16%) followed by 10^{-6} PIBs (40.76%) and 10^{-3} PIBs concentration (37.04%). The interaction effects between silkworm breeds/hybrids and PIBs concentrations were found to be significantly different. Among the improved breeds and hybrids, the effective rate of rearing was significantly maximum in $PM \times CSR_2$ (48.17%), which was on par with that of $MH_1 \times CSR_2$ (46.83%) at the viral dilution of 10^{-6} PIBs concentration, which was on par with 10^{-3} PIBs concentration (46.33%) in $PM \times CSR_2$ and MH_1 (45.17%) at the viral dilution of 10^{-6} PIBs concentration. The effective rate of rearing was significantly minimum in CSR_2 (24.66%) followed by CSR_4 (28.83%) at the viral dilution of 10^{-3} PIBs concentration and also in CSR_2 (30.17%) and CSR_4 (32.50%) at the viral dilution of 10^{-6} PIBs concentration.

The cocoon, pupal and shell weights were found to be non-significant except shell ratio in improved breeds and hybrids of silkworm. However, the cocoon weight was maximum in $PM \times CSR_2$ (1.50 g) and it was minimum in BL_{43} (1.29 g). The cocoon weight varied from 1.89 g ($CSR_4 \times CSR_2$) to 1.56 g (BL_{43}) in non-inoculated batches of silkworm. The pupal weight was maximum in $CSR_2 \times CSR_4$ (1.09 g), while it was minimum in MH_1 (0.96 g) (Fig.4). Among the non-inoculated breeds and hybrids, the pupal weight varied from 1.19 g (CSR_2) to 1.45 g ($PM \times CSR_2$). The shell weight was maximum in CSR_2 (0.323 g), whereas minimum in MH_1 (0.254 g). Similarly, the shell weight ranged between 0.331g (MH_1) to 0.461g (CSR_2) in non-inoculated batches of silkworm. The shell ratio was significantly maximum in CSR_2 (22.64%). It was significantly minimum in $PM \times CSR_2$ (18.60%). The shell ratio varied from 19.02 per cent ($PM \times CSR_2$) to 26.34 per cent (CSR_2) in control batches of silkworm. Among the PIBs concentrations tested, the cocoon (1.30 g) shell (0.271g) and pupal weights (0.92 g) and shell ratio (20.64%) were significantly maximum at viral dilution of 10^{-6} PIBs followed by 10^{-3} PIBs (1.17g, 0.216g, 0.86g, 18.60 per cent, respectively) compared to the control (1.75g, 0.39g, 1.29g and 22.03 per cent, respectively). Among the interactions, the cocoon, shell and pupal weights and shell ratio were found to be non-significant. The cocoon, shell and pupal weights and shell ratio were maximum at 10^{-6} PIBs in $PM \times CSR_2$ (1.42 g), CSR_2 (0.229 g), $CSR_2 \times CSR_4$ (1.00g) and $BL_{43} \times CSR_2$ (22.98%) at 10^{-3} PIBs. It was significantly minimum in MH_1 (1.09 g), BL_{43} (0.200 g), MH_1 (0.80g) and $MH_1 \times CSR_2$ (17.05%) at 10^{-3} PIBs. These findings indicated that the cocoon traits had decreased with the increased dose of the PIBs concentration. The variations with regard to cocoon traits might be due to genetic or physiological differences among silkworm breeds and hybrids.

These results are in close conformity with the findings of Satish and Govindan (1986) [4], who reported that infection with NPV from first to third day of fifth instar silkworm resulted in greater reduction in cocoon, pupal and shell weights and shell ratio in NB_4D_2 than in $PM \times NB_4D_2$. Rathna Sen *et al.* (1999) [2] reported reduced cocoon and shell

weights and shell ratio of bivoltine silkworm breeds in *Bm*NPV infection with different concentrations. Maximum reduction of cocoon traits due to *Bm*NPV infection *viz*. cocoon (26.46%), shell (51.72%) and pupal weights (26.17%) in NB_7. But the extent of reduction of cocoon weight was 22.53% in NP_2, shell weight (26.67%) and pupal weight (12.05%) were in C-Nichi (Usha, 1996) [5]. Ravikumar (2000) [3] also reported that fourth and fifth instar NPV inoculated lots significantly maximum reduction of cocoon weight (41.00 and 40.93%), pupal weight (39.60 and 39.58%) and shell weight (48.16 and 45.99%) in CSR_4, but which were on par with CSR_5 (40.81 and 40.80%, 39.77 and 39.66%; 48.89 and 46.42% respectively). The least reduction of cocoon weight (16.08 and 16.25%), pupal weight (15.27 and 15.18%) and shell weight (28.78 and 19.80%) was in KSO-1. Further, among the different dilutions tested highest per cent reduction of cocoon weight (8.77 and 9.81%), pupal weight (64.47 and 59.15%) and shell weight (71.16 and 62.64%) was observed in 10^{-2} PIBs compared to 10^{-32} (65.76 and 59.89%; 7.72 and 8.26%; 18.68 and 13.40%, respectively).

The shell ratio was significantly different in breeds and hybrids. Among the improved breeds and hybrids, the shell ratio ranged between 18.60 per cent to 22.64 per cent in PM × CSR_2 and CSR_2, respectively. The shell ratio was significantly maximum in CSR_2 (22.64%) which was on par with CSR_4 × CSR_2 (21.45%), BL_{43} × CSR_2 (20.48%), CSR_4 (20.46%), MH_1 × CSR_2 (20.28%), CSR_2 × CSR_4 (19.46%) and BL_{43} (19.37%). It was significantly minimum in PM × CSR_2 (18.60%) followed by MH_1 (19.07%). The shell ratio varied from 19.02 per cent (PM × CSR_2) to 26.34 per cent (CSR_2) in control batches of silkworm The shell ratio was significantly maximum in control (22.03%) followed by 10^{-6} (20.64%) and 10^{-3} PIBs concentration (18.60%). The interaction effect between silkworm breeds/hybrids and PIBs concentrations were non-significant in respect of shell ratio. The shell ratio ranged between 17.05 to 22.98 per cent in MH_1 × CSR_2 at viral dilution of 10^{-6} PIBs and BL_{43} × CSR_2 at viral dilution of 10^{-3} PIBs concentration, respectively. Among the improved breeds and hybrids, the shell ratio was maximum in BL_{43} × CSR_2 (22.98%) followed by CSR_2 (22.82%), CSR_4 × CSR_2 (21.48%) and MH_1X CSR_2 (21.45%) at 10^{-6} PIBs followed by CSR_4 × CSR_2 (20.66%) at 10^{-3} PIBs concentration. It was minimum in MH_1 × CSR_2 (17.05%) followed by PM × CSR_2 (17.42%), CSR_2 × CSR_4 (17.92%), BL_{43} (18.18%), CSR_4 (18.29%), MH_1 (18.35%) and CSR_2 (18.75%) at viral dilution of 10^{-3} PIBs. These results are in close conformity with the findings of Satish and Govindan (1986) [4], who reported that infection with NPV from first to third day of fifth instar silkworm resulted in greater reduction in shell ratio in NB_4D_2 than in PM × NB_4D_2. weight (26.67%) and pupal weight (12.05%) were in C-Nichi. (Table 4)

The ERR was significantly different among the improved breeds and hybrids. The ERR was significantly maximum in PM × CSR_2 (62.50%) which was on par with MH_1 × CSR_2 (61.11%) and CSR_4 × CSR_2 (56.56%). It was significantly minimum in CSR_2 (47.78%) followed by CSR4 (50.50%). The ERR ranged between 88.00 per cent (CSR_2 x CSR_4) to 93.00 per cent (PM × CSR_2) in non-inoculated batches. The ERR was significantly maximum in 10^{-6} PIBs (40.76%) and 10^{-3} PIBs (37.04%) compared to control (90.16%) batches. Among the interactions, the ERR was significantly maximum in PM × CSR_2 (48.17%), which was on par with that of MH_1 × CSR_2 (46.83%) at viral dilution of 10^{-6} PIBs concentration. The ERR was significantly minimum in CSR_2 (24.66%) followed by CSR_4 (28.83%) at the viral dilution of 10^{-3} PIBs (Table 5). The current observations indicated that ERR was significantly less in bivoltine breeds than the hybrids and multivoltine breeds. The ERR was significantly minimum in the breeds/hybrids with administration of highest *Bm*NPV concentration compared to the non-inoculated batches of silkworm. Therefore, hybrids were less sensitive to *Bm*NPV as evidenced by maximum ERR. These results are in confirmation with findings of Usha (1996) [5] and Ravikumar (2000) [3]. Hence these findings clearly indicated that CSR_2 and CSR_4 were highly sensitive to *Bm*NPV infection. Where as PM × CSR_2 and MH_1 × CSR_2 were highly tolerant to *Bm*NPV infection. Therefore tolerence of silkworm to *Bm*NPV is controlled by polygenes.

Table 1: Effect of *Bm*NPV infection on cocoon weight (g) in improved breeds and hybrids

Breeds/ Hybrids / PIBs Concentrations.	Cocoon weight (g)									
	CSR_2	**CSR_4**	**MH_1**	**BL_{43}**	**CSR_2 × CSR_4**	**CSR_4 × CSR_2**	**MH_1 × CSR_2**	**BL_{43} × CSR_2**	**PM × CSR_2**	**Mean**
10^{-3}	1.12	1.23	1.09	1.10	1.20	1.21	1.29	1.11	1.20	1.72
10^{-6}	1.31	1.40	1.20	1.20	1.34	1.35	1.31	1.24	1.42	1.30
Control	1.75	1.80	1.68	1.56	1.81	1.89	1.70	1.69	1.88	1.75
Mean	1.39	1.48	1.32	1.29	1.45	1.48	1.43	1.35	1.50	

Test of significance	F-test	SEm±	CD at 5%
Silkworm breeds and hybrids (A)	NS	0.088	0.249
PIBs concentrations (B)	*	0.051	0.144
Interaction (A × B)	NS	0.152	0.432

*: Significant at 5% NS: Non-significant

Table 2: Effect of *Bm*NPV infection on pupal weight (g) in improved breeds and hybrids

Breeds/ Hybrids / PIBs Concentrations.	Pupal weight (g)									
	CSR_2	**CSR_4**	**MH_1**	**BL_{43}**	**CSR_2 × CSR_4**	**CSR_4 × CSR_2**	**MH_1 × CSR_2**	**BL_{43} × CSR_2**	**PM × CSR_2**	**Mean**
10^{-3}	0.89	0.90	0.80	0.82	0.91	0.82	0.88	0.84	0.85	0.86
10^{-6}	0.92	0.95	0.88	0.91	1.00	0.93	0.90	0.92	0.91	0.92
Control	1.19	1.28	1.21	1.29	1.35	1.41	1.27	1.20	1.45	1.29
Mean	1.00	1.04	0.96	1.01	1.09	1.05	1.02	0.99	1.07	

Test of significance	F-test	SEm±	CD at 5%
Silkworm breeds and hybrids (A)	NS	0.057	0.161
PIBs concentrations (B)	*	0.033	0.093
Interaction (A × B)	NS	0.098	0.279

* : Significant at 5%, NS : Non-significant

Table 3: Effect of *Bm* NPV infection on shell weight (g) in improved breeds and hybrids

Breeds/Hybrids / PIBs Concentrations.	Shell weight (g)									
	CSR_2	CSR_4	MH_1	BL_{43}	$CSR_2 \times CSR_4$	$CSR_4 \times CSR_2$	$MH_1 \times CSR_2$	$BL_{43} \times CSR_2$	$PM \times CSR_2$	Mean
10^{-3}	0.210	0.225	0.201	0.200	0.215	0.250	0.200	0.231	0.209	0.216
10^{-6}	0.299	0.289	0.230	0.249	0.249	0.290	0.281	0.285	0.268	0.271
Control	0.461	0.404	0.331	0.341	0.396	0.420	0.380	0.400	0.367	0.39
Mean	0.323	0.306	0.254	0.263	0.287	0.320	0.287	0.305	0.281	

Test of significance	F-test	SEm±	CD at 5%
Silkworm breeds and hybrids (A)	NS	0.027	0.076
PIBs concentrations (B)	*	0.015	0.044
Interaction (A × B)	NS	0.046	0.131

* : Significant at 5%, NS : Non-significant

Table 4: Effect of *Bm*NPV infection on shell ratio (%) in improved breeds and hybrids

Breeds/ Hybrids / PIBs Concentrations.	Shell ratio (%)									
	CSR_2	CSR_4	MH_1	BL_{43}	$CSR_2 \times CSR_4$	$CSR_4 \times CSR_2$	$MH_1 \times CSR_2$	$BL_{43} \times CSR_2$	$PM \times CSR_2$	Mean
10^{-3}	18.75 (25.65)	18.29 (25.29)	18.35 (25.35)	18.18 (25.22)	17.92 (24.97)	20.66 (27.03)	17.05 (24.38)	20.81 (27.10)	17.42 (24.66)	18.60 (25.52)
10^{-6}	22.82 (28.49)	20.64 (27.02)	19.17 (25.94)	19.76 (26.36)	18.59 (27.59)	21.48 (27.60)	21.45 (27.59)	22.98 (28.64)	18.87 (25.74)	20.64 (27.22)
Control	26.34 (30.86)	22.44 (28.26)	19.70 (26.33)	20.18 (26.69)	21.88 (27.89)	22.22 (28.02)	22.35 (28.21)	23.66 (29.05)	19.52 (26.22)	22.03 (27.95)
Mean	22.64 (28.33)	20.46 (26.86)	19.07 (25.87)	19.37 (26.09)	19.46 (26.82)	21.45 (27.55)	20.28 (26.73)	22.48 (28.27)	18.60 (25.54)	

Test of significance	F-test	SEm±	CD at 5%
Silkworm breeds and hybrids (A)	*	0.721 (0.501)	2.05 (1.42)
PIBs concentrations (B)	*	0.416 (0.289)	1.18 (0.409)
Interaction (A × B)	NS	1.25 (0.868)	3.55 (2.47)

Figures in parentheses are in angular transformed values
*: Significant at 5% NS: Non-significant

Table 5: Effect of *Bm*NPV infection on effective rate of rearing (ERR%) in improved breeds and hybrids

Breeds/ Hybrids / PIBs Concentrations.	Effective rate of rearing (ERR%)									
	CSR_2	CSR_4	MH_1	BL_{43}	$CSR_2 \times CSR_4$	$CSR_4 \times CSR_2$	$MH_1 \times CSR_2$	$BL_{43} \times CSR_2$	$PM \times CSR_2$	Mean
10^{-3}	24.66 (29.56)	28.83 (32.48)	39.83 (39.13)	35.50 (36.57)	37.34 (37.66)	39.17 (38.74)	44.50 (41.83)	37.17 (37.56)	46.33 (42.89)	37.04 (37.38)
10^{-6}	30.17 (33.31)	32.50 (34.75)	45.17 (42.23)	40.17 (39.33)	40.00 (39.22)	41.83 (40.29)	46.83 (43.18)	42.00 (40.39)	48.17 (43.95)	40.76 (39.63)
Control	88.50 (71.53)	90.17 (71.75)	91.09 (72.44)	89.83 (71.41)	88.00 (69.77)	88.67 (70.37)	91.99 (73.51)	90.17 (72.20)	93.00 (75.10)	90.16 (72.01)
Mean	47.78 (44.80)	50.50 (46.33)	58.69 (51.27)	55.17 (49.10)	55.13 (48.89)	56.56 (49.81)	61.11 (52.84)	56.45 (50.05)	62.50 (53.98)	

Test of significance	F-test	SEm±	CD at 5%
Silkworm breeds and hybrids (A)	*	0.873 (0.673)	2.48 (1.91)
PIBs concentrations (B)	*	0.504 (0.388)	1.43 (1.10)
Interaction (A × B)	*	1.51 (1.17)	4.29 (3.31)

Figures in parentheses are angular transformed values
*: Significant at 5%

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