Animal Review
2014 Vol. 1, No. 4, pp. 57-64
ISSN(e): 2409-6490
ISSN(p): 2412-3382

STUDIES ON SEASONAL PERFORMANCE OF NEWLY DEVELOPED BIVOLTINE SILKWORM (*BOMBYX MORI* L) HYBRIDS TOLERANT TO *BM*NPV AND EFFECT OF TEMPERATURE ON DISEASE INDUCTION

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ABSTRACT

Season and region specific studies of silkworm Bombyx mori L. are of greater importance in identifying and understanding the adaptability of silkworm genotypes, which are largely influenced by climatic factors. The performance of the newly developed hybrid combinations was evaluated for twelve economic traits during pre-monsoon, monsoon and post-monsoon seasons of the year to understand genotype and environment interactions and their stability under fluctuating tropical environmental conditions with Bombyx mori Nuclear Polyhedro Virus (BmNPV) inoculation @ $2x10^6$ PIB's/larva. The data was subjected to two way analysis of variance and season-wise relative merit of individual hybrids was computed following Multiple Trait Evaluation Index method. The index values indicated that the hybrids BNR9 x BNR10 and BNR9 x BNR4 with higher average cumulative EI values were ranked I and II and establishing their superiority over multiple traits. Further the larvae soon after fourth moult were kept at room temperature (25ºC), low temperature (5ºC) and high temperature (32ºC) without feed for 6 hours and then inoculated perorally with BmNPV. The newly ecdysed fifth instar larvae when exposed to low temperature of 5ºC and starved enhanced larval susceptibility to BmNPV while the hybrids BNR9 x BNR10 and BNR9 x BNR4 exhibited higher tolerance to BmNPV at 25ºC and moderate tolerance at 32ºC.

Keywords: Silkworm, *Bombyx mori* nuclear polyhedro virus (*Bm*NPV), Seasonal performance, Tropical environment, Temperature effect, Disease induction.

Contribution/ Originality

This study documents the evaluation of silkworm hybrids under *Bm*NPV inoculated conditions during different seasons of the year. The methodology includes the estimation of the tolerance of the hybrids to *Bm*NPV during various seasons influencing the physiological activities affecting their growth and development as well as the expression of economic characters.

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1. 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 [1-3]. 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.

As epidemic of the viral diseases in silkworm is observed to have close link with climatic factors such as temperature, humidity, air current, food and the environment, to which the insect is exposed also plays an important role in the manifestation of resistance. The rate of disease induction is perhaps controlled by the host's developmental stage, particularly moulting period [4], indicating that physiological changes in silkworms may also play an important role in the induction of viral diseases. Tropical sericulture beset with wide and sudden fluctuations in the environment coupled with poor quality of mulberry and management practices by the farmers requires more flexible hybrids with genetic plasticity to buffer these adverse situations. The productive commercial hybrids presently used by farmers are susceptible to grasserie disease and it is felt that there is an urgent need to develop bivoltine silkworm hybrids resistant to these conditions with increased productivity. Most of the small and marginal farmers in India are unable to practice complete disease management practices due to their socio-economic conditions where these breeds are better option and equipped to survive. In view of the above the present study was carried out at Andhra Pradesh State Sericulture Research Institute, Hindupur to evaluate the performance of newly developed bivoltine silkworm hybrids in various seasons under *Bm*NPV inoculated conditions to identify the most promising combinations for commercial use.

2. MATERIALS AND METHODS

The performance of ten new hybrid combinations *viz.*, BNR1 x BNR2, BNR1 x BNR6, BNR3 x BNR6, BNR3 x BNR8, BNR5 x BNR6, BNR7 x BNR2, BNR7 x BNR6, BNR9 x BNR4, BNR9 x BNR8 and BNR9 x BNR10 were evaluated under *Bm*NPV inoculated conditions in three seasons namely, pre-monsoon, monsoon and post-monsoon season were evaluated in three seasons namely, pre-monsoon (February - May), monsoon (June - October) and post-monsoon (November - January) seasons along with the existing bivoltine hybrids *viz.*, APS45 x APS12 and CSR2 x CSR4 as controls. All the hybrids, their parents along with control breeds and hybrids were reared under inoculated conditions with *Bm*NPV polyhedra @ 2 x 10^6 PIB's /ml in each season with three replications of 300 larvae per replication after 3rd moult. The larvae were fed with V_1 mulberry leaf. The rearings were carried out in Randomized Block Design and data for twelve traits namely, fecundity, yield per 10,000 larvae by number, yield per 10,000 larvae by weight, survival rate, cocoon weight, cocoon shell weight, cocoon shell ratio and filament length, non-breakable filament length, raw silk percentage, denier and neatness was analysed using two way analysis of variance [5] for three seasons separately to find out genotype-environment interaction. The merit of the individual hybrids and their relative superiority was computed following the Multiple Trait Evaluation Index [6]. The combination which recorded highest average cumulative evaluation index value was assigned 1st rank and subsequent ranks were assigned for the combinations in descending order. Effect of temperature on induction of disease in hybrids was studied by exposing inoculated larvae to low temperature (5^{0}C), room temperature (25^{0}C) and high temperature (32^{0}C) for 6 hours on first day of fifth instar within 2 hours of out of fourth moult. Ten hybrid combinations along with controls with 25 basic number of larvae in three replications were inoculated with *Bm*NPV polyhedra @ 20,000 PIBs/ml (0.25ml/25 larvae). After 6 hours all the larvae were kept back at room temperature (25^{0}C) and reared under normal conditions. The rate of viral multiplication at different temperatures and mortality due to *Bm*NPV was recorded up to pupal stage and the data was subjected to statistical analysis of variance.

3. RESULTS

The data on mean of three seasons pertaining to twelve economic traits of the ten hybrids *viz*., BNR9 x BNR4, BNR1 x BNR6, BNR3 x BNR8, BNR9 x BNR10, BNR5 x BNR6, BNR7 x BNR2, BNR1 x BNR2, BNR9 x BNR8, BNR7 x BNR6 and BNR3 x BNR6 with control hybrids, APS45 x APS12 and CSR2 x CSR4 under *Bm*NPV inoculated conditions are presented in Table 1. Analysis of variance results for hybrids are depicted in Table 2 and evaluation index values for each of twelve traits are presented in Table 3. The data related to the effect of temperature on disease induction is depicted in Table 4.

Fecundity (no.): The overall performance for the trait over the three seasons revealed that BNR9 x BNR4 and BNR5 x BNR6 recorded the maximum (505) and minimum (467) eggs/laying with an average of 484 eggs/laying.Cocoon Yield per 10,000 Larvae by Number: The overall performance of the hybrids for cocoon yield per 10,000 larvae by number indicate that maximum (8787) and minimum (8196) cocoon yield was recorded for BNR9 x BNR7 and BNR1 x BNR2 respectively. Cocoon yield per 10,000 larvae by weight (kg): Comparison of the newly evolved hybrids for overall performance indicate that maximum (16.98 kg) and minimum (13.79 kg) cocoon yield was recorded for BNR9 x BNR10 and BNR1 x BNR2 respectively.

Survival rate (%): Considering the overall performance over the three seasons, BNR9 x BNR10 recorded the maximum (85.20 %) value followed by BNR9 x BNR4 (83.89 %) and BNR7 x BNR2 (83.79 %).Cocoon weight (g): Comparison of the newly evolved hybrids over the three seasons indicate that maximum (1.812 g) and minimum (1.560 g) values were recorded for BNR9 x BNR10 and BNR1 x BNR2 respectively. Cocoon shell weight (g): The overall performance of the hybrids over three seasons together revealed that BNR9 x BNR10 and BNR3 x BNR6 recorded the maximum (0.404 g) and minimum (0.333 g) values respectively.

Cocoon shell ratio (%): The overall performance of the hybrids for the trait indicate that BNR9 x BNR10 and BNR3 x BNR6 recorded the maximum (22.30 %) and minimum (21.06 %) values respectively.

Filament length (m): Comparison of the newly evolved hybrids over the three seasons of the year expressed maximum (977 m) and minimum (806 m) values for BNR9 x BNR10 and BNR3 x BNR6 respectively.

Non-breakable Filament length (m): Comparison of the newly evolved hybrids over the three seasons of the year revealed maximum (866 m) and minimum (694 m) values for BNR9 x BNR10 and BNR3 x BNR6 and BNR5 x BNR6 respectively.

Raw Silk Percentage (%): Considering the overall performance over the three seasons, BNR9 x BNR10 recorded the maximum (18.09 %) value followed by BNR7 x BNR6 (17.19 %) and BNR3 x BNR8 (16.88 %)

Denier (d): The overall performance of the hybrids over three seasons together revealed that BNR7 x BNR6 and BNR7 x BNR6 recorded maximum (2.39) and minimum (2.31) values respectively.

Neatness (p): The overall performance of the hybrids for the trait neatness indicate that BNR7 x BNR2 and BNR1 x BNR6 recorded maximum (93 p) and minimum (90 p) values respectively.

Table-1. Mean performance of hybrid combinations

Combination	Fecun-dity (No.)	Yield /10,000 Larvae No.	Yield /10,000 Larvae Wt. (kg)	Pupa- tion Rate (%)	Cocoon Weight (g)	Shell Weight (g)	Shell Ratio (%)	Fila-ment Length (m)	NBFL (m)	Raw Silk (%)	Denier (d)	Neat-ness (p)
BNR1 X BNR2	469	8196	13.79	78.66	1.560	0.336	21.47	814	726	15.39	2.35	91
BNR1 X BNR6	489	8353	14.18	79.52	1.593	0.342	21.47	824	706	16.41	2.37	90
BNR3 X BNR6	494	8390	14.30	79.88	1.584	0.333	21.06	806	694	16.68	2.38	91
BNR3 X BNR8	493	8549	15.58	82.44	1.634	0.355	21.67	849	730	16.88	2.32	92
BNR5 X BNR6	467	8484	14.45	81.40	1.619	0.349	21.88	813	694	15.73	2.37	91
BNR7 X BNR2	477	8647	15.38	83.79	1.617	0.354	21.79	850	731	16.84	2.31	93
BNR7 X BNR6	483	8619	15.81	82.55	1.721	0.376	21.85	893	763	17.19	2.39	91
BNR9 X BNR4	505	8685	16.80	83.89	1.773	0.389	21.95	952	814	16.84	2.32	91
BNR9 X BNR8	478	8589	15.24	82.80	1.654	0.351	21.11	784	625	16.36	2.34	91
BNR9 X BNR10	500	8787	16.98	85.20	1.812	0.404	22.30	977	832	18.09	2.34	91
APS45 X APS12(C)	469	6498	11.09	25.98	1.436	0.328	20.86	739	632	16.20	2.37	90
CSR2 X CSR4(C)	477	2538	4.22	22.30	1.417	0.283	19.97	642	554	14.97	2.31	90
Average	484	7861	13.99	72.37	1.618	0.350	21.45	828	708	16.46	2.35	91
S.D.	13	1782	3.44	22.62	0.118	0.031	0.62	90	79	0.84	0.03	0.8
S.E	4.85	674	1.30	8.55	0.04	0.01	0.24	34	30	0.32	0.01	0.3
CV (%)	2.65	22.67	24.62	31.26	7.28	8.93	2.91	11	11	5.07	1.20	0.9

Values represent mean of 3 seasons
Avg = Average, S.D = Standard Deviation, S.E = Standard Error, C.V = Coefficient of Variation

Table-2. Analysis of variance of hybrid combinations

Source of variation	DF	Fecun-dity (No.)	Yield /10,000 Larvae No.	Yield /10,000 Larvae Wt.(kg)	Pupation Rate (%)	Cocoon Weight (g)	Shell Weight (g)	Shell Ratio (%)	Filament Length (m)	NBFL (m)	Raw Silk (%)	Denier (d)	Neat-ness (p)
Hybrids	11	1479.737**	28592.00*	106.721**	2862.285**	0.100**	0.009**	3.587**	78976.720**	5654.95**	4.4854**	0.0123**	5.0665**
Seasons	2	5940.934**	83001.800**	104.189**	65.308**	0.826**	0.065**	15.374**	5824.800**	4176.2935**	20.7801**	0.0061**	59.082**
Hybrids * Seasons	22	10.024**	86553.260**	2.309**	10.024**	0.016**	0.001**	2.055**	1009.770**	7026.996**	2.2602**	0.0078**	2.361
Error (B)	70	25.212	8403.223	0.003	0.468	0.000	0.000	0.032	20.509	155.107	0.493	0.001	1.921
Total	107	438.733	29823.000	13.396	298.510	0.029	0.002	1.106	21106	1516.943	1.646	0.003	3.404

Values represent mean of 3 seasons
df = degree of freedom; * Significant (p< 0.05); ** Significant (p< 0.01)

The overall mean performance of the hybrids reveals that BNR9 x BNR10 and BNR9 x BNR4 recorded higher performance. The ANOVA evaluated for the hybrids revealed highly significant differences ($p<0.01$) between hybrids, seasons and season x hybrid interactions and non-significant interaction between season x hybrid in neatness.

Evaluation Index: The relative merit as represented by average cumulative index value recorded for the overall performance of the hybrids and presented in descending order showed that highest EI value for BNR9 x BNR10 (61.33) followed by BNR9 x BNR4 (567.48), BNR7 x BNR6 (56.13), BNR7 x BNR2 (52.88), BNR3 x BNR8 (52.87), BNR3 x BNR6 (50.63), and BNR1 x BNR6 (50.28) indicate their economic superiority over others including control hybrids APS45 x APS12 and CSR2 x CSR4 which recorded average cumulative index values of 41.13 and 31.10 respectively over twelve multiple traits evaluated.

Table-3. Evaluation index of hybrids

Combination	Fecun-dity	Yield /10,000 Larvae		Pupa-tion Rate	Cocoon Weight	Shell Weight	Shell Ratio	Fila-ment Length	NBFL	Raw Silk	Denier	Neat-ness	Avg. EI	Rank
		No.	Wt.											
BNR1 X BNR2	38.97	51.87	49.44	52.78	45.03	45.40	50.34	48.33	52.20	37.16	49.53	45.69	47.23	X
BNR1 X BNR6	54.22	52.76	50.57	53.16	47.86	47.56	50.37	49.44	49.73	49.33	58.76	39.53	50.28	VII
BNR3 X BNR6	58.49	52.97	50.91	53.32	47.07	44.47	43.74	47.46	48.12	52.58	61.92	46.53	50.63	VI
BNR3 X BNR8	57.57	53.86	54.64	54.45	51.28	51.66	53.52	52.32	52.75	54.95	40.52	56.88	52.87	V
BNR5 X BNR6	37.05	53.49	51.34	53.99	50.05	49.57	56.90	48.24	48.16	41.21	57.46	47.09	49.55	VIII
BNR7 X BNR2	44.80	54.41	54.04	55.05	49.92	51.22	55.43	52.36	52.90	54.45	34.94	75.07	52.88	IV
BNR7 X BNR6	49.71	54.25	55.29	54.50	58.72	58.26	56.50	57.24	56.93	58.66	63.57	49.88	56.13	III
BNR9 X BNR4	66.88	54.62	58.17	55.10	63.16	62.60	58.03	63.82	63.47	54.44	41.00	48.48	57.48	II
BNR9 X BNR8	46.06	54.08	53.65	54.61	53.02	50.44	44.66	45.04	39.36	48.75	47.67	55.48	49.40	IX
BNR9 X BNR10	63.19	55.19	58.71	55.67	66.46	67.35	63.63	66.56	65.75	69.51	48.50	55.48	61.33	I
APS45 X APS12(C)	38.51	42.35	41.60	29.49	34.54	42.89	40.58	40.04	40.32	46.86	58.75	37.57	41.13	XI
CSR2 X CSR4(C)	44.55	20.13	21.64	27.87	32.91	28.57	26.31	29.14	30.31	32.11	37.38	42.33	31.10	XII

Avg. EI= Average Evaluation Index

Effect of Temperature on Disease Induction: The pupation rate was observed 0.00 % when the *Bm*NPV inoculated larvae exposed to 5^{0}C. The larvae when exposed to 25^{0}C highest pupation rate was observed in BNR9 x BNR10 (88.00 %) followed by BNR7 x BNR6 (86.67 %) and BNR9 x BNR4 (85.33 %). The larvae when exposed to high temperature (32^{0}C) highest pupation rate was observed in BNR9 x BNR10 (54.67 %) followed by BNR7 x BNR6 (52.00 %) and BNR9 x BNR4 (50.67 %). The analysis of variance results indicate highly significant ($p<0.01$) values for pupation.

Table-4. Effect of temperature on disease induction in Hybrids

Breed	Mortality due to BmNPV at various temperatures											
	5°C				25°C				32°C			
	Larval Morta-lity (%)	Mount-age Morta-lity (%)	Pre - Pupal Morta-lity (%)	Pupa-tion (%)	Larval Morta-lity (%)	Mount-age Morta-lity (%)	Pre - Pupal Morta-lity (%)	Pupa-tion (%)	Larval Morta-lity (%)	Mount-age Morta-lity (%)	Pre - Pupal Morta - lity (%)	Pupa-tion (%)
BNR1 X BNR2	94.67	4.00	1.33	0.00	4.00	6.67	6.67	82.67	13.33	20.00	21.33	45.33
BNR1 X BNR6	93.33	6.67	0.00	0.00	5.33	5.33	4.00	85.33	17.33	22.67	12.00	48.00
BNR3 X BNR6	98.67	1.33	0.00	0.00	6.67	6.67	5.33	81.33	23.33	25.67	15.67	35.33
BNR3 X BNR8	93.33	4.00	2.67	0.00	8.00	1.33	8.00	82.67	18.67	17.33	20.00	44.00
BNR5 X BNR6	98.67	1.33	0.00	0.00	4.00	5.33	6.67	84.00	20.00	14.67	16.00	49.33
BNR7 X BNR2	84.00	9.33	4.00	2.67	1.33	4.00	9.33	85.33	20.00	21.33	9.33	49.33
BNR7 X BNR6	90.67	2.67	4.00	2.67	1.33	5.33	6.67	86.67	18.67	12.00	17.33	52.00
BNR9 X BNR4	98.67	1.33	0.00	0.00	1.33	6.67	6.67	85.33	20.00	18.67	10.67	50.67
BNR9 X BNR8	93.33	6.67	0.00	0.00	4.00	6.67	5.33	84.00	24.00	20.00	6.67	49.33
BNR9 X BNR10	84.00	6.67	4.00	5.33	1.33	2.67	8.00	88.00	18.67	12.00	14.67	54.67
APS45 X APS12(C)	97.33	2.67	0.00	0.00	22.67	18.67	29.33	24.67	38.67	20.00	30.67	10.67
CSR2 X CSR4(C)	98.67	1.33	0.00	0.00	13.33	12.00	10.67	31.33	26.67	21.33	21.33	19.67

Values represent mean of three replications

4. DISCUSSION

Success of silkworm breeds/hybrids depends largely on their adaptability to the environment in which it is destined to be reared as opined by Sato [7]. It is clear from the results, that under *Bm*NPV inoculated conditions the survival rate and cocoon characters of newly evolved hybrids are significantly superior to that of controls during different seasons of the year. The peer analysis of the data on the performance of hybrids in pre-monsoon, monsoon and post-monsoon seasons of the year revealed maximum expression of economic traits during favourable post-monsoon season followed by monsoon and pre-monsoon season. These findings corroborate with [8-19]. However, the performance of the hybrids during three different seasons revealed marginal differences in the expression of economic traits.

The higher survival rate and quantitative traits under *Bm*NPV inoculated conditions were noticed in the newly evolved hybrids even during unfavourable months. The higher cocoon weight observed in the new hybrids, which is positively co-related with the cocoon yield indicates higher productivity under diversified environmental and *Bm*NPV inoculated conditions reflecting their overall superiority and cocoon crop stability. The overall mean performance of the hybrids reveals that BNR9 x BNR10 and BNR9 x BNR4 recorded higher performance. The ANOVA evaluated for the hybrids revealed highly significant differences ($p<0.01$) between hybrids, seasons and season x hybrid interactions and non-significant interaction between season x hybrid in neatness. Further the evaluation index also confirmed the superiority of BNR9 x BNR10 (61.33) and BNR9 x BNR4 (57.48).

Extensive work has been done on the induction of viral diseases. Among them, the important physical agents, which induce diseases in silkworms, are temperature and pathogenic virulence. Temperature is the most important external physical factor for both silkworm susceptibility and multiplication of viruses in the host. Most silkworm varieties have been adapted to rearing at 25^{0}C, which is most suitable for their development. Accordingly, temperatures much higher or lower then 25^{0}C tend to act as a stress and increase the larval susceptibility to viral infections [4]. Exposure of silkworm larvae to low temperature (5^{0}C) before peroral infection with NPV enhanced susceptibility to each virus [20]. Many workers were also successful in inducing NPV with cold treatment soon after the ecdysis of IV instar [21]. However, in an experiment the virus concentration below 103 particles/ml injected into V instar larvae at 25ºC went hidden (occult) but were reactivated by cold treatment [22]. It has also been observed that the incidence of nuclear polyhedro virus (NPV) was higher in sudden changes from room temperature to low temperature, *i.e.*, 5ºC than in gradual changes [23, 24]. The results of the present study on disease induction corroborates with the above findings showing that the newly ecdysed fifth instar larvae when exposed to low temperature of 5ºC and starved enhanced larval susceptibility to *Bm*NPV. Environmental factor such as low temperature would activate the latent virus and once the occult virus reaches the infective state, the virus multiplication proceeds, in the same manner as in the case of normal/natural infections [25]. Changes in the resistance to the infection with NPV was investigated by subjecting 4th an 5th instar larvae to low temperature (5^{0} and 10°C) and high temperature (33^{0}, 35^{0} and 37°C) treatments for various periods of time immediately after ecdysis [26]. In this study, the larvae when exposed to 25^{0}C highest pupation rate was observed in BNR9 x BNR10 (88.00 %) followed by BNR7 x BNR6 (86.67 %) and BNR9 x BNR4 (85.33 %) exhibiting higher level of tolerance at optimum temperature. The larvae when exposed to high temperature (32^{0}C) highest pupation rate was observed in BNR9 x BNR10 (54.67 %) followed by BNR7 x BNR6 (52.00 %) and BNR9 x BNR4 (50.67 %) expressing moderate tolerance showing that increase in temperature will decrease tolerance to *Bm*NPV.

Based on the mean, index values, stability parameters and temperature effect on disease induction the newly evolved hybrids BNR9 × BNR10 and BNR9 × BNR4 tolerant to *Bombyx mori* Nuclear Polyhedro Virus (*Bm*NPV) performed well for most of the characters and found suitable to rear throughout the year were recommended for commercial exploitation under tropical environmental conditions of Andhra Pradesh.

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