Effect of Different Doses of *Beauveria bassiana* (Bals.-Criv.) Vuill. Inoculation on Survival Parameters in a Few Thermotolerant Bivoltine Breeds and their Hybrids

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ABSTRACT

To know the effect of *Beauveria bassiana* inoculation on survival parameters in four thermo tolerant bivoltine breeds *viz*., B1, B4, B6, B8 and their hybrids *viz*., B1× B4, B1× B6, B1× B8, B4× B1, B4× B6, B4× B8, B6× B1, B6× B4, B6× B8, B8× B1, B8× B4 and B8× B6, the fifth instar silkworms were topically inoculated with different dilutions of the fungal spore suspension of B. *bassiana viz*., stock (3.17×10^5 spores / ml), 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8}). The results revealed that the thermo tolerant bivoltine silkworm hybrid B1×B8 showed highest LC_{50} of (9.04×10^4 spores / ml). The breed B8 showed better performance for LT_{50}, Effective Rate of Rearing (ERR), larval duration and pupation rate, while B1 was better for larval weight. Among the hybrids B1× B8 performed significantly better for the above mentioned traits followed by B1× B4 for all the fungal spore dilutions. Thus, these B1 breeds and B1× B8 and B1× B4 hybrids can be explored as a source of breeding material to impart muscardine tolerance and to identify genetic and molecular mechanism for dual stress tolerance in silkworms.

Keywords: Silkworm, Thermo tolerance, Bivoltine hybrids, Muscardine, Survival parameters

IN India, silk industry has made significant progress in global silk production securing second position, China being the leader (Anonymous, 2018). Silkworm breeding with the development of bivoltine hybrids has played a vital role in this perspective. However, silkworm crops are lost due to silkworm diseases, rather than to unfavourable weather conditions alone, that lead to poor harvest of mulberry leaves. Therefore, prevention of silkworm diseases and evolving silkworm breeds with high productivity are important aspects in commercial sericulture.

In tropical countries, temperature has a major role on productivity and growth of silkworm among abiotic factors. It is well-established fact that unlike polyvoltine, bivoltine are more susceptible to various stresses, namely, poor leaf quality, hot climatic conditions of tropics, and improper management during summer. To overcome this CSR&TI, Mysore (Suresh Kumar *et al*., 2003) and APSSRDI, Hindupur (Ramesh Babu *et al*., 2005) have evolved thermotolerant bivoltine silkworm breeds adoptable to high temperature and high temperature fluctuations prevailing in summer.

Fungal diseases are recognized as muscardine or mycoses. Silkworm is attacked by more than a dozen genera of fungi and white muscardine caused by entomopathogenic fungi, *B. bassiana* is most prevalent. The susceptibility in silkworm breeds to *B. bassiana* is genetically determined by two major genes, 'mus' gene located in the 11th chromosome and 'cal' gene located in the 7th chromosome (Shimada, 1999). However, muscardine disease resistance in silkworm is known to show quantitative inheritance (Zafar *et al*., 2013). Much of the work has been done on resistance of silkworms to viral diseases like NPV, CPV, IFV and DNV. There are some reports that, the resistance to CPV infection is generally controlled by polygene but, highly resistant strain like Diazo, possesses a major dominant gene controlling CPV resistance (Watanabe, 1986). Quantitative genetic studies on the resistance of silkworm to diseases other than viral diseases are limited.

Though thermotolerant breeds have been developed for tolerance to high temperature and temperature fluctuations, they have some inherent resistance to viral diseases which are mainly encountered under high temperature conditions. In addition, a few thermotoleran bivoltine breeds have also shown relatively better tolerance to white muscardine disease (Keerthana, 2018). If such breeds are used as parents in hybridization programme, genetic analysis and possible heterotic expression of quantitative traits in response to muscardine disease may throw light on multiple stress tolerance in mulberry silkworm.In this background an attempt was made to analyse the association between muscardine disease tolerance and high temperature tolerance through hybridization of selected thermotolerant bivoltine silkworms and look into the possibilities of evolving multiple stress resistance in silkworms.

Material and Methods

Effect of *B. bassiana* inoculation on survival parameters among a few thermotolerant bivoltine breeds and their hybrids was studied at the Department of Sericulture, UAS, GKVK, Bengaluru from September - December 2018.

Four, thermotolerant bivoltine silkworm breeds B1, B4, B6 and B8 from CSRTI, Mysore which were found to be relatively tolerant to muscardine in earlier studies (Keerthana, 2018 and Keerthana *et al*., 2019) were hybridized in a Diallele cross to develop sixteen combinations comprising of four parents and 12 hybrids *viz*., B1, B4, B6, B8, B1× B4, B1× B6, B1× B8, B4× B1, B4× B6, B4× B8, B6× B1, B6× B4, B6× B8, B8× B1, B8× B4 and B8× B6. Rearing of each breed and hybrid was conducted up to fourth instar by following standard rearing practices, on V1 mulberry leaves (Dandin *et al*., 2001). Newly ecdysed fifth instar larvae (50 worms per replication in three replications each) were topically inoculated with different dilutions of the fungal spore suspension *i.e*., stock (3.17×10^5 spores / ml), 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8} dilutions, at the rate of 0.5 ml per worm by spraying with an automizer (Venkataramana Reddy, 1978). High relative humidity of 95±5 per cent and temperature of 25±1°C were maintained in the rearing room. Control batch of entire cross combinations without any fungal inoculation was also maintained.

Observations on LC_{50}, LT_{50}, larval weight, larval duration, ERR and pupation rate were recorded. The data obtained were analysed using completely randomized design (Sundarraj *et al*., 1972). The per cent data was analysed after transformation by using the formula $\sin^{-1}\sqrt{p/100}$. The zero values in the data obtained were analysed after normalizing the distribution by$\sqrt{x+1}$ transformation. The mean values of the experiments were compared by Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

LC_{50}

Four thermotolerant silkworm breeds and their hybrids when treated immediately after fourth moult with different dilutions of *B.bassiana* spores, B1 × B8 hybrid showed highest LC_{50} value (9.03×10^4 spores / ml), followed by B1×B4 (7.41×10^4 spores / ml) and B8 (7.12×10^4spores / ml). Whereas, B6 (3.91×10^4 spores/ml) and B6 × B8 (3.97×10^4 spores/ml) showed lowest LC_{50} values. The LC_{50} estimation of thermotolerant bivoltine silkworm hybrids revealed that, the hybrid B1×B8 (9.04×10^4 spore per ml) was relatively more tolerant to *B. bassiana* infection among all the hybrids studied.

The lethal concentration of the fungus, *B. bassiana* required for 50 per cent mortality was maximum in C-nichi during both fourth (7.71×10^2 spores / ml) and fifth (4.73×10^2 spores / ml) instars, while the same was minimum in NB_{18} (1.66×10^2 and 0.65×10^2 spores / ml during fourth and fifth instars, respectively) indicating their varied degree of susceptibility to the infection The present findings are inline with the findigs of Raghavaiah and Jayaramaiah(1990). Three Indian silkworm strains when infected with *B. bassiana* during fifth instar showed seven fold differences in LC_{50} values between most susceptible and tolerant strains (Lakshmi *et al*., 2005). In the present study, the thermotolerant bivoltine hybrids revealed varied degree of tolerance to *B. bassiana* being 2.3 folds more tolerance in the hybrid B1×B8 and 1.2 fold in B1×B4 hybrid compared to most susceptible B6 breed, thus indicating the thermotolerant hybrids B1×B8 and B1×B4 are relatively more tolerant to the fungal infection. (Table 1)

LT_{50}

Time taken for 50 per cent mortality (LT_{50}) among four thermotolerant bivoltine breeds and their 12 hybrids inoculated by *B. bassiana* at different doses showed significant differences (Table 1). Among the breeds, B1 showed significantly highest LT_{50} value (7.54 days) at stock concentration, while in all the other dilutions, B8 breed showed highest LT_{50} values (8.32 days at 10^{-2}, 8.66 days at 10^{-4}, 9.91days at 10^{-6} and 11.61days at 10^{-8} dilutions). Similarly, among hybrids, B1 × B8 showed highest LT_{50} value at stock and all dilutions (8.47 days, 8.81 days, 9.96 days, 10.60 days and 13.43 days at stock , 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8} dilutions, respectively). Over the dilutions, highest LT_{50} was observed in B1 × B8 (10.25 days) while lowest LT_{50} was noticed in B6 breed and B6×B4 hybrid (7.62 and 7.63 days, respectively).

The hybrid PM × KA when treated with different dilution of *B. bassiana i.e.*, 10^{-9} to 10^{-2} showed highest LT_{50} (9.94 days) at 10^{-9} spore dilution and lowest LT_{50} (4.95 days) at 10^{-2} spore dilution (Venkataramana Reddy, 1978). Thermotolerant bivoltine silkworm breeds B4, B8 and B7 showed higher LT_{50} values at all spore dilutions (stock, 10^{-1} to 10^{-5}) (Keerthana *et al.*, 2019). It was found that LT_{50} increases with decrease in spore concentrations used to inoculate different larval instars. In the present study, similar trend was observed wherein, the thermotolerant

TABLE 1

LC_{50} and LT_{50} of thermotolerant bivoltine silkworm breeds and their hybrids inoculated with different doses of *B. bassiana* spores

Parents / Hybrids	LC_{50}			Regression equation	LT_{50}					
	Spores/ml	Upper limit (Spores/ml)	Lower limit (Spores/ml)		Stock	10^{-2}	10^{-4}	10^{-6}	10^{-8}	Mean
B1	$5.59×10^4$	$6.61×10^4$	$4.74×10^4$	y = 0.3852x+3.4826	7.54^{cde}	7.93^{bcd}	8.24^{abc}	8.78^{a}	9.51^{ab}	8.40
B4	$4.75×10^4$	$5.67×10^4$	$3.97×10^4$	y = 0.4246x+3.4684	6.67^{abc}	7.74^{abc}	7.96^{abc}	8.54^{a}	9.05^{a}	7.99
B6	$3.91×10^4$	$4.64×10^4$	$3.29×10^4$	y = 0.9381x+1.5746	6.2^{1a}	7.38^{ab}	7.52^{a}	8.34^{a}	8.67^{a}	7.62
B8	$7.12×10^4$	$8.35×10^4$	$6.07×10^4$	y = 0.3485+ 3.4207	6.98^{abc}	8.32^{cde}	8.66^{bc}	9.91^{b}	11.61^{c}	9.09
B1×B4	$7.41×10^4$	$8.63×10^4$	$6.36×10^4$	y = 0.3309x+3.4874	8.20^{de}	8.46^{de}	8.94^{cd}	10.17^{b}	11.26^{bc}	9.41
B1×B6	$4.32×10^4$	$5.11×10^4$	$3.64×10^4$	y = 0.5890x+2.1498	7.04^{abc}	7.78^{abc}	7.74^{ab}	8.28^{a}	8.64^{a}	7.89
B1×B8	$9.03×10^4$	$10.63×10^4$	$7.68×10^4$	y = 0.2894x+3.5145	8.47^{e}	8.81^{e}	9.96^{d}	10.60^{b}	13.43^{d}	10.25
B4×B1	$4.80×10^4$	$5.82×10^4$	$3.95×10^4$	y = 0.3380x+3.8146	6.74^{abc}	7.72^{abc}	8.03^{abc}	8.56^{a}	8.89^{a}	7.98
B4×B6	$5.05×10^4$	$5.92×10^4$	$4.32×10^4$	y = 0.4977x+3.1196	6.85^{abc}	7.79^{abc}	8.15^{abc}	8.64^{a}	9.08^{a}	8.10
B4×B8	$4.93×10^4$	$5.86×10^4$	$4.15×10^4$	y = 0.4480x+3.3206	7.12^{abc}	7.72^{abc}	7.93^{abc}	8.60^{a}	8.99^{a}	8.07
B6×B1	$4.36×10^4$	$5.12×10^4$	$3.71×10^4$	y = 1.3017x -0.0426	6.63^{abc}	7.72^{abc}	7.67^{ab}	8.29^{a}	8.58^{a}	7.78
B6×B4	$4.15×10^4$	$4.88×10^4$	$3.52×10^4$	y=1.3540x -0.2111	6.45^{ab}	7.25^{a}	7.54^{a}	8.32^{a}	8.58^{a}	7.63
B6×B8	$3.97×10^4$	$4.79×10^4$	$3.30×10^4$	y= 0.5260x+ 3.2647	7.23^{abcd}	7.90^{bcd}	7.86^{abc}	8.43^{a}	8.47^{a}	7.98
B8×B1	$4.28×10^4$	$5.01×10^4$	$3.65×10^4$	y = 0.8255x+1.9037	7.34^{bcd}	7.43^{ab}	7.54^{a}	8.29^{a}	8.64^{a}	7.85
B8×B4	$4.25×10^4$	$5.06×10^4$	$3.57×10^4$	y = 0.5081x+3.3032	6.94^{abc}	7.49^{ab}	7.82^{ab}	8.33^{a}	8.45^{a}	7.81
B8×B6	$4.12×10^4$	$4.87×10^4$	$3.48×10^4$	y = 0.7391x+2.3316	6.74^{abc}	7.24^{a}	7.74^{ab}	8.36^{a}	8.31^{a}	7.68
F-test	NA	NA	NA	NA	*	*	*	*	*	NA
SEm±	-	-	-	-	0.198	0.12	0.21	0.15	0.34	-
CD at 5%	-	-	-	-	0.57	0.35	0.59	0.44	0.98	-
CV%	-	-	-	-	4.85	2.66	4.42	3.03	6.28	-

NA- Not analysed; *- Significant at 5%; NS- Non significant; figures with same superscript are statistically on par; Stock – $3.17 × 10^5$ spores/ml(Allign)

TABLE 2

ERR and fifth instar larval weight of thermotolerant bivoltine silkworm breeds and their hybrids inoculated by different doses of *B. bassiana* spores

Parents/ hybrids	ERR (%)							Fifth instar larval weight (g / 10 worms)						
	Stock	10^{-2}	10^{-4}	10^{-6}	10^{-8}	Mean	Control	Stock	10^{-2}	10^{-4}	10^{-6}	10^{-8}	Mean	Control
B1	22 ^{def} (4.74)	30.67 ^{dev} (40.00)	44.67 ^{def} (41.94)	45.33 ^{bcd} (42.32)	48.00 ^{abc} (43.85)	38.13	100	28.86 ^{d}	29.38 ^{e}	33.99 ^{j}	32.34 ^{d}	32.19 ^{def}	31.35	42.34 ^{j}
B4	17.33 ^{cde} (4.20)	24.67 ^{bcd} (31.41)	34.00 ^{bcd} (35.66)	40.00 ^{ab} (39.23)	52.67 ^{cd} (46.53)	33.73	100	29.09 ^{d}	27.73 ^{cd}	29.14 ^{de}	30.66 ^{bc}	33.57 ^{g}	30.04	41.27 ^{h}
B6	0 ^{a} (0.71)	19.33 ^{abcd} (26.04)	24.00 ^{ab} (29.28)	33.33 ^{a} (35.25)	42.67 ^{ab} (40.78)	23.86	100	23.81 ^{a}	27.35 ^{cd}	29.16 ^{def}	29.24 ^{a}	30.50 ^{ab}	28.01	38.35 ^{ab}
B8	33.33 ^{ef} (5.81)	40.67 ^{ef} (39.62)	40.00 ^{de} (39.23)	52.67 ^{cde} (46.53)	61.33 ^{de} (51.56)	45.60	100	26.58 ^{bc}	29.70 ^{e}	30.83 ^{hi}	33.98 ^{e}	34.95 ^{h}	31.21	42.18 ^{j}
B1×B4	32.66 ^{ef} (5.76)	41.33 ^{ef} (26.08)	48.67 ^{ef} (44.24)	54.00 ^{de} (47.30)	60.00 ^{de} (50.77)	47.33	100	31.21 ^{e}	31.40 ^{f}	31.68 ^{i}	35.28 ^{f}	35.95 ^{h}	33.10	43.47 ^{k}
B1×B6	4.66 ^{abc} (2.21)	19.33 ^{abcd} (43.47)	26.00 ^{abc} (30.65)	33.33 ^{a} (35.26)	43.33 ^{abc} (41.66)	25.33	100	26.13 ^{b}	27.53 ^{cd}	29.54 ^{efg}	29.74 ^{ab}	30.14 ^{a}	28.62	39.75 ^{e}
B1×B8	42.00 ^{f} (6.51)	47.33 ^{f} (30.65)	52.67 ^{f} (46.53)	60.00 ^{e} (50.78)	66.00 ^{e} (54.37)	53.60	100	30.78 ^{e}	33.15 ^{g}	34.90 ^{j}	36.31 ^{f}	37.09 ^{i}	34.45	40.19 ^{f}
B4×B1	21.33 ^{def} (4.65)	26.00 ^{bcd} (29.75)	34.67 ^{bcd} (36.06)	40.00 ^{ab} (39.23)	46.67 ^{abc} (43.09)	33.73	100	26.91 ^{bc}	29.38 ^{e}	30.12 ^{fgh}	31.70 ^{cd}	32.24d ^{ef}	30.07	41.56 ^{i}
B4×B6	12.66 ^{bcd} (3.36)	27.33 ^{cd} (30.15)	35.33 ^{cd} (36.41)	42.67 ^{abcd} (40.77)	50.00 ^{bc} (45.00)	33.59	100	28.18 ^{cd}	29.19 ^{e}	30.56 ^{h}	31.74 ^{d}	32.27d ^{ef}	30.39	40.28 ^{f}
B4×B8	16.66 ^{cde} (4.14)	25.33 ^{bcd} (30.07)	34.00 ^{bcd} (35.62)	41.33 ^{abc} (39.99)	48.00 ^{abc} (43.85)	33.06	100	27.83 ^{cd}	28.56 ^{de}	30.35 ^{gh}	31.67 ^{cd}	33.28 ^{fg}	30.34	40.33 ^{f}
B6×B1	0 ^{a} (0.71)	25.33 ^{bcd} (30.07)	26.67 ^{abc} (31.08)	36.00 ^{ab} (36.85)	44.00 ^{abc} (41.54)	26.40	100	26.92 ^{bc}	27.81 ^{cd}	29.33 ^{def}	30.44 ^{b}	31.82 ^{cde}	29.26	39.84 ^{e}
B6×B4	0 ^{a} (0.71)	17.33 ^{abc} (24.55)	25.33 ^{abc} (30.19)	34.67 ^{ab} (36.07)	43.33 ^{abc} (41.17)	24.13	100	23.87 ^{a}	25.85 ^{ab}	28.40 ^{cd}	29.09 ^{a}	31.72 ^{cde}	27.77	39.44 ^{d}

(Table 2 continued)

Parents/ hybrids	ERR (%)							Fifth instar larval weight (g / 10 worms)						
	Stock	10^{-2}	10^{-4}	10^{-6}	10^{-8}	Mean	Control	Stock	10^{-2}	10^{-4}	10^{-6}	10^{-8}	Mean	Control
B6×B8	6.66 ^{abc} (2.39)	16.00 ^{ab} (23.37)	26.00 ^{abc} (30.50)	32.67 ^{a} (34.70)	44.00 ^{abc} (41.55)	25.06	100	25.90 ^{b}	26.81 ^{abc}	27.13 ^{ab}	30.07 ^{ab}	30.38 ^{ab}	28.06	38.16 ^{a}
B8×B4	8.66 ^{bcd} (3.02)	18.67 ^{abc} (25.59)	26.67 ^{abc} (31.09)	32.67 ^{a} (34.85)	39.33 ^{a} (38.83)	25.20	100	25.40 ^{ab}	27.11 ^{bc}	27.54 ^{bc}	30.45 ^{b}	30.99 ^{abc}	28.30	40.79 ^{b}
B8×B6	3.33 ^{ab} (1.79)	16.00 ^{abc} (23.55)	27.33 ^{abc} (31.52)	36.67 ^{ab} (37.26)	44.00 ^{abc} (41.55)	25.47	100	23.84 ^{a}	25.53 ^{a}	26.46 ^{a}	30.55 ^{b}	31.41 ^{bcd}	27.56	38.50 ^{g}
F-test	*	*	*	*	*	NA	NS	*	*	*	*	*	NA	*
SEm±	0.42	1.50	1.33	1.26	1.03	-	-	0.32	0.26	0.19	0.21	0.21	-	0.05
CD at (5%)	1.21	4.33	3.82	3.62	2.98	-	-	0.91	0.74	0.53	0.59	0.62	-	0.14
CV (%)	22.23	8.69	6.58	5.49	4.04	-	-	2.03	1.56	1.07	1.13	1.14	-	0.21

NA- Not analysed; *- Significant at 5%, NS- Non significant; figures with same superscript are statistically on par; Stock – 3.17×10^{5} spores/ml

bivoltine silkworm breeds and hybrids showed LT_{50} value ranging from 6.63 to 8.47 days at stock, 7.24 to 8.81, 7.52 to 9.96, 8.29 to 10.60 and 8.31 to 13.43 days at 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8} dilutions, respectively. Higher LT_{50} values indicate the ability of the strain to resist the infection for longer time. Thus, B8 among parents and B1 × B8 among hybrids could resist fungal infection for longer time before they succumb to the disease.

Effective Rate of Rearing (%)

The effective rate of rearing based on fifth instar initial larval number showed significant differences among the thermotolerant bivoltine silkworm breeds and hybrids treated with different doses of *B. bassiana* (Table 2).The breed B1 showed significantly highest ERR (44.67 %) at 10^{-4} dilution, while in stock and all the other dilutions B8 breed showed highest ERR values (33.33 % at stock, 40.67 % at 10^{-2}, 52.67 % at 10^{-6} and 61.33 % at 10^{-8} dilutions). Similarly, among hybrids B1 × B8 showed highest ERR at both stock and all dilutions (42.00 %, 47.33 %, 52.67 %, 60.00 % and 66.00 % at stock, 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8} dilutions, respectively). While, lowest ERR was noticed in B6 (23.86 %) among breeds and in B8×B4 (25.20 %) among hybrids over all the dilutions. In control batches *i.e.*, without fungal inoculation cent per cent ERR was recorded in all the parents and hybrids (Plate 1 to 4).

In earlier studies, when eleven thermotolerant breeds were challenged with six conidial concentrations (stock, 10^{-1} – 10^{-5}dilutions) the breed B4 recorded highest ERR (12.67 %, 14.67 %, at 10^{-3} and 10^{-4} dilutions, respectively), followed by B1(10 % at 10^{-3}) and B8 (28.00 % at 10^{-5}) and significantly least ERR was recorded in CSR_2 (2.67 % at 10^{-3}), followed by APS_{12} (1.33 % at 10^{-4}) and APS $_{45}$ (6.66 % at 10^{-5}) (Keerthana *et al.*, 2019). From the present results, it was clear that, compared to parents, the performance of hybrids was superior with respect to ERR and that the hybrids B1×B8 and B1×B4 recorded higher ERR at different fungal dilutions indicating these could be better tolerant than parents to the fungal infection and B6 scored lowest ERR in all the dilutions which indicates its susceptibility towards fungal infection.

Plate 1: Cocoons of B1 breed and its hybrids inoculated with 9.03×10^4 spores / ml of *B. bassiana* and high temperature treatment at 36 ± 1°C & 85 ±5 % RH

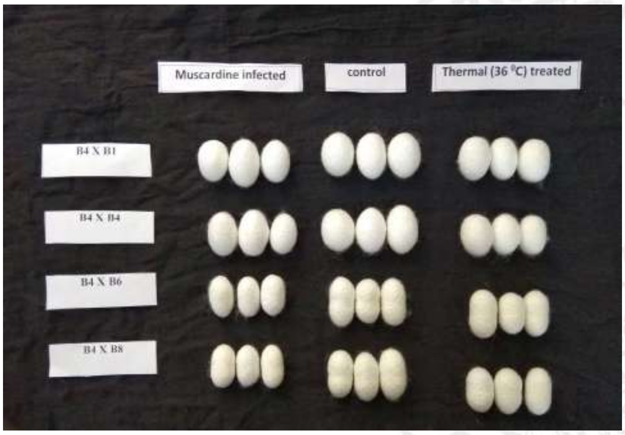

Plate 2: Cocoons of B4 breed and its hybrids inoculated with 9.03×10^4 spores / ml of *B. bassiana* and high tempera ture treatment at 36 ± 1°C & 85 ±5 % RH

Plate 3: Cocoons of B6 breed and its hybrids inoculated with 9.03×10^4 spores / ml of *B. bassiana* and high temperature treatment at 36 ± 1°C & 85 ±5 % RH

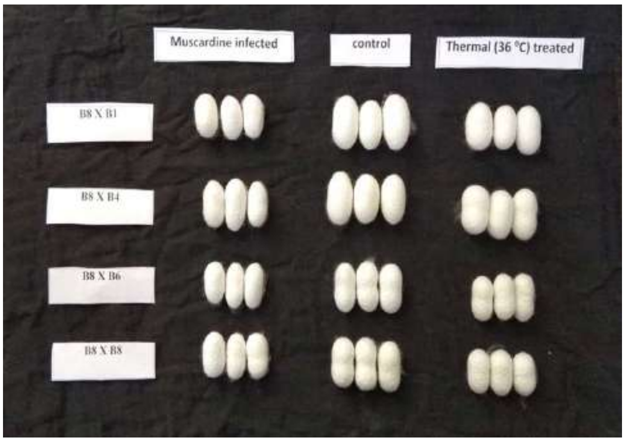

Plate 4: Cocoons of B8 breed and its hybrids inoculated with 9.03×10^4 spores / ml of *B. bassiana* and high temperature treatment at 36 ± 1°C & 85 ±5 % RH

Fifth Instar Larval Weight (g / 10 worms)

Significant differences were observed for fifth instar larval weight of thermotolerant bivoltine silkworm breeds and their hybrids inoculated with different dilutions of *B. bassiana* (Table2). The breed B4 showed significantly highest larval weight (29.09 g/ 10 worms) at stock concentration, while at 10^{-4} dilution it was highest in the breed B1 (33.99 g/ 10 worms). The breed B8 showed significantly highest larval weight at 10^{-2}, 10^{-6} and 10^{-8} dilutions (29.70g/ 10 worms, 33.98g/ 10 worms and 34.95g/ 10 worms, respectively). Similarly, among hybrids B1×B4 showed significantly highest larval weight (31.21g) at stock concentration, while in all the other dilutions B1×B8 recorded significantly highest larval weight (33.15g/ 10 worms at 10^{-2}, 34.90g/ 10 worms at 10^{-4}, 36.31g/ 10 worms at 10^{-6} and 37.09g/ 10 worms at 10^{-8} dilutions). Over the dilutions, significantly least larval weight was recorded in B8 × B6 (27.56g/ 10 worms) hybrid, followed by B6 × B4 breed (27.77g/ 10 worms). In control batch, significantly maximum larval weight was recorded in B1 among the breeds (42.34g/ 10 worms) and in B1×B4 among the hybrids (43.47g/ 10 worms), while least larval weight was recorded in B6 (38.35 g/ 10 worms) among breeds and in B6×B8 (38.16 g/ 10 worms) among hybrids.

The muscardine inoculated larvae were found to lose their body weight from third day onwards due to cessation of feeding (Venkataramana Reddy, 1978).

Reduction in the grown up larval weight in fifth instar of *B. mori*, infected with *B. bassiana* may be due to the consequence of fungal infection that led to decrease in food consumption, digestion, relative consumption rate and efficiency of conversion of ingested food. Rajitha and Savithri (2015) reported that silkworm hybrid PM × CSR_2 treated with sub lethal concentration of *B. bassiana* conidial suspension (2.15 × 10^6 spores / ml), exhibited significant reduction in mature larval weight (2.08g) compared to control (2.58g). Even in the present study, larval weight was significantly reduced as the concentration of fungal spore increased. Among parents the tolerant breed B8 lost its body weight from 42.18g (control) to 26.58 g (stock: 3.17 × 10^5spores/ml) and among hybrids B1×B8 lost its body weight from 40.19g (control) to 30.78g (stock: 3.17 × 10^5spores/ml), which were the least reduction in larval body weight due to fungal infection.

Fifth Instar Larval Duration (days)

Significant differences were observed for fifth instar larval duration among thermotolerant bivoltine silkworm breeds and their hybrids inoculated with different dilutions of *B. bassiana* (Table 3). The breed B8 recorded significantly shorter larval duration at stock concentration, 10^{-2}, 10^{-4} and 10^{-6} dilutions (10.33, 10.00, 9.00 and 9.00 days, respectively), while at 10^{-8} dilution, it was shorter in both B1 and B8 breeds (9 days each). Similarly, among hybrids both B1×B4 and B1×B8 recorded significantly shorter larval duration at stock concentration, 10^{-4} and 10^{-6} dilutions (10.00, 9.00 and 9.00 days each, respectively), while, at 10^{-2} dilution it was shorter in B1×B4, B1×B8 and B4×B8 hybrids (10.00 days each) and at 10^{-8} dilution, the hybrids B1×B4, B1×B8, B4×B6 and B4×B8 showed significantly shorter larval duration (8.67 days each). Significantly longer larval duration was observed in B6 (10.58 days) breed and in both B6×B1 and B6×B8 (10.50 days) hybrids over all the dilutions. In control batches *i.e.*, without any fungal treatment, non-significant difference was observed for larval duration. However, among parents, shorter larval duration was noticed in B8 (8.53 days), while longer duration was observed in both B6 and B1 (8.70 days each) breeds. Among the hybrids shorter larval duration was recorded in B8×B1 (8.50 days) and longer duration was recorded in B6×B4 and B8×B6 (8.70 days) hybrids.

Inoculation with different doses of *Bm*NPV to six different genotypes of silkworm resulted in significant increase in total larval duration as compared to control. Total larval duration of Pure Mysore increased from 26.47 days in absolute control batch to 30.30 days in the batch administered with 8.55 × 10^7 PIBs/ml of *Bm*NPV (stock). Similar trend was observed in other genotypes *viz*., Nistari, C-nichi, Diazo, CSR_2 and CSR_4 when inoculated with *Bm*NPV (Manjunath Gowda *et al*., 2011). Under *B. bassiana* infection also the fifth instar larval duration was extended and the extent of prolongation was higher in susceptible hybrids *i.e.,* B1×B6 and B8×B4 from 8.58 and 8.66 days respectively in absolute control batch to 11.67 days in the batches treated with 3.17 × 10^5 spores/ml (stock) and the extent of prolongation was minimum in tolerant hybrids *i.e*., B1 × B8 and B1 × B4 (8.53 and 8.55 days in control, respectively to 10.00 days in both the hybrids at stock concentration of *B. bassiana*).

Pupation Rate (%)

At stock concentration, cent per cent mortality was recorded in B6 breed and in B6×B1 and B6×B4 hybrids hence, pupation rate could not be recorded. The breed B8 showed significantly highest pupation rate at stock concentration, 10^{-2}, $10^{-4}$and 10^{-6} dilutions (56.67 %, 63.33 %, 90.00 % and 100.00 %, respectively). Similarly, among hybrids B1×B8 recorded significantly highest pupation rate at stock, 10^{-2} and 10^{-4} dilutions (70.00 %, 73.33 % and 96.67 %, respectively), while at 10^{-6} dilution, pupation rate was significantly higher in B1×B4, B1×B8, B4 × B6 and B4 × B8 (96.67 % each). At 10^{-8} dilution, cent per cent pupation rate was observed in almost all parents and hybrids except B6, B1×B6, B6×B1, B6×B4, B6×B8 and B8×B4, where pupation rate varied from 86.67 to 96.67 per cent. Over the dilutions lowest pupation was noticed in B8 × B1 (53.33 %), followed by B1 × B6 (54.00 %) hybrids. In un-inoculated control batches non-significant difference was observed for pupation rate, which varied from 90 per cent to 100 per cent in different hybrids (Table 3).

TABLE 3

Larval duration and pupation rate of thermotolerant bivoltine silkworm breeds and their hybrids inoculated by different doses of *B. bassiana* spores

Parents/ hybrids	Larval duration (days)							Pupation rate (%)						
	Stock	10^{-2}	10^{-4}	10^{-6}	10^{-8}	Mean	Control	Stock	10^{-2}	10^{-4}	10^{-6}	10^{-8}	Mean	Control
B1	10.67 ^{b}	10.33 ^{a}	9.67 ^{a}	9.33 ^{a}	9.00 ^{ab}	9.80	8.70 ^{a}	43.33 ^{cde} (6.61)	66.67 ^{e} (54.78)	73.33 ^{abcd} (59.21)	93.33 ^{bcd} (77.71)	100.00 ^{a} (90.00)	75.33	100 ^{a} (90.00)
B4	10.67 ^{b}	10.33 ^{a}	10.00 ^{a}	9.67 ^{a}	9.33 ^{ab}	10.00	8.62 ^{a}	36.67 ^{bcde} (5.94)	43.33 ^{bcd} (41.15)	73.33 ^{abcd} (59.00)	86.67 ^{abcd} (68.86)	100.00 ^{a} (90.00)	68.00	100 ^{a} (90.00)
B6	0.00 ^{a}	11.33 ^{a}	10.67 ^{a}	10.33 ^{a}	10.00 ^{b}	10.58	8.70 ^{a}	0.00 ^{a} (0.71)	23.33 ^{a} (28.78)	46.67 ^{a} (43.08)	66.67 ^{a} (54.78)	86.67 ^{a} (72.29)	55.84	100 ^{a} (90.00)
B8	10.33 ^{b}	10.00 ^{a}	9.00 ^{a}	9.00 ^{a}	9.00 ^{ab}	9.47	8.53 ^{a}	56.67 ^{de} (7.55)	63.33 ^{de} (52.78)	90.00 ^{def} (71.57)	100.00 ^{d} (90.00)	100.00 ^{a} (90.00)	82.00	100 ^{a} (90.00)
B1×B4	10.00 ^{b}	10.00 ^{a}	9.00 ^{a}	9.00 ^{a}	8.67 ^{a}	9.33	8.55 ^{a}	53.33 ^{cde} (7.29)	70.00 ^{e} (56.79)	93.33 ^{ef} (77.71)	96.67 ^{cd} (83.86)	100.00 ^{a} (90.00)	82.67	100 ^{a} (90.00)
B1×B6	11.67 ^{b}	11.00 ^{a}	9.67 ^{a}	9.67 ^{a}	9.33 ^{ab}	10.27	8.58 ^{a}	20.00 ^{bcd} (4.43)	43.33 ^{bcd} (41.15)	50.00 ^{a} (45.00)	66.67 ^{a} (54.99)	90.00 ^{a} (75.00)	54.00	100 ^{a} (90.00)
B1×B8	10.00 ^{b}	10.00 ^{a}	9.00 ^{a}	9.00 ^{a}	8.67 ^{a}	9.33	8.53 ^{a}	70.00 ^{e} (8.38)	73.33 ^{e} (59.00)	96.67 ^{f} (83.86)	96.67 ^{cd} (83.86)	100.00 ^{a} (90.00)	87.33	100 ^{a} (90.00)
B4×B1	10.67 ^{b}	10.33 ^{a}	9.67 ^{a}	9.67 ^{a}	9.33 ^{ab}	9.93	8.63 ^{a}	43.33 ^{cde} (6.61)	53.33 ^{cde} (46.92)	83.33 ^{cde} (66.14)	83.33 ^{abc} (66.14)	100.00 ^{a} (90.00)	72.66	96.67 ^{a} (83.86)
B4×B6	10.67 ^{b}	10.33 ^{a}	9.33 ^{a}	9.33 ^{a}	8.67 ^{a}	9.67	8.54 ^{a}	33.33 ^{bcde} (5.72)	43.33 ^{bcd} (41.15)	86.67 ^{cdef} (68.86)	96.67 ^{cd} (83.86)	100.00 ^{a} (90.00)	72.00	100 ^{a} (90.00)
B4×B8	10.67 ^{b}	10.00 ^{a}	9.33 ^{a}	9.33 ^{a}	9.00 ^{ab}	9.67	8.56 ^{a}	33.33 ^{bcde} (5.75)	43.33 ^{bcd} (41.15)	80.00 ^{bcde} (63.43)	96.67 ^{cd} (83.86)	100.00 ^{a} (90.00)	70.67	100 ^{a} (90.00)
B6×B1	0.00 ^{a}	11.33 ^{a}	10.67 ^{a}	10.33 ^{a}	9.67 ^{ab}	10.50	8.65 ^{a}	0.00 ^{a} (0.71)	36.67 ^{abc} (37.14)	56.67 ^{ab} (48.85)	73.33 ^{ab} (59.21)	90.00 ^{a} (75.00)	64.17	100 ^{a} (90.00)
B6×B4	0.00 ^{a}	11.00 ^{a}	10.00 ^{a}	10.00 ^{a}	9.00 ^{ab}	10.00	8.70 ^{a}	0.00 ^{a} (0.71)	33.33 ^{abc} (35.22)	50.00 ^{a} (45.00)	70.00 ^{a} (57.00)	96.67 ^{a} (83.86)	62.50	100 ^{a} (90.00)

(Table 3 continued)

Parents/ hybrids	Stock	10^{-2}	10^{-4}	10^{-6}	10^{-8}	Mean	Control	Pupation rate (%) Stock	10^{-2}	10^{-4}	10^{-6}	10^{-8}	Mean	Control
B6×B8	11.50 ^{ab}	11.00 ^{a}	10.33 ^{a}	10.00 ^{a}	9.67 ^{ab}	10.50	8.60 ^{a}	20.00 ^{abc} (3.92)	43.33 ^{bcd} (41.15)	53.33 ^{ab} (46.92)	70.00 ^{ab} (56.79)	86.67 ^{a} (72.29)	54.67	100 ^{a} (90.00)
B8×B1	11.50 ^{ab}	10.67 ^{a}	9.67 ^{a}	9.33 ^{a}	8.67 ^{a}	9.97	8.50 ^{a}	16.67 ^{abcd} (4.10)	26.67 ^{ab} (30.79)	50.00 ^{a} (45.00)	73.33 ^{ab} (59.00)	100.00 ^{a} (90.00)	53.33	96.67 ^{a} (83.86)
B8×B4	11.67 ^{b}	10.67 ^{a}	9.33 ^{a}	9.33 ^{a}	9.00 ^{ab}	10.00	8.66 ^{a}	33.33 ^{bcde} (5.72)	40.00 ^{abc} (39.23)	56.67 ^{ab} (48.85)	76.67 ^{abc} (61.71)	96.67 ^{a} (83.86)	60.67	93.33 ^{a} (81.14)
B8×B6	11.50 ^{ab}	10.33 ^{a}	10.00 ^{a}	9.33 ^{a}	9.00 ^{ab}	10.03	8.70 ^{a}	10.00 ^{ab} (2.83)	40.00 ^{abc} (39.15)	63.33 ^{abc} (52.78)	83.33 ^{abc} (66.14)	100.00 ^{a} (90.00)	59.33	90.00 ^{a} (75)
F-test	*	*	*	NS	*	NA	NS	*	*	*	*	*	NA	NS
SEm±	1.68	0.32	0.36	0.31	0.25	-	0.06	0.69	2.36	3.21	4.28	4.72	-	3.67
CD at (5%)	4.84	0.93	1.05	0.90	0.72	-	0.16	1.98	6.79	9.25	12.33	13.59	-	10.58
CV (%)	35.84	5.30	6.48	5.66	4.75	-	1.11	24.70	9.52	9.62	10.71	9.59	-	7.25

NA- Not analysed; *- Significant at 5%; NS- Non significant; figures with same superscript are statistically on par; Stock – 3.17×10^5 spores/ml(Allign)

Biabani *et al*. (2005) evaluated 20 commercial hybrids of silkworm for their resistance to *Bm*NPV, among which 113k×114k hybrid recorded highest pupation of 90.67 per cent, followed by 113k×108k (89.33%) and 107k×124k (88.69%) hybrids. The hybrid 111k×114k with 36.34 per cent pupal survival rate showed the lowest resistance to this pathogen. Chandrasekharan and Nataraju (2008) have observed reduced pupation rate in silkworms infected with *Bm*NPV, which was lesser at lower dose and comparatively higher at higher dose, supporting the trend observed in the present study. Sivaprasad *et al*. (2003) defined apparent tolerance as inoculated larvae completing the larval period and forming cocoons but not pupating into pupae. If larvae completed their larval period, pupated into pupae and moths emerged from the cocoons, it was defined as the real tolerance group. In the present study pupation was observed even at higher doses *i.e.*, 3.17×10^5 spores/ml where one of the hybrid B1×B8 exhibited 70 per cent pupation suggesting real tolerance could be there in this thermotolerant bivoltine silkworm hybrid for *B. bassiana* infection.

Lipids in the epicuticle of the silkworm B. mori inhibit the invasion of *B. bassiana*. The living integument and exuviae of resistant Chinese silkworm race has a greater antifungal action than that of susceptible Japanese strain. Similarly differences in antifucngal activity of the integument in different thermotolerant breeds and hybrids could be the cause for differences in tolerance to *B. bassiana* infection (David, 1967).

With these findings it could be therefore, inferred that thermotolerant bivoltine silkworm breeds B8 and B1 and hybrids B1×B8 and B1×B4 showed relatively better tolerance to *B. bassiana* infection indicating that there could be possibility of dual tolerance for high temperature and muscardine infection. Further, they can be explored as a source of breeding material to impart muscardine tolerance to other productive thermotolerant breeds and to understand the genetic and molecular mechanism for dual tolerance in silkworm breeds and hybrids.

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