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Investigating the performance of thermotolerant bivoltine silkworm breeds and hybrids infected with *Beauveria bassiana*

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Abstract

A study was conducted to know the rearing performance of the thermotolerant bivoltine silkworm breeds and hybrids under *Beauveria bassiana* infection at the Department of Sericulture, UAS, GKVK, Bengaluru during 2021-22. Thermotolerant bivoltine breeds viz., B1, B4 and B8 and their hybrids with CSR4 i.e., B1×CSR4, B4×CSR4 and B8×CSR4 along with Pure Mysore as a muscardine resistant and CSR4 as a muscardine susceptible breeds were used in this study. A batch of silkworms of all the breeds and hybrids were inoculated with 9.04×10^4 spores/ml of *B. bassiana* @ 0.5 ml per silkworm immediately after 4th moult and another batch was reared under normal condition. The rearing performance revealed that B1 breed and B1×CSR4 hybrid had performed better under control conditions, where as Pure Mysore breed for the survival characters, while B4 breed and B4×CSR4 hybrid for economic characters performed better under infected conditions. Hence, we can say that B4 breed and B4×CSR4 hybrid are exhibiting good disease resistance.

Keywords: Silkworm, thermotolerant, muscardine and bivoltine

Introduction

The success of sericulture industry primarily depends on the successful harvest of cocoon crops. India being a tropical country where we encounter marginal sub-tropical and temperate sericulture zones, predominantly multivoltine × bivoltine hybrids are reared. But the raw silk produced from these hybrids do not satisfy the international standards as it is not as superior as that produce by bivoltine × bivoltine hybrids. Thus, there is great need and scope for improving the bivoltine sericulture in the country, and more emphasis needs to be given to bivoltine sericulture for producing international grade raw silk (Jayashree *et al.*, 2020) [1]. But the major problem for bivoltine sericulture is the high incidence of diseases. The major diseases affecting mulberry silkworm are muscardine, pebrine (both fungal disease), flacherie (bacterial diseases) and grasserie (viral diseases). Silkworm crop loss occurs in all the silkworm growing areas of the world, but the type of severity varies. They differ from region to region, crop to crop and even from farmer to farmer. Leaving aside minor variations, it has been found that crop loss is generally more in the tropics than in the temperate regions.

An estimated 30 to 40 percent of India's annual cocoon output is thought to be lost due to different silkworm diseases, with white muscardine disease accounting for 10 to 40 percent of the loss (Janakiraman, 1961; Chandrasekharan and Nataraju, 2008) [2, 3]. More than 700 species of fungi are known to cause mycosis in insects, making them the largest group of illnesses. The majority of infections induced by the fungus that cause silkworm disease are caused by members of the genera *Metarhizium* and *Beauveria*. *Beauveria bassiana* (Bals. - Criv.) Vuill., the fungus that causes the common disease known as white muscardine, is one of the most dangerous to the silkworm *Bombyx mori* L.

Achieving the aim of silk output and acquiring high-quality silk both depend on the upbringing of bivoltine silkworm breeds. Bivoltines are more susceptible to numerous pressures in tropical environments than multivoltines because they evolved in temperate regions. Therefore, the Central Sericulture Research and Training Institute in Mysore and the Andhra Pradesh State Sericulture Research and Development Institute in Hindupur have

developed thermotolerant bivoltine silkworm breeds which able to adapt to temperature fluctuations and the high temperatures prevailing during summer in an effort to develop bivoltine silkworm breeds for tropical environments. Availability of multiple stress tolerant silkworm breeds will be of immense value so that they can be reared under different weather conditions and then throughout the year to get sustainable higher yield. Although those breeds can be raised in the summer, it is unknown if they can tolerate the winter time during white muscardine disease is prevalent (Lakshmi *et al.*, 2011) ^[4]. Additional investigations into the reaction of thermotolerant bivoltine breeds to *B. bassiana* infection have demonstrated

that strains B1, B2, B4, and B8 exhibit a degree of resistance to fungal attacks (Keerthana, 2018) ^[5].

Materials and Methods

Silkworm breeds used in the experiment

Five breeds B1, B4, B8, CSR4 and Pure Mysore and three hybrids B1×CSR4, B4×CSR4 and B8×CSR4 were used. The breeds and hybrids characteristics are given in the Table 1. These breeds and hybrids were reared in two sets with three replications, one which is control and other set under muscardine inoculation by following appropriate rearing practices (Dandin and Giridar, 2014) ^[6].

Table 1: Larval and cocoon parameters of the thermotolerant breeds and hybrids used in the experiment

Sl. No.	Breed/Hybrid	Breed Characters
1.	B1	Plain larvae spinning oval shaped cocoons
2.	B4	Plain larvae spinning oval shaped cocoons
3.	B8	Marked larvae spinning peanut shaped cocoons
4.	CSR4	Plain larvae spinning peanut shaped cocoons
5.	PURE MYSORE	Plain larvae spinning spindle shaped cocoons
6.	B1×CSR4	Plain larvae spinning oval shaped cocoons
7.	B4×CSR4	Plain larvae spinning oval shaped cocoons
8.	B8×CSR4	Marked larvae spinning peanut shaped cocoons

Appropriate dilutions were done so as to make the final concentration to 90,400 spores/ml, which was based on earlier work done in the department with same breeds (Keerthana, 2018) ^[5]. The fifth instar silkworms immediately after fourth moult were topically infected with the above mentioned concentration at the rate of 0.5 ml per silkworm by spraying uniformly with an atomizer. High relative humidity of 95 ± 5 percent and a temperature of 25 ± 1 °C were maintained in the rearing room. White muscardine incidence was recorded up to ten days post inoculation. Untreated worms of fifth instar were taken as the control.

Observations like fifth instar larval duration (h), fifth instar larval weight (g), single cocoon weight (g), larval mortality (%), pupation percentage, effective rate of rearing (ERR), pupal weight (g), cocoon shell weight (g), cocoon shell ratio (%), cocoon yield by number and weight and filament length were recorded.

$$\text{Larval mortality (\%)} = \frac{\text{Number of dead larvae in a batch}}{\text{Total number of treated larvae in the batch}} \times 100$$

$$\text{ERR (\%)} = \frac{\text{Number of cocoon harvested}}{\text{Total number of worms treated}} \times 100$$

$$\text{Cocoon shell ratio (\%)} = \left[\frac{\text{Weight of cocoon shell}}{\text{Weight of whole cocoon}} \right] \times 100$$

Filament length (m)

A sample of five cocoons per replication was randomly drawn and stifled in a hot air oven at 70° C for three hours. The cocoons were kept in open for three hours to remove the moisture content and were dried under shade. The cocoons were cooked individually in boiling water for three minutes to soften the sericin layer. These cooked cocoons were reeled on an eprouvette. Length of the silk filament was determined from the number of revolutions recorded and converted into meters by the formula,

$$L = R \times 1.125 \text{ m}$$

Where,

L = Length of the silk filament (m)

R = Number of revolutions

1.125 m = Circumference of the eprouvette.

Statistical analysis

The data obtained were analysed using completely randomized block design (Sundarraj *et al.*, 1972) ^[7]. The mean values of the experiments were compared by using Duncan's Multiple Range Test (DMRT) (Duncan, 1955) ^[8] and presented below.

Results and Discussion

Fifth instar larval duration (h)

The larval duration was determined from first day of fifth instar till spinning. Significant differences were observed among the breeds under control as well as under *B. bassiana* inoculation. Fifth instar larval duration varied significantly among the parents, CSR4 breed has shown highest fifth instar larval duration of 197.52 h under control conditions, which was followed by Pure Mysore and B8 breeds with (195.64 h and 194.32 h respectively). B4 with 190.40 h has shown the lowest fifth instar larval duration among the parental breeds, which was followed by B1 (193.60 h). Among the hybrids, B4×CSR4 with 180.88 h has shown highest fifth instar larval duration, followed by B1×CSR4 and B8×CSR4 (178.64 h and 173.20 h, respectively).

In the fungal infected batch, CSR4 breed among the parents has shown highest fifth instar larval duration (213.76 h), which was followed by Pure Mysore and B1 (209.04 h and 207.84 h respectively). Least fifth instar larval duration was recorded in B4 (197.76h), which was followed by B8 (203.44 h). Highest percent increase in fifth instar larval duration was observed in CSR4 breed (8.22%) and the least increase in fifth instar larval duration was observed in B4 breed (3.87%) over their respective controls. Among hybrids, B4×CSR4 has showed highest fifth instar larval duration of 192.32 h, which was followed by B8×CSR4 and B1×CSR4 (183.92 h and 182.64 h, respectively). Highest increase in fifth instar larval duration due to fungal infection

was observed in B4×CSR4 (6.32%). Lowest increase in fifth instar larval duration was recorded in B1×CSR4 hybrid (2.24%) (Table 2).

Four thermotolerant silkworm breeds (B1, B4, B6 and B8) and their hybrids were inoculated with different dilutions (10^{-2} , 10^{-4} , 10^{-6} , 10^{-8}) of *B. bassiana* wherein B6 (10.58 days) breed, B6 X B1 and B6 X B8 (10.50 days each) hybrids showed prolonged larval duration over all the dilutions compared to control (Jayashree *et al.*, 2020) [1]. Results of the current study are also in conformity with the findings of Manjunath Gowda *et al.* (2011) [9] under *BmNPV* infection and Keerthana *et al.* (2019) [10] and Sahana *et al.* (2021) [11] under the muscardine infections.

Fifth instar larval weight (g/10 larvae)

Fifth instar larval weight varied significantly among the parents, B1 breed has shown highest larval weight of 37.92 g/10 larvae under control batch, which was followed by B4, CSR4 breeds with (36.40 g/10 larvae and 31.82 g/10 larvae respectively). Pure Mysore with 19.55 g/10 larvae has shown the lowest larval weight among the parental breeds, which was followed by B8 (28.04 g/10 larvae). Among the hybrids under control condition, B1×CSR4 with 40.35 g/10 larvae has shown highest larval weight, followed by B4×CSR4 and B8×CSR4 (39.22 g/10 larvae and 32.10 g/10 larvae, respectively).

In fungal infected batch, B4 and B1 are on par with each other with larval weight of 27.53 g/10 larvae and 27.26 g/10 larvae, respectively. Least larval weight was recorded by PM (17.00 g/10 larvae), which was followed by CSR4 and B8 (19.35 g/10 larvae and 21.25 g/10 larvae). Least percent reduction was observed in Pure Mysore breed (13.04%). Highest reduction in larval weight was observed in CSR4 breed (39.19%) and the over their respective controls. Among the hybrids, B4×CSR4 has showed highest larval weight of 32.92 g/10 larvae, which was followed by B1×CSR4 and B8×CSR4 (31.77 g/10 larvae and 24.24 g/10 larvae, respectively). Least reduction in larval weight due to fungal infection was recorded in B4×CSR4 hybrid (16.06%), while highest reduction in larval weight was observed in B8×CSR4 hybrid (24.48%) (Table 2).

According to studies of Srijeeth (2019) [12] B2, B3, B1 and B5 breeds showed higher fifth instar larval weight under fungal pathogen stress. In earlier studies B4, B2 and B1 exhibited highest larval weight (21.35 g/10 larvae, 20.78

g/10 larvae and 20.50 g/10 larvae, respectively) under *B. bassiana* inoculation (Keerthana *et al.*, 2020) [13] which supports the present findings. Jayashree *et al.* (2020) [1] recorded among parents the tolerant breed B8 lost its body weight from 42.18 g (control) to 26.58 g (fungal infection) and among hybrids B1×B8 lose its body weight from 40.19 g (control) to 30.78 g (fungal infection), which was the least reduction in larval body weight.

Single cocoon weight (g)

Significant differences were observed among the thermotolerant silkworm breeds and their hybrids with CSR4 breed for single cocoon weight in both control and *B. bassiana* inoculated batches. In the control batch, among the breeds B1 has shown highest single cocoon weight of 1.89 g, which was followed by B4 and CSR4 (1.82 g and 1.75 g, respectively). Lowest single cocoon weight was recorded in Pure Mysore with 1.31 g, which was followed by B8 with 1.48 g. Among the hybrids, B1×CSR4 has shown highest single cocoon weight (2.01 g) under control, which was followed by B4×CSR4 and B8×CSR4 hybrids (1.95 g and 1.56 g, respectively).

In fungal inoculation batch, B4 breed has shown highest single cocoon weight of 1.00 g, which was followed by Pure Mysore and B1 (1.06 g and 0.97 g, respectively). Least single cocoon weight was recorded in B8 (0.83 g). Highest percent reduction in cocoon weight due to fungal infection over control was observed in CSR4 breed (58.28%) and it was lowest in Pure Mysore breed (19.08%). Among the hybrids, B4×CSR4 hybrid recorded highest single cocoon weight of 1.41g, which was followed by B1×CSR4 and B8×CSR4 hybrids (1.27 g and 0.89 g, respectively). Least percent reduction in cocoon weight due to fungal infection over control was observed in B4×CSR4 hybrid (27.65%), while highest percent reduction in cocoon weight was observed in B8×CSR4 hybrid (42.95%) (Table 2).

The results of Raghavaiah (1986) [14] indicated that, NB₇ breed spun cocoons with maximum cocoon weight (1.027 g) compared to NB₁₈ (0.940 g) when infected with muscardine fungus. Keerthana *et al.* (2020) [13] have also recorded maximum cocoon weight of 1.16 g, 1.06 g and 1.05 g in B4, B1 and B8 breeds, respectively under *B. bassiana* inoculation which supports the present finding with respect to the performance of B4 and B1 breeds.

Table 2: Fifth instar larval duration (h), fifth instar larval weight (g) and Single Cocoon weight (g) as affected by muscardine inoculation among selected thermotolerant bivoltine silkworm breeds and hybrids

Breeds and hybrids		Fifth instar larval duration (h)		Fifth instar larval weight (g)		Single Cocoon weight (g)	
		Control	Muscardine inoculation	Control	Muscardine inoculation	Control	Muscardine inoculation
Breeds	Pure Mysore	195.64 ^a	0.98 ^g	0.98 ^g	17.00 ^e	1.31 ^f	1.06 ^{cd}
	CSR4	197.52 ^a	1.43 ^c	1.43 ^c	19.35 ^f	1.75 ^d	0.73 ^f
	B1	193.60 ^{ab}	1.38 ^d	1.38 ^d	27.26 ^c	1.89 ^{bc}	0.97 ^{cd}
	B4	190.40 ^b	1.20 ^e	1.20 ^e	27.53 ^c	1.82 ^{cd}	1.00 ^c
	B8	194.32 ^{ab}	1.43 ^c	1.43 ^c	21.25 ^e	1.48 ^e	0.83 ^e
Hybrids	B1×CSR4	178.64 ^c	1.56 ^a	1.56 ^a	31.77 ^b	2.01 ^a	1.27 ^b
	B4×CSR4	180.88 ^c	1.50 ^b	1.50 ^b	32.92 ^a	1.95 ^{ab}	1.41 ^a
	B8×CSR4	173.20 ^d	1.10 ^f	1.10 ^f	24.24 ^d	1.56 ^e	0.89 ^d
F test		*	*	*	*	*	*
S.Em ±		1.32	2.155	0.992	0.895	0.03	0.022
CD @5%		3.99	6.516	2.788	2.707	0.09	0.067
CV (%)		1.216	1.877	4.814	6.162	3.004	3.956

Figures with same superscript are statistically on par; *-Significant @ 5%

Larval mortality (%)

There was no larval mortality among both the thermotolerant breeds and hybrids with CSR4 along with Pure Mysore in the control batch. In the fungal infected batch, CSR4 breed has recorded maximum larval mortality of 62.68 percent, which was followed by B8 and B1 (58.57% and 51.42%, respectively). Least larval mortality was recorded in Pure Mysore *i.e.*, 4.35 percent, which was followed by B4 (44.63%).

In *B. bassiana* inoculated batch, among the hybrids B8×CSR4 has shown highest larval mortality of 42.56

percent. Least larval mortality was recorded in B4×CSR4 with 27.35 percent, while B1×CSR4 hybrid has recorded 32.84 percent larval mortality (Fig 1)

Sreejith (2019) ^[12], studied that when eight thermotolerant bivoltine silkworm breeds *i.e.*, B1, B2, B3, B4, B5, B6, B7 & B8 were inoculated with *B. bassiana*, maximum larval mortality was recorded in the breed B3 (75%), followed by B8 (72%), B5 and B6 (70% each) and B7 (67%) and minimum larval mortality was recorded by breed B1 (41%) and B2 (57%).

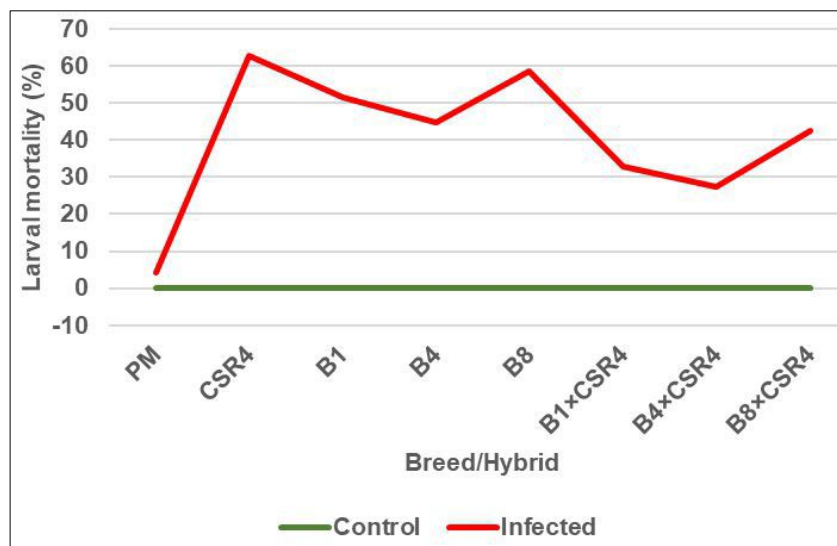


Fig 1: Larval mortality (%) among thermotolerant bivoltine breeds and hybrids

Pupation rate (%)

Pupation percentage was not affected under control, as cent percent pupation was observed in all the breeds and hybrids studied.

In *B. bassiana* inoculated batch, Pure Mysore breed recorded highest pupation percentage of 98.12, which was followed by B1 and B4 breeds (65.21% and 62.15% respectively). CSR4 breed has shown lowest pupation percentage of 52.31, which was followed by B8 breed (57.06%). Highest percent reduction in pupation rate over their respective controls was seen in CSR4 breed (47.69%) and lowest percent reduction was seen in Pure Mysore breed (2.00%). Among the hybrids, B4×CSR4 hybrid has recorded

higher pupation percentage of 77.35, which was followed by B1×CSR4 hybrid (73.22%). B8×CSR4 hybrid has shown lower pupal weight of 68.16 among the hybrids. Highest percent reduction in pupation rate over control was observed in B8×CSR4 hybrid (31.84%) and it was lowest in B4×CSR4 hybrid (22.65%) (Fig 2).

According to Jayashree (2019) ^[15], B4 breed and its hybrids showed highest pupation rate under muscardine inoculation. In the present study also, same trend was observed in B4 breed and B4×CSR4 hybrid had exhibited higher pupation rate. Studies of Sahana *et al.* (2021) ^[11] revealed that Pure Mysore and B1 breeds recorded cent percent pupation followed by B8 (93.33%) and B2 (86.67%) breeds.

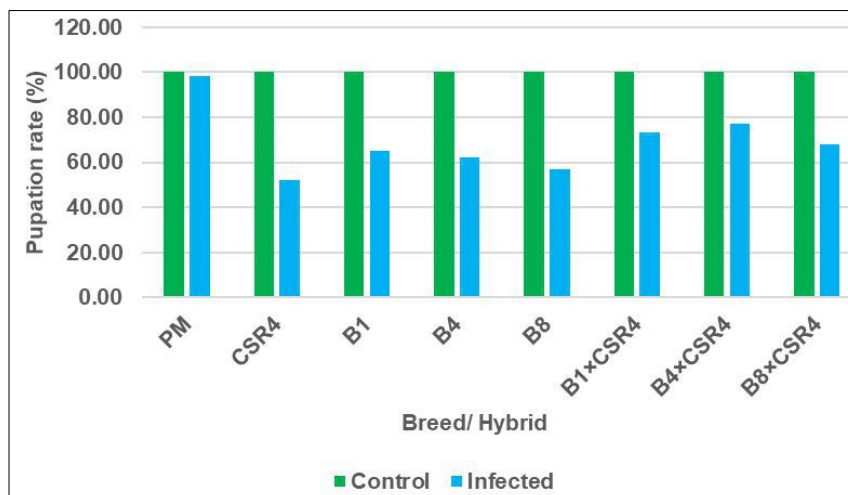


Fig 2: Pupation rate (%) among thermotolerant bivoltine breeds and hybrids

Effective rate of rearing (%)

There was a significant difference in effective rate of rearing due to the infection of *B. bassiana* but not in untreated ones. All the breeds and hybrids in control batch recorded 100 percent ERR.

But, in *B. bassiana* inoculated batch, ERR showed significant difference among silkworm breeds inoculated with fungal spores. Highest ERR was recorded in Pure Mysore breed with 95.65 (%), followed by B4 and B1 breeds (55.37% and 48.58% respectively). Least ERR was exhibited by CSR4 breed with 37.32 percent, which was followed by B8 (41.43%). Least percent reduction in ERR was due to fungal infection in Pure Mysore (4.35%) and higher percent reduction in ERR was in CSR4 breed (62.68%) over their respective controls. Among the hybrids, B4×CSR4 hybrid exhibited higher ERR of 72.65 percent,

which was followed by B1×CSR4 hybrid (67.16%). Least ERR percentage was exhibited by B8×CSR4 hybrid with 57.45 percent. Least percent reduction in ERR due to fungal infection was seen in B4×CSR4 (27.35%) and higher percent reduction in ERR was seen in B8×CSR4 breed (42.56%) over their respective controls (Fig 3).

Previously, when eight races of silkworms viz., Pure Mysore, Hosa Mysore II, *C. nichii*, HS6, NN6D, NB4D₂, KA and J122 were inoculated with nine conidial concentrations (10^1 - 10^9 spores/ml) of *B. bassiana*, variation in ERR over spore concentration and between the breeds was observed (Venkataramana Reddy, 1978) [16]. Similarly, Sahana *et al.* (2021) [11] were found that, in *B. bassiana* inoculated batch, cent percent ERR was recorded in Pure Mysore, followed by B2 (84.00%), B1 (77.33%) and B4 (74.00%).

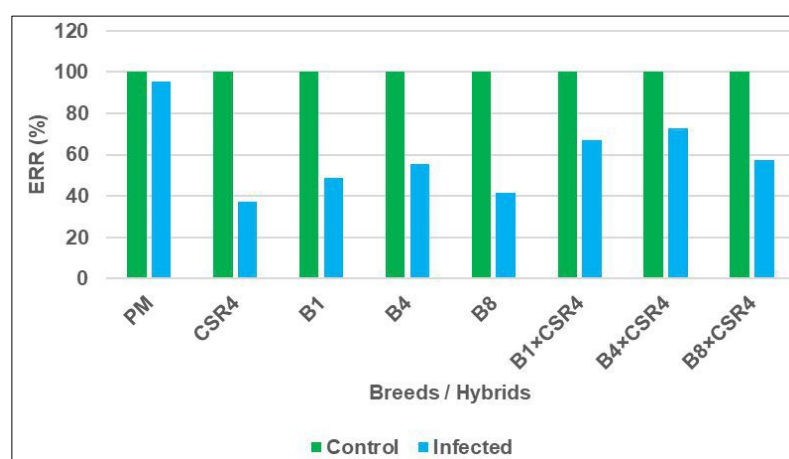


Fig 3: Effective rate of rearing (%) among thermotolerant bivoltine breeds and hybrids

Pupal weight (g)

In control batch, among the breeds, B1 and CSR4 has shown highest pupal weight of 1.43 g, which was followed by B4 (1.38 g). Pure Mysore has shown the lowest pupal weight of 0.98 g, which was followed by B8 (1.20 g). Among the hybrids, B1×CSR4 hybrid has shown highest pupal weight with 1.56 g, which was followed by B4×CSR4 hybrid (1.50 g). Least pupal weight was recorded in B8×CSR4 hybrid (1.10 g).

In *B. bassiana* inoculated batch, Pure Mysore breed has recorded highest pupal weight of 0.80 g, which was followed by B4 and B1 breeds (0.74 g and 0.65 g respectively). CSR4 breed has shown lowest pupal weight of 0.58 g, which was followed by B8 breed (0.63 g). Least percent reduction in pupal weight due to fungal infection over their respective controls was seen in Pure Mysore (18.36%), while highest percent reduction in pupal weight was seen in CSR4 breed (59.44%). B4×CSR4 hybrid has recorded higher pupal weight of 1.09 g, which was followed by B1×CSR4 hybrid (0.94 g). B8×CSR4 hybrid has shown lower pupal weight of 0.67 g among the hybrids. Least percent reduction in pupal weight due to fungal infection over control was observed in B4×CSR4 hybrid (27.33%) and it was highest in B1×CSR4 hybrid (39.74%) (Table 3).

Reduction in pupal weight in cross breed (PM X CSR2) silkworm was observed when treated with sub-lethal concentration of *B. bassiana* conidial suspension (Rajitha and Savithri, 2015) [17]. The present findings were also supported by the results of Keerthana *et al.* (2020) [13],

wherein B4 breed recorded maximum pupal weight of 0.92 g followed by B1, B6, B7 and B8 breeds (0.87 g each).

Cocoon shell weight (g)

In control batch, among the breeds, B1 recorded highest shell weight of 0.45 g, which was followed by B4 and CSR4 breeds (0.43 g and 0.38 g respectively). Pure Mysore has shown the lowest shell weight of 0.14 g, which was followed by B8 (0.34 g). Among the hybrids, B1×CSR4 hybrid has recorded highest shell weight with 0.47 g, which was followed by B4×CSR4 hybrid (0.46 g). Least shell weight was recorded in B8×CSR4 hybrid (0.30 g).

In *B. bassiana* inoculated batch, B1 and B4 breeds have recorded highest shell weight of 0.23 g, which was followed by B8 breed (0.19 g). Pure Mysore breed has shown lowest shell weight of 0.11 g, which was followed by CSR4 breed (0.16 g). Highest percent reduction over their respective controls in shell weight was seen in CSR4 breed (57.89%) and lowest percent reduction was seen in Pure Mysore (21.43%). Among the hybrids, B4×CSR4 hybrid has recorded higher shell weight of 0.33 g, which was followed by B1×CSR4 hybrid (0.28 g). B8×CSR4 hybrid showed lower shell weight of 0.22 g among the hybrids. Highest percent reduction in shell weight over control was observed in B1×CSR4 hybrid (40.42%) and it was lowest in B4×CSR4 hybrid (28.26%) over their respective controls (Table 3).

Topical application of *B. bassiana* spores to ten thermotolerant silkworm breeds recorded the highest cocoon shell weight in B4 (0.24 g) and B1 (0.19 g) breeds

(Keerthana *et al.*, 2020) ^[13]. Similarly, Sreejith (2019) ^[12] recorded significantly maximum shell weight in breed B4 (0.24), followed by B1 (0.18), B5 (0.17) and B2 (0.16), while Sahana *et al.* (2021) recorded maximum shell weight in B4 (0.24 g) and B1(0.21 g) breeds.

In the present study, B4 breed and B4×CSR4 hybrid was found to yield higher shell weight under fungal inoculation.

Cocoon shell ratio (%)

In control batch, among the breeds B4 breed has shown highest shell ratio of 24.31 percent, which was followed by B1 and CSR4 breeds (23.46% and 21.64% respectively). Pure Mysore has showed the lowest shell ratio of 12.36 percent among the breeds, which was followed by B8 (21.34%). Among the hybrids, B4×CSR4 and B1×CSR4 hybrids have shown shell ratio percentage (23.12% and 22.98%), which are on par with each other. Least shell ratio was recorded by B8×CSR4 (20.66%) hybrid.

In *B. bassiana* inoculated batch, B4 breed has recorded highest shell ratio of 21.86 percent, which was followed by B1 (20.43%). Pure Mysore breed has shown lowest shell ratio of 11.53 percent, which was followed by B8 and CSR4

breeds (16.02% and 13.92%). Highest percent reduction in shell weight over the respective controls was seen in CSR4 breed (35.56%) and lowest percent reduction in shell weight was seen in Pure Mysore breed (6.72%). Among the hybrids, B4×CSR4 hybrid has recorded higher shell ratio of 24.63 percent, which was followed by B1×CSR4 hybrid (23.35%). B8×CSR4 hybrid has shown lower shell ratio of 19.23 percent among the hybrids. Highest percent increase in shell ratio over control was observed in B4×CSR4 hybrid (6.54%) due to decrease in pupal weight under inoculated conditions. It was lowest in B1×CSR4 hybrid (1.62%) over its control (Table 3).

In earlier studies, NB₄D₂ produced highest shell ratio compared to NB₇, KA and NB₁₈ (Venkataramana Reddy, 1978) ^[16] and crossbreed, PM X CSR₂ recorded reduced cocoon shell ratio of under *B. bassiana* inoculation compared to control (Seema *et al.*, 2019) ^[18]. According to Keerthana (2018), among the ten thermotolerant bivoltine breeds (B1 to B8, APS12 and APS45), B4 scored significantly highest shell ratio under *B. bassiana* infection (20.71%) while, B3 recorded highest shell percentage under high temperature treatment (21.83%).

Table 3: Cocoon parameters Pupal weight (g), shell weight (g) and shell ratio (%) as affected by muscardine inoculation among selected thermotolerant bivoltine silkworm breeds and hybrids

Breeds and hybrids		Pupal weight (g)		Shell weight (g)		Shell ratio (%)	
		Control	Muscardine inoculation	Control	Muscardine inoculation	Control	Muscardine inoculation
Breeds	Pure Mysore	0.98 ^g	0.80 ^g	0.14 ^{de}	0.11 ^f	12.36 ^e	11.53 ^e
	CSR4	1.43 ^c	0.58 ^c	0.38 ^c	0.16 ^e	21.64 ^{bcd}	13.92 ^{de}
	B1	1.43 ^c	0.65 ^{ef}	0.45 ^a	0.23 ^c	23.46 ^{ab}	20.43 ^{bc}
	B4	1.38 ^d	0.74 ^d	0.43 ^b	0.23 ^c	24.31 ^a	21.86 ^{abc}
	B8	1.20 ^e	0.63 ^f	0.34 ^d	0.19 ^d	21.34 ^{cd}	16.02 ^d
Hybrids	B1×CSR4	1.56 ^a	0.94 ^b	0.47 ^a	0.28 ^b	22.98 ^{abc}	23.35 ^{ab}
	B4×CSR4	1.50 ^b	1.09 ^a	0.46 ^a	0.33 ^a	23.12 ^{abc}	24.63 ^a
	B8×CSR4	1.10 ^f	0.67 ^e	0.30 ^e	0.22 ^{cd}	20.66 ^d	19.23 ^c
F test		*	*	*	*	*	*
S.Em ±		0.014	0.01	0.007	0.008	0.579	0.935
CD @ 5%		0.042	0.031	0.023	0.026	1.751	2.826
CV (%)		1.843	2.46	3.47	6.755	4.723	8.578

Figures with same superscript are statistically on par; *-Significant @5%

Cocoon yield by number (No./10,000 larvae)

In the controlled batch of thermotolerant breeds and their hybrids with CSR4 along with Pure Mysore has shown no difference in cocoon yield by number.

In *B. bassiana* inoculated batch, cocoon yield by number showed significant difference among silkworm breeds. Highest cocoon yield by number was recorded in Pure Mysore breed with 9565.00/10,000 larvae, followed by B4 and B1 breeds (5537.00/10,000 larvae and 4858.00/10,000 larvae, respectively). Least cocoon yield by number was exhibited by CSR4 breed with 3732.00/10,000 larvae, which was followed by B8 (4143.00/10,000 larvae). Least percent reduction in cocoon yield by number due to fungal infection was seen in Pure Mysore (4.35%) and higher percent reduction in cocoon yield by number was seen in CSR4 breed (62.68%) over their respective controls. Among the hybrids, B4×CSR4 hybrid exhibited higher cocoon yield by number with 7265.00/10,000 larvae, which was followed by B1×CSR4 hybrid (6716.00/10,000 larvae). Least cocoon yield by number was in B8×CSR4 hybrid with 5744.00/10,000 larvae (Table 3).

Keerthana *et al.*, (2020) ^[13] recorded significantly highest number of cocoons in breed B4 (620/1,000 worms) followed by B1 (500.00/1,000 worms) under *B. bassiana* inoculation. The present findings are in line with the above findings where the hybrids of B1×CSR4 and B4×CSR4 recorded maximum cocoon yield by number than any other silkworm breeds and hybrids under *B. bassiana* infected condition. Jayashree (2019) ^[15] recorded that in the study of thermotolerant breeds and hybrids, among parents B8 (613.33/1000 worms) and among hybrids B1 × B8 (660.00/1000 worms) recorded significantly highest cocoon yield by number at 10⁻⁸ spore concentration and no cocoons were formed at stock in B6, B6 × B1 and B6 × B4.

In the present study, Pure Mysore breed alone could perform better with respect to cocoon yield by number under both control and *B. bassiana* inoculation conditions. Among hybrids B4×CSR4 and B1×CSR4 performed better under control and fungal inoculated conditions.

Cocoon yield by weight (kg/10,000 larvae)

In this study, cocoon yield by weight was significantly affected among thermotolerant breeds and hybrids with

CSR4 along with Pure Mysore due to the treatment of *B. bassiana* compared to that of untreated ones.

Under control conditions, cocoon yield by weight varied significantly among the parents with B1 breed recording highest cocoon yield of 18.93 kg/10,000 larvae, which was followed by B4 and CSR4 breeds with 18.20 kg/10,000 larvae and 17.50 kg/10,000 larvae, respectively. Pure Mysore with 13.13 kg/10,000 larvae recorded the least cocoon yield by weight among the parental breeds, which was followed by B8 (14.83 kg/10,000 larvae). Among the hybrids under control condition, B1×CSR4 with 20.10 kg/10,000 larvae has shown highest cocoon yield, followed by B4×CSR4 and B8×CSR4 (19.53 kg/10,000 larvae and 15.60 kg/10,000 larvae, respectively).

In the fungal infected batch, Pure Mysore breed has shown highest cocoon yield by weight with 8.55 kg/10,000 larvae among breeds, which was followed by B4 and B1 with cocoon yield of 5.54 kg/10,000 larvae and 4.71 kg/10,000 larvae respectively, and both were on par. Least cocoon yield was recorded by CSR4 (2.74 kg/10,000 larvae), which was followed by B8 (3.46 kg/10,000 larvae). Least percent reduction in cocoon weight due to fungal infection was observed in Pure Mysore breed (34.88%) and highest reduction in cocoon yield by weight was observed in CSR4 breed (84.34%) over their respective controls.

Among the hybrids, B4×CSR4 has shown highest cocoon yield of 9.21 kg/10,000 larvae, which was followed by B1×CSR4 and B8×CSR4 (8.13 kg/10,000 larvae and 7.09 kg/10,000 larvae, respectively). Least reduction in cocoon yield due to fungal infection was recorded with B4×CSR4 hybrid (52.84%). Highest reduction in cocoon yield by weight was observed in B8×CSR4 (54.55%) (Table 3).

According to Sreejith (2019) ^[12], significantly highest cocoon yield was recorded in B4 (1007.50 g/1000 worms), followed by B1 (971.00 g/1000 worms) under *B. bassiana* infection. Sahana *et al.* (2021) ^[11] in their study of thermotolerant breeds performance under muscardine infection among 10 breeds, B4 breed was observed to be better performer under both high temperature treatment and *B. bassiana* inoculation with respect to cocoon yield by weight.

In present study B1 and B4 and its hybrids has performed better under both control and fungal stress with respect to cocoon yield by weight.

Filament length (m)

In control batch, among the breeds, B1 has shown longest filament length of 1113.76 m, which was followed by B4 and CSR4 (1094.01 m and 1012.59 m, respectively). Pure Mysore has shown the shortest filament length of 554.87 m among the breeds, which was followed by B8 (910.13 m). Among the hybrids B1×CSR4 and B4×CSR4 have shown longest filament length with 1262.44 m and 1211.36 m, which were found to be statistically on par with each other. Shortest filament length was exhibited by B8×CSR4 hybrid (976.13 m).

In *B. bassiana* inoculated batch, B4 breed has recorded longest filament length of 668 m, which was followed by B1 and B8 breeds (550.22 m and 455.85 m respectively). CSR4 breed has shown shortest filament length of 295.94 m, which was followed by Pure Mysore (398.98 m). Highest percent reduction in filament length was exhibited by CSR4 (70.77%). Lowest percent reduction in filament length was seen in Pure Mysore (28.09%) over their respective controls. Among the hybrids, B4×CSR4 hybrid has been recorded longest filament length of 870.86 m, which was followed by B1×CSR4 (742.66 m). B8×CSR4 hybrid has shown shortest filament length of 464.21 m among the hybrids. Highest percent reduction in filament length due to fungal infection was observed in B8×CSR4 hybrid (48.99%) and it was lowest in B4×CSR4 hybrid (28.11%), over their respective controls (Table 3).

Under *B. bassiana* inoculation, Keerthana *et al.* (2020) ^[13] recorded significantly longest filament length in B4 breed (695.47 m), followed by B8 (657.47 m) and B7 (629.33 m) breeds and significantly lowest filament length was noticed in B2 breed (480.00 m). Jayashree *et al.* (2020) ^[11] observed that, filament length was significantly reduced from 1015.50 m in control to 768.94 m at 10⁻⁸ dilution, to 726.54 m at 10⁻⁶ dilution, to 643.10 m at 10⁻⁴ dilution, to 512.79 m at 10⁻² dilution and to 493.22 m at stock concentration of fungal spore suspension. The findings of Sahana *et al.* (2021) ^[11] revealed highest value for average filament length in B4 breed under *B. bassiana* infected condition.

Table 4: Cocoon yield by number (per 10,000 worms), weight (kg/10,000 worms) and filament length (m) as affected by muscardine inoculation among selected thermotolerant bivoltine silkworm breeds and hybrids.

Breeds and hybrids		Cocoon yield by no. (per 10,000 worms)		Cocoon yield by weight (kg/10,000 worms)		Filament length (m)	
		Control	Muscardine inoculation	Control	Muscardine inoculation	Control	Muscardine inoculation
Breeds	Pure Mysore	10000.00	9,565.00	13.13 ^f	8.55 ^{ab}	554.87 ^f	398.98 ^f
	CSR4	10000.00	3,732.00	17.50 ^d	2.74 ^e	1113.76 ^b	550.22 ^d
	B1	10000.00	4,858.00	18.93 ^{bc}	4.71 ^d	1094.01 ^{bc}	668.65 ^c
	B4	10000.00	5,537.00	18.20 ^{cd}	5.54 ^d	910.13 ^e	455.85 ^{ef}
	B8	10000.00	4,143.00	14.83 ^e	3.46 ^e	1012.59 ^{cd}	295.94 ^g
Hybrids	B1×CSR4	10000.00	6,716.00	20.10 ^a	8.13 ^b	1262.44 ^a	742.66 ^b
	B4×CSR4	10000.00	7,265.00	19.53 ^{ab}	9.21 ^a	1211.36 ^a	870.86 ^a
	B8×CSR4	10000.00	5,744.00	15.60 ^e	7.09 ^c	976.13 ^{de}	464.21 ^e
F test		NA	*	*	*	*	*
S.Em ±		-	141.799	0.299	0.311	31.238	20.022
CD @5%		-	428.772	0.904	0.942	94.456	60.542
CV (%)		-	4.131	3.004	8.732	5.32	6.238

Figures with same superscript are statistically on par; *-Significant @5%; NA- not analysed

Conclusion

The results of the study provide important light on how thermotolerant bivoltine silkworm hybrids and breeds respond to *B. bassiana* infection stress. According to the results, the B4 breed and its hybrid, B4×CSR4, are more resistant under disease condition. Together with resilience in terms of pupation rate, weight of a single cocoon, and larval weight, these strains outperformed other breeds and hybrids in terms of effective rate of rearing, or ERR.

Overall, the B4 breed and B4×CSR4 hybrid stand out for their ability to sustain better economic traits and survival rates under fungal stress. Future breeding programs could focus on enhancing the disease resistance of bivoltine silkworms by incorporating the traits observed in B4 and B4×CSR4, aiming to achieve both higher silk yield and quality under adverse conditions.

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References

- Jayashree, Manjunath Gowda, Narayanaswamy KC, Narayanareddy R. Response of a few thermotolerant bivoltine breeds and their hybrids to *Beauveria bassiana* (Bals.-Criv.) Vuill. infection in terms of yield and economic parameters of cocoon. J Entomol Zool Stud. 2020;8(3):1367-1373.
- Janakiraman AT. Disease affecting the Indian silkworm races. Indian J Seric. 1961;13:91-101.
- Chandrasekharan K, Nataraju B. Studies on white muscardine disease of mulberry silkworm *Bombyx mori* L. in India. Indian J Seric. 2008;47(2):136-154.
- Lakshmi H, Chandrashekharaiyah, Babu MR, Raju PJ, Saha AK, Bajpai AK. HTO5 × HTP5, the new bivoltine silkworm (*Bombyx mori* L.) hybrid with thermotolerance for tropical areas. Int J Plant Anim Environ Sci. 2011;1(2):88-104.
- Keerthana A. Studies on response of thermotolerant bivoltine silkworm breeds to white muscardine infection. M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Bangalore, India. 2018;1-96.
- Dandin SB, Giridhar K. Handbook of sericulture technologies. CSB Publications; c2010. p. 13.
- Sundararaj N, Nagaraju S, Venkataramu MN, Jagannath MK. Designs of analysis of field experiments. University of Agricultural Sciences, Bangalore; c1972. p. 424.
- Duncan DB. Multiple range test and multiple 'F' test. Biometrics. 1955;11:1-42.
- Manjunath Gowda, Narayanaswamy KC, Shailaja Hittalamani. Genetics of tolerance to BmNPV infections among some popular breeds of the silkworm *Bombyx mori* L. Indian J Ecol. 2011;38(2):242-252.
- Keerthana A, Gowda M, Narayanaswamy KC, Amaranatha N. Performance of thermotolerant bivoltine silkworm breeds for larval growth and cocoon yield parameters under *Beauveria bassiana* infection. Mysore J Agric Sci. 2019;53(1):19-26.
- Sahana KP, Manjunath Gowda, Narayanaswamy KC, Chandrashekhar S. Response of identified thermotolerant bivoltine silkworm breeds for *Beauveria bassiana* (Bals.-Criv.) Vuill. infection: A source for thermal and fungal dual stress resistance. Mysore J Agric Sci. 2021;55(3):59-68.
- Sreejith V. Biochemical changes in thermotolerant bivoltine breeds during high temperature treatment and muscardine disease. M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Bangalore, India. 2019;1-154.
- Keerthana A, Manjunath Gowda, Narayanaswamy KC, Amaranatha N. Some thermotolerant bivoltine silkworm breeds tolerate white muscardine diseases caused by *Beauveria bassiana* (Bals.-Criv.) Vuill. infection. Int J Chem Stud. 2020;8(4):86-94.
- Raghavaiah G. Studies on the eco-pathology and management of the white muscardine disease of the silkworm, *Bombyx mori* L. caused by the entomogenous fungus *Beauveria bassiana* (Bals.) Vuill. Ph.D. Thesis, University of Agricultural Sciences, Bangalore, India. c1986; p. 134.
- Jayashree. Studies on the response of thermotolerant bivoltine hybrids of *Bombyx mori* L. to *Beauveria bassiana* (Bals.-Criv.) Vuill. infection. M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Bangalore, India. c2019;1-131.
- Venkataramana Reddy MR. Studies on the white muscardine disease of silkworm, *Bombyx mori* L. M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Bangalore, India. c1978;1-89.
- Rajitha K, Savithri G. Studies on symptomological and economic parameters of silk cocoons of *Bombyx mori* inoculated with *Beauveria bassiana* (Bals.) Vuill. Int J Curr Microbiol Appl Sci. 2015;4(2):44-54.
- Seema KD, Priti MG, Shubhangi SP, Vitthalrao BK. The influence of infection of *Beauveria bassiana* (Bals.) Vuill, a fungal species (Family: Clavicipitaceae) on quality of the cocoons spun by the larval instars of *Bombyx mori* L. (Race: PM x CSR2). J Bacteriol Mycol. 2019;7(1):14-18.