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Response of Identified Thermotolerant Bivoltine Silkworm Breeds for *Beauveria bassiana* (Bals-Criv.) Vuill. Infection : A Source for Thermal and Fungal Dual Stress Resistance

K. P. Sahana, Manjunath Gowda, K. C. Narayanaswamy and S. Chandrashekhar
Department of Sericulture, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
e-Mail : sahanaprakashuasb11@gmail.com

Abstract

An investigation was conducted using ten different silkworm breeds to assess their performance under two different stress conditions *i.e.,* high temperature treatment (36 ± 1 p C) and *Beauveria bassiana* (Bals-Criv.) Vuill. (LC_{50} 6.86 x 10^4 spore / ml) inoculation. Among the ten, seven (B1, B2, B4, B6, B8, APS12 and APS45) were thermotolerant bivoltine silkworm breeds, two were popular bivoltine (CSR 2 and CSR 4) and one was popular multivoltine (pure Mysore) breed. All the ten breeds were exposed to aforementioned two stress conditions. The results revealed that, the breeds B4, APS45 and B1 performed better under thermal treatment as they exhibited maximum ERR, single cocoon weight, cocoon shell weight, pupal weight and cocoon shell ratio. With respect to *B. bassiana* inoculation, the thermotolerant silkworm breeds B1, B2 and B4 exhibited higher ERR, pupation percentage, single cocoon weight, cocoon shell weight, pupal weight and shell percentage. Further, the breeds B4 and B1 were found to show commonality in exhibiting better survival and quantitative cocoon parameters under both high temperature treatment and *B. bassiana* inoculation. Hence, the breeds B1 and B4 can be used as potential dual stress resistant breeds against high temperature conditions and *B. bassiana* infection.

Keywords : Bivoltine, *Bombyx mori*, Cumulative survival index, Muscardine, Thermotolerance

India occupies the second position in global silk production next only to China. Sericulture in India is being practiced predominantly in tropical regions and to limited extent in temperate region. The existing tropical situation in the country provides scope for the exploitation of multivoltine breeds / hybrids as these breeds show the inherent capacity to perform well under varied and / fluctuating environmental conditions. But the quality of multivoltine silk is low compared to the existing International standards. To meet this standard it is necessary to shift to bivoltine sericulture which assures the production of quantitatively and qualitatively superior cocoons. It is a well established fact that, unlike multivoltine silkworms, bivoltines are more vulnerable to different stresses under tropical condition as these bivoltines have originated from temperate region. It is, therefore, imperative to evolve bivoltine silkworm breeds which can give stable yields under different stress conditions. Keeping this in view, the study was envisaged to know the phenotypic assessment of few thermotolerant bivoltine silkworm breeds under two different stress conditions *i.e.,* high temperature treatment and *Beauveria bassiana* infection. Ten thermotolerant silkworm breeds *viz.,* B1, B2, B3, B4, B5, B6, B8, APS12 and APS45 were evaluated under muscardine infection in our previous study and results revealed that, B4 breed performed better with respect to single cocoon weight, pupal weight, shell weight, cocoon shell ratio, filament length and filament weight, followed by B1 and B8 breeds (Keerthana *et al.,* 2019b).

Material and Methods

Phenotypic performance of ten selected silkworm breeds under high temperature treatment and *Beauveria bassiana* inoculation was studied at the Department of Sericulture, University of Agricultural Sciences, GKVK, Bengaluru during, 2020-2021. Seven thermotolerant silkworm breeds namely, B1, B2, B4, B6 and B8 and APS12 and APS45 were procured from Central Sericultural Research and Training Institute, Mysore and Andhra Pradesh State Sericulture

Research and Development Institute, Hindupur, respectively. Along with these seven breeds, three popular silkworm breeds *viz.,* CSR2 (productive bivoltine), CSR4 (susceptible bivoltine) and Pure Mysore (resistant multivoltine) were also used for investigation. Silkworm rearing was conducted during July to August 2020. Silkworms were reared in bulk up to fourth moult following the standard rearing practices given by Dandin and Giridhar (2014) and V-1 mulberry leaves were fed to silkworm till spinning.

Newly ecdysed fifth instar silkworms (50 silkworms per replication in three replication each) were used to impose the treatment. All the ten breeds were maintained in two sets for two different treatments *i.e.,* high temperature and *B. bassiana* inoculation. One set of all the ten breeds were treated with high temperature *i.e.,* 36 ± 1 p C and 85 ± 5 per cent relative humidity using BOD incubator. The treatment was given for first six days of fifth instar for duration of six hours daily (10.00 to 16.00 hours) (Sudhakara Rao *et al.,* 2006) and the silkworms were fed twice a day using leaf feeding method. Another set of all the breeds were topically inoculated with *B. bassiana* spore suspension with 6.86 x 10^4 spores per ml at the rate of 0.5 ml per silkworm, based on the previous studies conducted at the department (Keerthana *et al.,* 2019a) and silkworms were fed thrice a day using leaf feeding method. Simultaneously, a control batch of all the ten breeds was also maintained.

Observations on fifth instar larval duration, fifth instar larval weight, effective rate of rearing (ERR), per cent pupation, single cocoon weight, cocoon shell weight, cocoon shell ratio and pupal weight were recorded. The data obtained were analysed using completely randomized block design (Sundarraj *et al.,* 1972). Cumulative survival index (CSI) was calculated using the formula :

CSI=[(ERR%/100) x (per cent pupation/100)] x 100

where,

CSI- Cumulative Survival Index

ERR- Effective Rate of Rearing.

Further, the thermotolerant silkworm breeds were ranked utilizing the CSI so obtained and the mean of CSI was employed to identify the superior breeds separately for high temperature treated and *B. bassiana* inoculated batches.

RESULTS AND DISCUSSION

Fifth Instar Larval Duration (hrs)

Significant differences were observed among the breeds utilized for the study under both high temperature treatment and *B. bassiana* inoculation with respect to larval duration. The larval duration was determined from first day of fifth instar till spinning.

In high temperature treated batch, APS45 recorded maximum larval duration of 234.67 hr, followed by B2 (223.67 hr) and it was minimum in B8 (197.00 hr) and APS 12 (201.00 hr). At the treated temperature, B2 breed showed increased larval duration of 2.60 per cent over control, whereas the highest reduction in the same was observed in APS45 (2.63 %) (Table 1). In *B. bassiana* inoculated batch, B4 breed showed maximum larval duration of 219.33 hr, followed by B2 and B1 (217.50 hr each) and the same was found to be minimum in Pure Mysore and APS12 (195.00 hr each) and B8 (197 hr). In the pathogen inoculated batch, increased larval duration was observed in B4 breed (0.61 %) and APS45 breed showed the highest reduction (9.96 %) over control (Table 1). In a similar study, 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).

Fifth instar larval weight (g)

Fifth instar larval weight was affected significantly among all the silkworm breeds due to high temperature treatment and *B. bassiana* inoculation. In thermal treated batch, maximum larval weight was recorded in B2 breed (31.27 g) followed by B4 (30.89 g) and B1 (30.33 g) breeds. The least larval weight was

TABLE 1

Effect of thermal treatment and *B. bassiana* infection on growth and survival of selected thermotolerant bivoltine silkwormbreeds

BREEDS	Larval duration (hrs)			Larval weight (g / 10 larvae)			Effective rate of rearing (%)			Per cent pupation		
	Control	Thermal treated	*B. bassiana* infected	Control	Thermal treated	*B. bassiana* infected	Control	Thermal treated	*B. bassiana* infected	Control	Thermal treated	*B. bassiana* infected
B1	218.50	218.75 (+0.11)	217.50 (-0.46)	36.34	30.33 (-16.54)	18.07 (-50.27)	100.00	83.33 (-16.67)	77.33 (-22.67)	100.00	100.00 (0)	100.00 (0.00)
B2	218.00	223.67 (+2.60)	217.50 (-0.23)	37.03	31.27 (-15.56)	18.59) (-49.81)	100.00	48.00 (-52.00)	84.00 (-16.00)	100.00	100.00 (0)	86.67 (-13.33)
B4	218.00	220.33 (+1.07)	219.33 (+0.61)	34.24	30.89 (-9.79)	16.83 (-50.84)	100.00	90.67 (-9.33)	74.00 (-26.00)	100.00	100.00 (0)	66.67 (-33.33)
B6	217.17	218.75 (+0.73)	215.75 (-0.65)	31.11	24.41 (-21.53)	15.04 (-51.65)	100.00	90.00 (-10.00)	59.33 (-40.67)	100.00	100.00 (0)	56.67 (-43.33)
B8	199.67	197.00 (-1.34)	197.00 (-1.34)	27.12	22.82 (-15.84)	12.92 (-52.36)	100.00	82.22 (-17.78)	64.44 (-35.56)	100.00	100.00 (0)	93.33 (-6.67)
APS12	197.00	201.00 (+2.03)	195.00 (-1.02)	31.83	26.15 (-17.83)	15.50 (-51.31)	100.00	90.00 (-10.00)	48.89 (-51.11)	100.00	100.00 (0)	76.67 (-23.33)
APS45	241.00	234.67 (-2.63)	217.00 (-9.96)	30.42	30.22 (-0.66)	14.56 (-52.13)	100.00	86.67 (-13.33)	54.67 (-45.33)	100.00	100.00 (0)	73.33 (-26.67)
CSR2	218.50	218.75 (+0.11)	216.00 (-1.14)	36.12	29.01 (-19.69)	15.32 (-57.59)	100.00	91.11 (-8.89)	70.83 (-29.17)	100.00	100.00 (0)	66.67 (-33.33)
CSR4	218.75	220.83 (+0.95)	216.00 (-1.26)	29.88	23.08 (-22.76)	10.09 (-66.23)	100.00	85.33 (-14.67)	60.00 (-40.00)	100.00	100.00 (0)	56.67 (-43.33)
Pure Mysore	215.25	215.25 (0.00)	195.00 (-9.41)	19.47	18.89 (-3.00)	16.50 (-15.25)	100.00	100.00 (-0.00)	100.00 (-0.00)	100.00	100.00 (0)	100.00 (0.00)
SEm±	0.87	2.41	0.94	0.57	0.53	0.29	0.00	2.00	4.44	0.00	0.00	3.94
CD at 5%	2.56	7.10	2.78	1.69	1.56	0.86	0.00	5.91	13.08	0.00	0.00	11.63
CV (%)	0.70	1.92	0.78	3.17	3.42	3.28	0	4.09	11.08	0	0	8.80
F-test	*	*	*	*	*	*	NA	*	*	-	-	*

Note :
- ✓ Positive and negative figures in the parenthesis indicate per cent increase (+) or decrease (-) over control, respectively
- ✓ * - Significant at 5 %; NA - Not analysed
- ✓ *B. bassiana* inoculation 6.86 X 10^4 spores per ml @ 0.5 ml per worm; High temperature treatment @ 36±1 °C for 6 hrs per day from 1st to 6th day of fifth instar

recorded in Pure Mysore (18.89 g), followed by B8 (22.82 g) and CSR4 (23.08 g). Highest reduction in larval weight was observed in CSR4 breed (22.76 %) and the least per cent reduction was observed in APS45 breed (0.66 %) over control (Table 1). Reduction in larval weight in high temperature treated silkworms might be attributed to low feeding activity of the silkworms (Pillai and Krishnaswamy, 1980). Our findings were also supported by Keerthana *et al.* (2020) where they recorded highest larval weight in B1 and B4 breeds (38.57 g / 10 silkworms each) after being exposed to high temperature of 36 ± 1 p C.

In *B. bassiana* inoculated batch, B2 breed recorded maximum larval weight of 18.59 g, followed by B1 and B4 breeds (18.07 g and 16.83 g, respectively) and minimum weight was found in CSR4 (10.09 g) and B8 (12.92 g). In the pathogen inoculated batch, highest reduction in larval weight was observed in CSR4 breed (66.23 %), followed by CSR2 breed (57.59 %) with the lowest reduction of 15.25 per cent in Pure Mysore breed over their respective control (Table 1). Decrease in body weight in *B. bassiana* infected silkworms is due to cessation of feeding, decrease in food consumption, digestion, relative consumption rate and efficiency of conversion of ingested food (Venkataramana Reddy, 1978 and Cai, 1989). In earlier studies B4, B2 and B1 exhibited highest larval weight (21.35 g, 20.78 g and 20.50 g, respectively) under *B. bassiana* inoculation (Keerthana *et al.,* 2020) which supports the present findings.

Effective Rate of Rearing (ERR) (%)

In this study ERR was affected significantly due to high temperature treatment and *B. bassiana* treatment.

In high temperature treated batch Pure Mysore showed cent per cent ERR followed by CSR2 (91.11 per cent), B4 (90.67 per cent) and B6 and APS12 breeds (90.00 per cent each). The least ERR was observed in B2 breed (48.00 per cent). Highest reduction of 52.00 per cent ERR was exhibited by B2 breed as against no change in Pure Mysore breed over control (Table 1). In earlier studies, the breeds NB_4D_2, NP2, KSO1, CSR_2 and CSR_4 resulted in cent per cent mortality after being exposed to a threshold temperature of 45 ºC (Vasudha *et al.*, 2006). Pillai and Krishnaswamy (1980) reported that, the low survival rate in the silkworms exposed to high temperature in fifth instar is attributed to low feeding activity of the silkworm resulting in the physiological imbalance and poor health of the larvae. The present findings were also in line with the results of Keerthana *et al.* (2020) where they recorded significantly highest ERR in B4 breed (84.67 %), followed by APS45, B8 and APS12 breeds (81.33, 76.67 and 74.00 per cent, respectively).

In *B. bassiana* inoculated batch, cent per cent ERR was recorded in Pure Mysore, followed by B2 (84 %), B1 (77.33 %) and B4 (74.00 %). Minimum ERR was recorded in APS12 (48.89 %) followed by APS45 (54.67 %), B6 (59.33 %) and CSR4 (60.00 %). APS12 breed showed highest (51.11 %) reduction in ERR, followed by APS45 breed (45.33 %), but no change was observed in Pure Mysore breed over control (Table 1). Previously, when eight races of silkworms *viz.,* Pure Mysore, Hosa Mysore II, C. Nichi, HS6, NN6D, NB_4D_2, KA, 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). Infection of the thermotolerant silkworm breeds with *B. bassiana* resulted in highest ERR in B4 (54.67 %) than B8 (42.67 %) and B1 (40.00 %) breeds (Keerthana *et al.*, 2020). Similarly, the breed B4 performed better with respect to ERR under both high temperature treatment and *B. bassiana* inoculation in our study.

Per cent Pupation

Per cent pupation was not affected due to high temperature treatment, as cent per cent pupation was observed in all the breeds (Table 1). Whereas, in the *B. bassiana* inoculated batch significant differences were observed for the same. Under *B. bassiana* inoculation Pure Mysore and B1 breeds recorded cent per cent pupation followed by B8 (93.33 %) and B2 (86.67 %) breeds. But, the same was observed to be least in CSR4 and B6 breeds (56.67 % each), followed by B4 and CSR2 (66.67 % each). Highest reduction in pupation percentage was observed in CSR4 and B6 breeds (43.33 % each), while no change in pupation percentage in Pure Mysore and B1 breeds over their respective control was observed (Table 1). These results corroborate with the findings of Suresh Kumar *et al*. (2002) who found that, CSR18 and CSR19 thermotolerant bivoltine silkworm breeds resulted in better pupation rate (92.30 & 92.00 %, respectively) than control breeds KA and NB4D2 (76.60 & 88.20 %, respectively). Presence of saturated fatty acids namely, capric acid and caprylic acid in the pupal cuticle were found to show antifungal activity against *B. bassiana* infection (Koidsumi, 1957; Chandrasekharan and Nataraju, 1998). This could be a possible reason to show increased per cent pupation in Pure Mysore, B1, B8 and B2.

Single Cocoon Weight (g)

Significant differences were observed among the silkworm breeds for single cocoon weight in both high temperature treated and *B. bassiana* inoculated batches. In high temperature treated batch, maximum cocoon weight of 1.79 g was recorded in CSR2 followed by B4 (1.76 g) and APS45 (1.73 g) breeds and it was minimum in Pure Mysore (1.21 g) followed by B8 (1.40 g) and CSR4 (1.52 g) breeds. An increase in cocoon weight over control was observed in Pure Mysore (6.04 %), APS45 (2.84 %) and CSR2 (2.78 %) and it decreased in other breeds. Highest reduction in the same was observed in CSR4 breed (9.46 %) followed by B6 breed (8.35 %) (Table 2). Previously, the thermotolerant silkworm breeds CSR46 and CSR47 yielded single cocoon weight in the same range (1.48 g and 1.34 g, respectively) when reared at 36 ± 1 p C (Suresh Kumar *et al.,* 2006). Also, when the thermotolerant silkworm breeds were treated with high temperature of 36 ± 1 p C, B8 breed (1.62 g) recorded the maximum cocoon weight, followed by B4 and APS45 (1.60 g each) (Keerthana *et al.,* 2020).

In *B. bassiana* inoculated batch, single cocoon weight was least affected in B4 (1.41 g), B1 (1.03 g) and B6 (1.00 g) breeds and it was most affected in CSR4 (0.71 g), B8 (0.83 g), APS12 and APS45 (0.85 g each) breeds. Highest per cent reduction in cocoon weight over control was observed in CSR4 breed (58.00 %) and it was lowest in Pure Mysore breed (18.00 %) (Table 2). The results of Raghavaiah (1986) in similar studies revealed 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) have also recorded the maximum cocoon weight of 1.16 g, 1.06 g and 1.05 g in B4, B1 and B8 breeds, respectively under *B. bassiana* infection which supports the present finding with respect to the performance of B4 and B1 breeds.

Cocoon shell weight (g)

The breeds utilized for the study showed significantly different cocoon shell weights when they were subjected to thermal treatment and *B. bassiana* inoculation. In high temperature treated batch, highest cocoon shell weight of 0.39 g was recorded in APS45 breed, followed by CSR2 (0.38 g) and B1 (0.37 g). Whereas, Pure Mysore, B8 and B6 breeds exhibited lower cocoon shell weight of 0.14 g, 0.28 g and 0.29 g, respectively. Cocoon shell weight was increased by 14.82 per cent over control in APS45 breed and it was decreased by 18.10 per cent in CSR4 breed (Table 2). The results are consistent with earlier findings as in CSR18 and CSR19 thermotolerant bivoltine silkworm breeds which produced shell weight of 0.30 g and 0.23 g when reared at 36±1 p C and 85±5 per cent relative humidity (Suresh Kumar *et al.,* 2002) and APS12 which produced shell weight of 0.39 g at 32 ± 1 p C (Lakshmi and Chandrashekaraiah, 2006). Similarly, highest cocoon shell weight of 0.34 g was recorded in B4 breed, followed by B3, B6 and APS45 breeds (0.32 g each) (Keerthana *et al.,* 2020).

In *B. bassiana* inoculated batch, significantly highest cocoon shell weight was recorded in B4 (0.24 g), B1 (0.21 g), B6 and APS45 breeds (0.17 g each). Pure Mysore recorded the lowest cocoon shell weight of 0.11 g followed by B8 (0.13 g), B6 and APS12 (0.17 g each). Maximum reduction in cocoon shell weight over control was observed in CSR4 breed (72.23 %), followed by CSR2 breed (65.79 %) (Table 2). In earlier

TABLE 2

Effect of thermal treatment and B. bassiana infection on cocoon parameters of selected thermotolerant bivoltine silkworm breeds

BREEDS	Single cocoon weight (g)			Cocoon shell weight (g)			Pupal weight (g)			Cocoon shell ratio (%)		
	Control	Thermal treated	*B. bassiana* infected	Control	Thermal treated	*B. bassiana* infected	Control	Thermal treated	*B. bassiana* infected	Control	Thermal treated	*B. bassiana* infected
B1	1.69	1.59 (-6.07)	1.03 (-39.08)	0.37	0.37 (+0.45)	0.21 (-44.12)	1.30	1.22 (-6.39)	0.81 (-37.86)	21.92	23.40 (+6.75)	20.10 (-8.27)
B2	1.72	1.66 (-3.68)	0.88 (-48.86)	0.39	0.35 (-9.30)	0.16 (-57.58)	1.37	1.20 (-12.08)	0.74 (-45.77)	22.49	21.18 (-5.83)	18.66 (-17.04)
B4	1.84	1.76 (-4.70)	1.14 (-37.97)	0.39	0.35 (-9.15)	0.24 (-38.46)	1.42	1.33 (-6.35)	0.89 (-37.41)	21.18	20.18 (-4.72)	21.01 (-0.80)
B6	1.74	1.59 (-8.35)	1.00 (-42.54)	0.32	0.29 (-10.00)	0.17 (-46.88)	1.51	1.28 (-15.74)	0.88 (-41.61)	18.70	18.34 (-1.91)	17.29 (-7.54)
B8	1.42	1.40 (-1.06)	0.83 (-41.67)	0.26	0.28 (+5.42)	0.13 (-50.00)	1.11	1.13 (+1.63)	0.66 (-40.41)	18.66	19.88 (+6.52)	15.99 (-14.29)
APS12	1.66	1.54 (-7.23)	0.85 (-48.91)	0.35	0.30 (-15.25)	0.17 (-52.94)	1.26	1.22 (-2.83)	0.67 (-46.67)	21.28	19.49 (-8.41)	19.60 (-7.88)
APS45	1.68	1.73 (+2.84)	0.85 (-49.46)	0.34	0.39 (+14.82)	0.14 (-59.46)	1.24	1.36 (+9.41)	0.67 (-46.26)	20.13	22.49 (+11.71)	16.15 (-19.79)
CSR2	1.74	1.79 (+2.78)	0.86 (-50.79)	0.39	0.38 (-3.27)	0.13 (-65.79)	1.31	1.39 (+6.15)	0.69 (-47.71)	22.34	20.98 (-6.06)	15.53 (-30.49)
CSR4	1.68	1.52 (-9.46)	0.71 (-58.00)	0.35	0.29 (-18.10)	0.10 (-72.23)	1.44	1.20 (-16.35)	0.61 (-57.84)	20.93	18.94 (-9.53)	13.84 (-33.88)
Pure Mysore	1.14	1.21 (+6.04)	0.93 (-18.00)	0.13	0.14 (+0.74)	0.11 (-19.25)	0.97	1.04 (+7.23)	0.80 (-17.52)	11.85	11.29 (-4.73)	11.67 (-1.52)
SEm±	0.04	0.04	0.02	0.01	0.01	0.01	0.03	0.03	0.02	0.90	0.63	0.78
CD at 5 %	0.13	0.11	0.07	0.04	0.02	0.02	0.10	0.10	0.06	2.65	1.85	2.30
CV (%)	4.74	4.25	4.76	7.46	4.59	7.21	4.55	4.60	4.56	7.79	5.53	7.96
F-test	*	*	*	*	*	*	*	*	*	*	*	*

Note:
- ✓ Positive and negative figures in the parenthesis indicate per cent increase (+) or decrease (-) over control, respectively
- ✓ * - Significant at 5 %
- ✓ *B. bassiana* inoculation 6.86 X 10^4 spores per ml @ 0.5 ml per worm; High temperature treatment @ 36±1 °C for 6 hrs per day from 1st to 6th day of fifth instar

studies NB7 silkworm spun cocoons with maximum shell weight compared to NB4D2, KA and NB18 bivoltine silkworm breeds when they were subjected to *B. bassiana* infection (Venkataramana Reddy, 1978). 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).

Pupal weight (g)

Pupal weight was significantly affected among the thermotolerant silkworm breeds due to high temperature treatment and *B. bassiana* inoculation. In high temperature treated batch, CSR2 breed exhibited significantly maximum pupal weight of 1.39 g, followed by APS45 (1.36 g), B4 (1.33 g) and B6 (1.28 g) breeds.

Pupal weight was minimum in Pure Mysore (1.04 g), followed by B8 (1.13 g) and CSR4 (1.20 g) breeds. Pupal weight was increased by 9.41 per cent over control in APS45 breed and it was decreased by 16.35 per cent in CSR4 breed (Table 2). Comparably, other thermotolerant silkworm breeds SR1 and SR4 recorded pupal weight of 1.17 g and 1.22 g, respectively (Sudhakara Rao *et al.,* 2006). In a similar high temperature treatment by experiment, Keerthana *et al.* (2020) recorded the highest pupal weight of 1.31 g in B8 breed, followed APS45, APS12 and B4 breeds (1.28 g, 1.27 g & 1.26 g, respectively).

In *B. bassiana* inoculated batch, significantly highest pupal weight was recorded in B4 (0.89 g) breed, followed by B6 (0.88 g) and B1 breeds (0.81 g) and it was significantly lowest in CSR4 (0.61 g), B8 (0.66 g) APS12 and APS45 breeds (0.67 g each). CSR4 breed recorded highest (57.84 %) reduction in pupal weight over control and it was lowest in Pure Mysore breed (17.52 %) (Table 2). Reduction in pupal weight in cross breed (PM x CSR_2) silkworm was observed when treated with sub-lethal concentration of *B. bassiana* conidial suspension (Rajitha and Savithri, 2015). The present findings were also supported by results of Keerthana *et al.* (2020), wherein B4 breed recorded maximum pupal weight of 0.92 g followed by B1, B6, B7 and B8 (0.87 g each).

CocoonShell Ratio (%)

Cocoon shell ratio was significantly affected due to high temperature treatment and *B. bassiana* inoculation among the thermotolerant silkworm breeds. In high temperature treated batch, B1 breed showed significantly highest cocoon shell ratio of 23.40 per cent, followed by APS45 (22.49 %), B2 (21.18 %) and CSR2 (20.98 %) breeds. APS45 breed showed increase in cocoon shell ratio by 11.71 per cent and it was decreased by 9.53 per cent in CSR4 breed (Table 2). Significantly lowest shell percentage was recorded in Pure Mysore (11.29 %), followed by B6 (18.34 %) and CSR4 (18.94 %). Thermotolerant breeds HTO5 and HTP5 exhibited shell percentage of 21.30 per cent and 22.3 per cent, respectively when exposed to 32 ± 1 p C (Lakshmi *et al.,* 2011). When, 20 silkworm breeds were reared at 36 ± 1 p C, CSR2 and CSR17 could produce shell percentage of 18.24 per cent and 18.25 per cent, respectively (Chandrakanth *et al.,* 2015). Likewise, among the ten thermotolerant bivoltine silkworm breeds, B3 breed recorded highest shell percentage of 21.83 per cent, followed by B4 (21.80 %) and B6 (20.75 %) breeds (Keerthana *et al.,* 2020).

In *B. bassiana* inoculated batch, B4 breed exhibited significantly highest cocoon shell percentage of 21.04 per cent, followed by B1, APS12 and B2 breeds (20.10, 19.60 and 18.66 %, respectively). Pure Mysore exhibited significantly lowest cocoon shell ratio of 11.67 per cent, followed by CSR4 (13.84 %) and CSR2 (15.53 %) breeds. Cocoon shell ratio was highly affected in CSR4 breed as it recorded 33.88 per cent reduction over control and it was least affected in Pure Mysore breed (1.52 %) (Table 2). In earlier studies, NB_4D_2 produced highest shell ratio compared to NB_7, KA and NB_{18} (Venkataramana Reddy, 1978) and crossbreed, PM x CSR_2 recorded reduced cocoon shell ratio of 12.80 per cent under *B. bassiana* infection compared to control (16.43 %) (Seema *et al.,* 2019).

Cumulative Survival Index (CSI) (%)

ERR and pupation rate being of paramount importance in deciding the survivability of silkworm breeds, the

TABLE 3

Ranking of selected thermotolerant bivoltine silkworm breeds subjected to high temperature treatment and *B. bassiana* inoculation based on CSI

High temperature treated	*B. bassiana* infected
Pure Mysore	Pure Mysore
CSR2	B1
B4	B2
B6	B8
APS12	B4
APS45	CSR2
CSR4	APS45
B1	APS12
B8	CSR4
B2	B6

cumulative survival indices were calculated utilizing those values for each breed under both high temperature treatment and *B. bassiana* inoculation. The breeds were ranked (Table 3) using the cumulative survival index and depicted in Fig. 1.

Pure Mysore ranked first (CSI, 100.00 %) in both high temperature treatment and *B. bassiana* inoculation, indicating its hardiness. CSR2 ranked second (CSI, 91.11 %), followed by B4 (CSI, 90.67 %), B6 and APS12 (CSI, 90.00 % each) breeds and B2 breed ranked last (CSI, 48 %) under high temperature treatment (Table 3; Fig. 1). The multivoltine strains, C. Nichi and Pure Mysore showed better survival

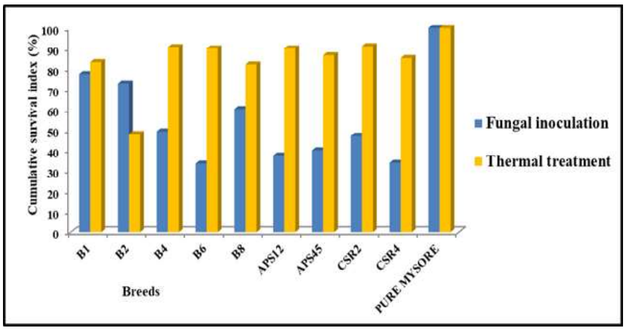

Fig. 1 : Cumulative survival index of selected thermotolerant bivoltine silkworm breeds subjected to high temperature (36±1 p C) treatment and inoculated with *B. bassiana*

rates than the bivoltine strain NB_4D_2, when exposed to 41 ºC and above (Omana and Gopinathan, 1995). The multivoltine breeds *viz.,* Pure Mysore and APM1 were less susceptible to *B. bassiana* infection compared to bivoltine race APS8 (Lakshmi *et al.*, 2005).

In *B. bassiana* inoculated batch, B1 breed ranked second (CSI, 77.33 %), followed by B2 (CSI, 72.80 %), B8 (CSI, 60.15 %) and B4 (49.33 %) breeds and the least rank was assigned to B6 and CSR4 breeds as they exhibited 33.62 per cent and 34.00 per cent of CSI, respectively (Table 3; Fig. 2). The thermotolerant bivoltine silkworm breeds which ranked above the mean CSI (84.73 % under high temperature treatment and 55.20 % under *B. bassiana* inoculation) were considered to perform better over other breeds. Therefore, the study results indicate that, among the thermotolerant bivoltine silkworm breeds selected for the study B4, B6, APS12, APS45 and CSR4 performed better under high temperature treatment. B1, B2 and B8 breeds were found to perform better under *B. bassiana* inoculation with respect to survivability.

The purpose of sericulture is to produce qualitatively and quantitatively superior cocoons which can be achieved through rearing of bivoltine silkworms. The present study results reveal that, the thermotolerant silkworm breeds B4, APS45 and B1 performed better under thermal treatment as they exhibited maximum ERR, single cocoon weight, cocoon shell weight, pupal weight and cocoon shell ratio. In *B. bassiana* inoculated breeds B1, B2 and B4 exhibited higher ERR, pupation percentage, single cocoon weight, cocoon shell weight, pupal weight and shell percentage. Results on quantitative and survival parameters obtained in the present study indicate that, B1 and B4 thermotolerant bivoltine silkworm breeds would be the potent sources with genetic plasticity to buffer against the dual stress conditions.

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REFERENCES

CAI, J., 1989, By scanning electron microscope, white cadaver skin bacteria invade the body of silkworm. *Chin. J. Nature*, **12**(11): 857-858.

CHANDRAKANTH, N., SHUNMUGAM MOORTHY, M. AND KANGAYAM PONNUVEL, 2015, Screening and classification of mulberry silkworm, *Bombyx mori* L. based on thermotolerance. *Int. J. Indust. Entomol.,* **31**(2) : 115 - 126.

CHANDRASEKHARAN, K. AND NATARAJU, B., 1998, *Beauveria bassiana* (Hyphomycetes:- Moniliales) infection during ecdysis of silkworm *Bombyx mori* (Lepidoptera: Bombycidae). *Mun. Entomol. Zool.*, **6**(1) : 312 - 316.

DANDIN, S. B. AND GIRIDHAR, K., 2014, *Handbook of Sericulture Technologies*. CSB publcations, p. 1 - 427.

JAYASHREE, MANJUNATH GOWDA, NARAYANASWAMY, K. C. AND NARAYANAREDDY, R., 2020, Effect of different doses of *Beauveria bassiana* (Bals.-Criv) Vuill. inoculation on survival parameters in a few thermotolerant bivoltine breeds and their hybrids, *Mysore J. Agri. Sci.,* **54**(2) : 67 - 76.

KEERTHANA, A, MANJUNATH GOWDA AND NARAYANASWAMY, K. C., 2019a, Performance of thermotolerant bivoltine silkworm breeds for larval growth and cocoon yield parameters under *Beauveria bassiana* infection. *Mysore J. Agri. Sci.,* **53** (1) : 19 - 26.

KEERTHANA, A, MANJUNATH GOWDA, NARAYANASWAMY, K. C. AND AMARANATHA, N., 2019b, Post cocoon traits of thermotolerant bivoltine silkworm breeds as affected by white muscardine, *Mysore J. Agri. Sci.,* **53**(2) : 1 - 8.

KEERTHANA, A, MANJUNATH GOWDA, NARAYANASWAMY, K. C. AND AMARANATHA, N., 2020, Some thermotolerant bivoltine silkworm breeds tolerate white muscardine diseases caused by *Beauveria bassiana* (Bals.-Criv) Vuill. infection. *Int. J. Chem. Stud.,* **8**(4) : 86 - 94.

KOIDSUMI, K., 1957, Antifungal action of cuticular lipids in insects. *J. Insect Physiol.*, **1** : 40 - 51.

LAKSHMI, H. AND CHANDRASHEKARAIAH, 2006, Identification of breeding resource material for the development of thermotolerant breeds of silkworm, *Bombyx mori* L. *J. Exp. Zool. India.*, **10**(1) : 55 - 63.

LAKSHMI, H., CHANDRASHEKHARAIAH, RAMESH BABU, M., RAJU, P. J., SAHA, A. K. AND BAJPAI, A. K., 2011, HTO5 × HTP5, the new bivoltine silkworm (*Bombyx mori* L.) hybrid with thermotolerance for tropical areas. *Int. J. Plant Anim. Environ. Sci.*, **1**(2) : 88 - 104.

LAKSHMI, V. V., JAMIL, K., BHARATHI, D. AND KUMAR, D. V. R., 2005, Differential susceptibility of silkworm, *Bombyx mori* L. strains to white muscardine disease caused by *Beauveria bassiana* (Bals.) Vuill. *Entomon*, **30** : 13 - 18.

OMANA, J. AND GOPINATHAN, K. P., 1995, Heat shock response in mulberry silkworm races with different thermotolerances. *J. Biosci.,* **20** : 499 - 513.

PILLAI, V. S. AND KRISHNASWAMI, S., 1980, Adoptability of silkworm *Bombyx mori* (L.) to tropical conditions: III. Studies on the effect of high temperature during later development stages of silkworm. *Indian J. Seric.*, **26 :** 63 - 71.

RAGHAVAIAH, G., 1986, 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, Bengaluru, India. p. 134.

RAJITHA, K. AND SAVITHRI, G., 2015, Studies on symptomological and economic parameters of silk cocoons of *Bombyx mori* inoculated with *Beauveria bassiana* (Bals.) Vuill. *Int. J. Curr. Microbiol. App. Sci.*, **4**(2) : 44 - 54.

SEEMA, K. D., PRITI, M. G., SHUBHANGI, S. P. AND VITTHALRAO, B. K., 2019, The influence of infection of *Beauveria bassiana* (Bals) Vuill, a fungal species (Family : *Clavicipitaceae*) on quality of the cocoons of spinned by the larval instars of *Bombyx mori* (L) (Race: PM x CSR_2). *J. Bacteriol. Mycol.,* **7**(1) : 14 - 18.

SUDHAKAR RAO, P., DATTA, R. K. AND BASAVARAJA, H. K., 2006, Evolution of a new thermotolerant bivoltine hybrid of the silkworm (*Bombyx mori* L.) for tropical climate. *Indian J. Seric.*, **45**(1) : 15 - 20.

SUNDARRAJ, N., NAGARAJU, S., VENKATARAMU, M. N. AND JAGANNATH, M. K., 1972, *Designs and Analysis of Field Experiments*. University of Agricultural Sciences, Bengaluru, India, p. 424.

SURESH KUMAR, N., BASAVARAJA, H. K., JOGE, P. G., MAL REDDY, N., KALPANA, G. V. AND DANDIN, S. B., 2006, Development of a new robust bivoltine hybrid (CSR46 × CSR47) of *Bombyx mori* L. for the tropics. *Indian J. Seric*., **45**(1) : 21 - 29.

SURESH KUMAR, N., BASAVARAJA, H. K., KISHOR KUMAR, C. M., MAL REDDY, N. AND DATTA, R. K., 2002, On the breeding of $CSR_{18} \times CSR_{19}$, a robust bivoltine hybrid of silkworm, *Bombyx mori* L. for the tropics. *Int. J. Indust. Entomol*., **5**(2) : 153 - 162.

VASUDHA, C. H., APARNA, S., GOWDA, S. AND MANJUNATHA, M. H., 2006, Impact of heat shock on heat shock proteins expression, biological and commercial traits of *Bombyx mori*. *Insect Sci.*, **13**(1) : 243.

VENKATARAMANA REDDY, M. R., 1978, Studies on the white muscardine disease of silkworm, *Bombyx mori* L. *M.Sc. (Agri.) Thesis*, University of Agricultural Sciences, Bengaluru, India, p. 89.

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