Mysore J. Agric. Sci., 58 (1) : 112-125 (2024)

Evaluation of New Foundation Crosses of Thermotolerant Bivoltine Silkworm (*Bombyx mori*) for their Tolerance to Muscardine Disease

Durgam Thrilekha[1], Manjunath Gowda[2], K. C. Narayanaswamy[3], Chikkalingaiah[4], S. Ramesh[5] and J. Seetharamulu[6]

[1,2,3&4]Department of Sericulture, [5]Department of Genetics and Plant Breeding, College of Agriculture, UAS, GKVK, Bengaluru - 560 065

[6]Director, Andhra Pradesh State Sericulture Research and Development Institute, Hindupur - 515 201

e-Mail : thrilekhareddydurgam@gmail.com

Authors Contribution

Durgam Thrilekha : Executed the research and analysed the data;

Manjunath Gowda & K. C. Narayanaswamy : Planned the research programme and arranged for the silkworm breeds;

Chikkalingaiah & S. Ramesh : Supported in designing the breeding schemes & data interpretation;

J. Seetharamulu : Supported in planning and data analysis

Corresponding Author :

Durgam Thrilekha

Received : November 2023

Accepted : December 2023

Abstract

An investigation was conducted to assess the performance of new bivoltine silkworm foundation crosses *viz.,* (B1 × B2), (B1 × B4), (B2 × B4) and (B6 × B8) under two different stress conditions *i.e.,* high temperature treatment (36 ± 1ºC) and *Beauveria bassiana* (Bals-Criv.) Vuill. (9.04×10^4 spores/ml @ 0.5 ml/worm) inoculation and evaluated along with commercial foundation crosses FC2 and FC1. The results from two seasons revealed that, under thermal treatment, B1 × B2 performed better for twelve out of fifteen parameters assessed [larval mortality (0.00%), ERR (100%), cocoon yield by number and weight (10,000/10,000 and 19.80 kg/10,000 larvae, respectively), grownup larval weight (33.00 g/10 larvae), single cocoon weight (1.65 g), shell weight (0.33 g), pupal weight (1.26 g) shell ratio (21.55%), filament length (1156.63 m), filament weight (0.27 g) and denier (2.10)]. Under *B. bassiana* inoculation, B1 × B4 showed better performance for thirteen out of fifteen traits [least larval mortality (41.33%), ERR (59.33%), fifth instar larval weight (32.00 g), pupation rate (50.00%), cumulative survival index (32.61%), cocoon yield by number and weight (5933.33 and 15.90 kg/ 10000 larvae, respectively), single cocoon weight (1.59 g), shell weight (0.31 g), pupal weight (1.15 g), shell ratio (21.00 %), filament length (724.39 m) and weight (0.24 g) and denier (2.71)]. Combining the performance of B1×B4 under both thermal and muscardine treatments, it showed superiority in seven out of fifteen parameters evaluated (fifth instar larval weight, cocoon yield by weight, single cocoon weight, shell weight, pupal weight, shell ratio and filament length). Whereas, B1×B2 hybrid was better for three parameters (least mortality, highest cocoon yield by number and ERR). Thus, these two foundation crosses *viz.,* (B1×B2), (B1×B4) can be used to develop double cross hybrids with dual stress tolerance.

Keywords : *Bombyx mori*, Bivoltine, Foundation crosses, High temperature, Muscardine disease, Thermotolerance

Indian sericulture holds significant cultural, economic and scientific importance. The industry is evolving to meet international quality standards and compete in the global market. To maintain and enhance the competitiveness of silk industry, there is a need to shift towards bivoltine sericulture, which is more productive than the traditional multivoltine sericulture. As these bivoltines have originated from temperate regions, bivoltine sericulture in tropics faces challenges such as poor leaf quality, susceptibility to diseases, hot climates and inadequate management during summer. To overcome these challenges, it is, therefore, imperative to identify bivoltine silkworm breeds and hybrids which can give stable yields under different climatic stress conditions. Accordingly, these bivoltine silkworms should be tolerant to diseases. Among the silkworm diseases, white muscardine disease, caused by *Beauveria bassiana* can cause significant losses (10-40%) in cocoon crops (Sreedhar and Reddy, 2017). Developing disease-resistant silkworm strains and implementing effective disease control measures are essential to mitigate these losses.

In order to evolve multi stress tolerant bivoltine silkworms, studies have revealed that, a few thermotolerant bivoltine breeds *viz.,* B1, B2 and B4 have shown relatively better tolerance to white muscadine disease caused by *B. bassiana* (Keerthana, 2018; Sahana *et al*., 2021; Chandrakala *et al*., 2022 and Manjunatha *et al.,* 2023). Similarly, among the hybrids of these thermotolerant bivoltine breeds, B1 × B4 and B1 × B8 exhibited better *per se* performance, while B4 × B6, B6 × B4 and B1 × B6 exhibited better heterotic performance under muscardine infection (Jayashree, 2019). In this background, an attempt was made to investigate the possibilities of dual stress tolerance for high temperature and muscardine disease in silkworm foundation crosses to be used as parents in developing robust double cross hybrids.

Material and Methods

Evaluation of six foundation crosses under high temperature treatment and *B. bassiana* inoculation was done at the Department of Sericulture, University of Agricultural Sciences, GKVK, Bengaluru during 2022-2023. Five thermotolerant silkworm breeds *viz.,* B1, B2, B4, B6 and B8 were procured from Central Sericultural Research and Training Institute, Mysore, which were identified to be muscardine resistant from the previous studies (Keerthana, 2018; Sahana *et al*., 2021; Chandrakala *et al*., 2022 and Manjunatha *et al.,* 2023). Accordingly, F_1's of oval cocoon spinning breeds *viz*., B1× B2, B1× B4 and B2× B4 and peanut cocoon spinning breeds *viz*., B6 × B8 were developed. Additionally, commercial foundation crosses *viz.,* FC2 (CSR2 × CSR27) and FC1 (CSR6 × CSR26), obtained from NSSO, were included in the evaluation. Three sets of the F_1 hybrids were reared for the study. One set was raised under normal conditions, the second set was inoculated with muscardine spores (9.04×10^4 spores/ml @ 0.5 ml/worm) immediately after the 4th moult and the third set was subjected to high temperature treatment at 36±1ºC and relative humidity of 85±5 per cent for 6 hours daily (10.00 to 16.00 hours) from the 3rd to 7th day during the fifth instar in BOD incubator (an environmental growth chamber with precise and automatic control facilities for uniform maintenance of temperature and relative humidity) (Keerthana, 2018). Each set had three replications with 200 worms for all the hybrids in all three sets of treatments.

Silkworm rearing was conducted during January - February 2023 and March - April 2023. Each foundation cross was 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. Rearing and cocoon parameters *viz*., larval mortality, fifth instar larval weight and larval duration, ERR, cocoon yield by number and weight, single cocoon, shell and pupal weights, shell ratio, pupation rate, cumulative survival index, average filament length, weight and denier were recorded. Mean data of two rearings was analysed using a completely randomized design (Sundarraj *et al*., 1972) and were compared using Duncan's Multiple Range Test (DMRT) (Duncan, 1955). To assess the overall survivability of silkworms, cumulative survival index (CSI) was calculated using the formula (Sahana *et al*., 2021).

$$CSI=[(ERR\%/100) \times (\text{per cent pupation}/100)] \times 100$$

where, CSI- Cumulative Survival Index;
ERR- Effective Rate of Rearing

Results and Discussion

Larval Mortality (%)

Significant differences were observed in larval mortality for all the foundation crosses under thermal treatment and muscardine inoculation (Table 1).

Upon high temperature treatment of 36 ± 1°C and 85 ± 5 per cent relative humidity, FC2 recorded significant maximum larval mortality of 4.66 per cent

TABLE 1

Larval growth parameters in thermotolerant bivoltine silkworm foundation crosses under thermal and muscardine treatments

Foundation crosses	Larval mortality (%)			Fifth instar larval duration (h)			Fifth instar larval weight (g/10 larvae)			Effective rate of rearing (%)		
	Control	Thermal treatment	Muscardine inoculation	Control	Thermal treatment	Muscardine inoculation	Control	Thermal treatment	Muscardine inoculation	Control	Thermal treatment	Muscardine inoculation
B1 × B2	0.00	0.00 ^{e}	40.67 ^{e}	178.00 ^{b}	183.30 ^{b}	176.62 ^{c}	35.56 ^{a}	33.00 ^{a}	31.55 ^{ab}	100.00	100.00 ^{a}	58.67 ^{a}
B1 × B4	0.00	2.00 ^{c}	41.33 ^{e}	180.67 ^{a}	186.67 ^{ab}	186.00 ^{a}	33.49 ^{b}	32.89 ^{a}	32.00 ^{a}	100.00	98.00 ^{abc}	59.33 ^{a}
B2 × B4	0.00	1.33 ^{d}	52.00 ^{c}	162.00 ^{d}	171.25 ^{c}	177.00 ^{c}	32.26 ^{bc}	31.55 ^{ab}	29.65 ^{bc}	100.00	98.67 ^{ab}	48.00 ^{c}
FC2	0.00	4.66 ^{a}	48.33 ^{d}	177.00 ^{b}	190.49 ^{a}	181.99 ^{b}	35.24 ^{a}	31.00 ^{abc}	30.21 ^{abc}	100.00	95.34 ^{c}	51.67 ^{b}
B6 × B8	0.00	0.00 ^{e}	63.33 ^{a}	168.00 ^{c}	167.30 ^{c}	159.10 ^{e}	30.47 ^{d}	30.16 ^{bc}	27.09 ^{d}	100.00	100.00 ^{a}	36.67 ^{e}
FC1	0.00	4.00 ^{b}	56.67 ^{b}	159.00 ^{e}	184.07 ^{b}	170.30 ^{d}	31.81 ^{cd}	29.00 ^{c}	28.00 ^{cd}	100.00	96.00 ^{bc}	43.33 ^{d}
F test	NA	*	*	*	*	*	*	*	*	NA	*	*
S.Em ±	-	0.101	0.852	0.544	1.617	1.145	0.507	0.701	0.691	-	0.933	1.131
CD @5%	-	0.316	2.653	1.696	5.037	3.568	1.580	2.183	2.154	-	2.907	3.523
CV (%)	-	10.716	3.262	0.552	1.551	1.132	2.651	3.881	4.025	-	1.900	4.377

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

Note : High-temperature treatment @ 36±1°C for 6 hours/day from 3rd day to 7th day during fifth instar; *B. bassiana* inoculation at 9.04×10^4 spores/ml @ 0.5 ml per worm.

followed by FC1 (4.00%). Whereas in B1 × B2 and B6 × B8 there was no larval mortality even under the temperature stress. Upon exposure to *B. bassiana* infection, B1 × B2 and B1 × B4 were statistically on par and exhibited significantly lowest larval mortality of 40.67 and 41.33 per cent, respectively, whereas B6 × B8 showed significantly highest larval mortality of 63.33 per cent.

Sreejith (2019) reported significantly least larval mortality in breed B8 (2%) followed by B7 (3%) and B6 (6%) when subjected to a temperature of 36 ± 1°C. The elevated susceptibility of fifth instar larvae to muscardine disease is in line with previous studies. In a similar study, least larval mortality was recorded in B4 (40.75%), followed by B1 (42.00%) under *B. bassiana* infection (Manjunatha *et al*., 2023) which supports the present findings as the hybrid (B1 × B4) of the same breeds recorded least mortality.

Fifth Instar Larval Duration (h)

Fifth instar larval duration showed significant variations across different foundation crosses under control, high temperature treatment and *B. bassiana* inoculation (Table 1).

In high temperature treatment of 36 ± 1°C and 85 ± 5 per cent relative humidity, FC2 recorded significantly longest fifth instar larval duration of 190.49 hours, followed by B1 × B4 (186.67 h). B6 × B8 recorded significantly shortest larval duration of 167.30 hours. Upon *B. bassiana* inoculation, B1 × B4 exhibited significantly longest fifth instar larval duration of 186.00 hours and recorded highest deviation of 8.63 per cent over the control, followed by FC2 (181.99 h). B6 × B8 showed significantly shortest fifth instar larval duration of 159.10 hours.

In high temperature treatment of 36 ± 1°C and 85 ± 5 per cent relative humidity, FC2 showed highest deviation of 11.32 per cent while B6 × B8 recorded least deviation of 3.19 per cent over the control in fifth instar larval duration. Under thermal stress, larval duration was prolonged in all the foundation crosses when compared to control except in B6 × B8. The results of Pandey and Tripathi (2006) corroborates the present findings, who reported extended larval duration under high temperature treatment and Jayashree *et al*. (2020a) reported similar findings in hybrids B6 × B1 (8.58 days) and B6 × B4 (8.56 days). Under fungal inoculation, FC1 recorded the least deviation of 2.85 per cent over the control in fifth instar larval duration. Fifth instar larval duration was prolonged in all the foundation crosses except in B1 × B2 and B6 × B8. This is consistent with the findings of Jayashree *et al*. (2020b) who showed that B6 × B4, B8 × B1 and B8 × B6 hybrids showed prolonged larval duration under *B. bassiana* infection. The prolongation of larval duration under stress conditions is due to the reduced metabolic activity of the silkworms (Janakiraman, 1961). This is because the silkworms divert their energy resources towards defence mechanisms to fight the stress.

Fifth Instar Larval Weight (g/10 Larvae)

Fifth instar larval weight showed significant variations across different foundation crosses under control, high temperature treatment and *B. bassiana* inoculation (Table 1).

Under high temperature treatment of 36 ± 1°C and 85 ± 5 per cent relative humidity, B1 × B2 and B1 × B4 showed significantly highest larval weight of 33.27 g/10 larvae and 32.89 g / 10 larvae, respectively. B6 × B8 showed significantly lowest larval weight of 29.00 g/10 larvae. Upon *B. bassiana* inoculation, B1 × B4 exhibited significantly highest larval weight of 32.00 g/10 larvae, followed by B1 × B2 (31.55 g/10 larvae). Whereas B6 × B8 recorded significantly lowest larval weight of 27.09 g/10 larvae.

The decrease in larval weight observed under high temperature treatment can be attributed to reduced feeding activity and digestibility of the silkworm (Pillai and Krishnaswamy, 1980). The present findings align with previous studies, where breeds B1, B2, B4 by (Keerthana *et al*., 2020) and (Sahana *et al*., 2021) and hybrids B1 × B4, B4 × B1 and B1 × B8 (Jayashree *et al*., 2020b) exhibited higher larval weight after exposure to a high temperature of 36±1°C for 6 hours daily during the third to seventh day of the fifth instar.

B1 × B4 showed the least deviation from the control under both thermal treatment (1.70%) and muscardine inoculation (4.46%). While, FC2 showed higher deviation from the control under both treatments, with 12.00 per cent under thermal treatment and 14.30 per cent under muscardine inoculation. The decrease in larval weight among *B. bassiana* infected silkworms could be attributed to multiple factors, including diminished feeding, reduced food consumption, impaired digestion and a decline in relative consumption rate and food conversion efficiency (Venkataramana Reddy, 1978 and Cai, 1989). Keerthana *et al*. (2019), Sahana *et al*. (2021) and Chandrakala *et al*. (2022) found that the breeds B4, B2 and B1 showed better fifth instar larval weight under *B. bassiana* inoculation. Similar results were obtained for the hybrids of these parents in the present study.

Effective Rate of Rearing (%)

Effective rate of rearing (ERR) showed significant differences among various foundation crosses under high temperature treatment and muscardine inoculation (Table 1).

Under high temperature treatment of 36 ± 1°C and 85 ± 5 per cent relative humidity, B1 × B2 and B6 × B8 showed a cent per cent ERR, indicating their exceptional thermotolerance. While FC2 recorded lowest ERR of 95.34 per cent. In the batch subjected to *B. bassiana* inoculation, B1 × B4 and B1 × B2 exhibited significantly highest ERR of 59.33 per cent and 58.67 per cent, respectively. Notably, least ERR was observed in B6 × B8 (36.67%).

FC2 and FC1 exhibited reduction of 4.6 and 4.0 per cent in ERR, respectively, compared to their respective controls under high temperature treatment. The present findings were in line with the results of Sahana *et al*. (2021) who reported that, breeds B4 (90.60%) and B6 (90.00%) resulted in maximum ERR. Similarly, among hybrids, B4 × B8 (78.00%) and B1 × B8 (74.67%) recorded highest ERR on exposure to high temperature of 36±1^{0}C for 6 h daily from third to seventh day of the fifth instar (Jayashree *et al*., 2020b). B6 × B8 and FC1 exhibited highest reduction in ERR (63.33 and 56.67%, respectively) compared to their respective controls indicating their susceptibility to *B. bassiana* infection. Present findings are also in conformity with the observations by (Keerthana *et al*., 2019), (Sahana *et al*., 2021) and (Chandrakala *et al*., 2022). These studies collectively affirm the enhanced performance of breeds B4 and B1, as well as hybrids B1 × B8 and B1 × B4 under *B. bassiana* infection (Jayashree *et al*., 2020b).

Cocoon Yield by Weight (kg/10,000 Larvae)

Cocoon yield of the thermotolerant silkworm foundation crosses exhibited significant variations under control, thermal treatment and muscardine inoculation (Table 2).

Under high temperature conditions of 36 ± 1°C and 85 ± 5 per cent relative humidity, both B1 × B2 and B1 × B4 were statistically on par with each other, recording significantly highest cocoon yield of 16.80 and 16.60 kg/10,000 larvae, respectively. B6 × B8 recorded least cocoon yield of 12.07 kg/10,000 larvae. Upon *B. bassiana* inoculation, B1 × B4 showed significantly highest cocoon yield of 15.90 kg/10,000 larvae, followed by B1 × B2 (14.96 kg/10,000 larvae. Lowest cocoon yield recorded significantly in B6 × B8 with 11.00 kg cocoons /10,000 larvae.

FC2 exhibited highest reduction of 15.54 per cent and B1 × B2 showed least reduction of 3.33 per cent in cocoon yield by weight over control upon exposure to high temperature treatment. Consistent results were also reported by Jayashree *et al*. (2020a), who found that B1 × B4 exhibited significantly higher cocoon yield (1,127.00 g/1,000 worms) followed by B1 × B8 (1,103.00 g/1,000 worms). Under muscardine inoculation, B6 × B8 exhibited highest reduction of 18.01 per cent and B2 × B4 showed least reduction of 13.6 per cent over control in cocoon yield. In a similar study, Jayashree *et al*. (2020a) reported that B1 × B8 hybrid (960.47 g/1000 worms) recorded significantly highest cocoon yield. The diverse responses in cocoon yield observed among thermotolerant bivoltine silkworm hybrids under stress conditions can be attributed to various factors *viz*., breed-specific characteristics, genetic predispositions and inherent immune responses.

TABLE 2

Productive parameters in thermotolerant bivoltine silkworm foundation crosses under thermal and muscardine treatments

Foundation crosses	Cocoon yield by weight(kg/ 10,000 larvae)			Single cocoon weight (g)			Shell weight (g)			Shell ratio (%)		
	Control	Thermal treatment	Muscardine inoculation	Control	Thermal treatment	Muscardine inoculation	Control	Thermal treatment	Muscardine inoculation	Control	Thermal treatment	Muscardine inoculation
B1 × B2	17.17 b	16.80 a	14.97 ab	1.72 b	1.65 a	1.49 b	0.34 ab	0.33 a	0.29 ab	1.26 a	1.32 b	1.26 a
B1 × B4	18.90 a	16.60 a	15.90 a	1.89 a	1.63 a	1.59 a	0.37 a	0.32 a	0.31 a	1.24 a	1.46 a	1.24 a
B2 × B4	14.97 c	13.90 c	12.93 cd	1.50 c	1.36 c	1.29 d	0.32 ab	0.30 a	0.26 bc	1.04 b	1.12 c	1.04 b
FC2	18.47 a	15.60 b	13.83 bc	1.85 a	1.55 b	1.38 c	0.33 ab	0.31 a	0.28 ab	1.23 a	1.49 a	1.23 a
B6 × B8	13.40 d	11.80 e	11.00 d	1.34 d	1.21 d	1.10 e	0.29 b	0.25 b	0.21 d	0.93 c	1.02 c	0.93 c
FC1	14.33 cd	13.10 d	12.43 cd	1.43 cd	1.31 c	1.24 d	0.30 b	0.26 b	0.23 cd	1.01 b	1.08 c	1.01 b
F test	*	*	*	*	*	*	*	*	*	*	*	*
S.Em ±	0.351	0.228	0.572	0.035	0.026	0.063	0.013	0.011	0.010	0.016	0.048	0.016
CD @5%	1.095	0.709	1.781	0.109	0.081	0.179	0.041	0.035	0.033	0.049	0.105	0.049
CV (%)	3.756	2.719	7.329	3.756	3.087	7.385	6.980	6.614	6.846	2.428	4.676	2.428

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

Note : High-temperature treatment @ 36±1°C for 6 hours/day from 3rd day to 7th day during fifth instar; *B. bassiana* inoculation at 9.04 x10^4 spores/ml @ 0.5 ml per worm

Single Cocoon Weight (g)

Significant differences were observed in single cocoon weight among various foundation crosses under control, thermal treatment and muscardine inoculation (Table 2 and Plate 1).

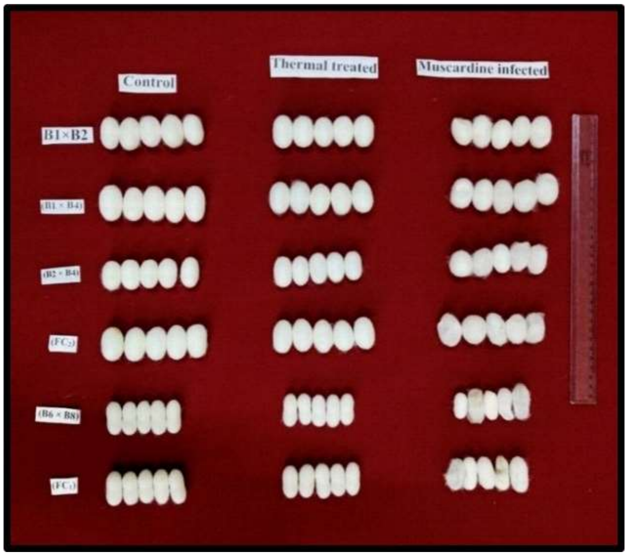

Plate 1 : Cocoons of silkworm foundation crosses reared under thermal and muscardine treatments in comparison with control

Under high temperature treatment of 36 ± 1°C and 85 ± 5 per cent relative humidity, B1 × B2 and B1 × B4 were statistically on par with each other and recorded significantly highest cocoon weight of 1.65 g and 1.63 g, respectively. B6 × B8 recorded significantly least cocoon weight (1.21 g). Upon *B. bassiana* inoculation, single cocoon weight was least affected in B1 × B4 (1.59 g) followed by B1 × B2 (1.49 g) and it was most affected in B6 × B8 (1.10 g) when compared to the control.

FC2 exhibited highest reduction (16.22 %) and B1 × B2 recorded least reduction of 4.07 per cent over the control in single cocoon weight under thermal treatment. In a similar study, Jayashree *et al*. (2020a) observed maximum cocoon weight of B1 × B4 recorded highest cocoon weight of 1.56 g, followed by B1 × B8 with 1.51 g upon exposure to high temperature. These results are consistent with the findings of Naseema Begum *et al*. (2003) in thermotolerant hybrids at 32°C observed cocoon weight ranging from 1.48 to 1.68g. Under muscardine inoculation, B1 × B2 showed highest reduction of 16.22 per cent and B2 × B4 showed least reduction of 4.07 per cent over control in single cocoon weight. The reduction in cocoon characters could be attributed to loss of appetite, physiological stress and lethargic conditions induced by the fungal pathogen (Lakshmi *et al.,* 2011). Present findings were also supported by Sahana *et al*. (2021) who reported highest single cocoon weight in B4 (1.41 g), followed by B1 (1.03 g). Among hybrids, elevated cocoon weight was observed in B1 × B8 (1.16 g), followed by B1 × B4 (1.13 g) (Jayashree *et al*., 2020a) under muscardine inoculation.

Shell Weight (g)

Significant differences were observed in shell weight among various foundation crosses under normal conditions, thermal treatment and muscardine inoculation (Table 2).

Under high temperature conditions of 36 ± 1°C and 85 ± 5 per cent relative humidity, B1 × B2 recorded significantly highest shell weight of 0.33 g, followed by B1 × B4, FC2 and B2 × B4 which were statistically on par, recording shell weight of 0.32 g, 0.31g and 0.30 g, respectively. B6 × B8 recorded significantly least shell weight (0.25 g). Upon *B. bassiana* inoculation, B1 × B4 exhibited significantly highest shell weight of 0.31 g, followed by B1 × B2 and FC2 (0.29 and 0.28 g, respectively). Notably, B6 × B8 showed significantly least shell weight of 0.21 g.

Least reduction of shell weight over the control in both treatments was observed in B1 × B4 (1.52 and 2.58 per cent in thermal and muscardine treatments, respectively) and highest reduction was found in B6 × B8 (7.58 and 9.68 per cent in thermal and muscardine treatments, respectively). Corroborative findings were also reported by Sahana *et al*. (2021) who observed that highest cocoon shell weight of 0.39 g in APS45 breed, followed by CSR2 (0.38 g) and B1 (0.37 g) breeds. Similarly, among the hybrids, highest shell weight was recorded in B1 × B4 (0.31 g), followed by B1 × B8 (0.30 g) under high temperature treatment (Jayashree *et al.,* 2020a). Under muscardine

TABLE 3

Survival parameters in thermotolerant bivoltine silkworm foundation crosses under thermal and muscardine treatments

Foundation crosses	Pupal weight (g)			Pupation rate (%)			CSI (%)		
	Control	Thermal treatment	Control	Control	Thermal treatment	Muscardine inoculation	Control	Thermal treatment	Muscardine inoculation
B1 × B2	1.32 [b]	1.26 [a]	1.32 [b]	100.00	96.33 [ab]	44.33 [a]	100.00	96.33 [ab]	28.85 [a]
B1 × B4	1.46 [a]	1.24 [a]	1.46 [a]	100.00	95.33 [ab]	50.00 [a]	100.00	93.45 [ab]	32.61 [a]
B2 × B4	1.12 [c]	1.04 [b]	1.12 [c]	100.00	92.00 [b]	28.00 [b]	100.00	90.79 [ab]	15.36 [bc]
FC2	1.49 [a]	1.23 [a]	1.49 [a]	100.00	82.00 [c]	33.33 [b]	100.00	78.22 [c]	21.43 [b]
B6 × B8	1.02 [c]	0.93 [c]	1.02 [c]	100.00	98.00 [a]	32.67 [b]	100.00	98.00 [a]	12.28 [c]
FC1	1.08 [c]	1.01 [b]	1.08 [c]	100.00	91.33 [b]	20.67 [c]	100.00	87.73 [b]	11.01 [c]
F test	*	*	*	NA	*	*	NA	*	*
S.Em ±	0.048	0.016	0.048	-	2.303	1.842	-	2.616	2.120
CD @5%	0.105	0.049	0.105	-	7.174	5.738	-	8.150	6.605
CV (%)	4.676	2.428	4.676	-	5.290	8.882	-	6.138	9.033

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

Note : High-temperature treatment @ 36±1°C for 6 hours/day from 3rd day to 7th day during fifth instar; *B. bassiana* inoculation at 9.04×10^4 spores/ml @ 0.5 ml per worm

inoculation, Sahana *et al.* (2022) reported significantly highest shell weight in B4 (0.24 g), B1 (0.21 g), B6 and APS12 breeds (0.17 g each) and among the hybrids, B1 × B8 showed highest shell weight of 0.21 g, followed by B1 × B4 (0.19 g) (Jayashree *et al.*, 2020a).

Pupal Weight (g)

Pupal weight showed significant differences among various foundation crosses under control, thermal treatment and muscardine inoculation (Table 3).

Under high temperature conditions of 36 ± 1°C and 85 ± 5 per cent relative humidity, B1 × B2, B1 × B4 were statistically on par with each other and recorded significantly highest pupal weight of 1.26g and 1.24g, respectively. B6 × B8 recorded significantly least pupal weight (0.93 g). Upon *B. bassiana* inoculation, B1 × B4 exhibited significantly highest pupal weight of 1.15 g, followed by B1 × B2 (1.04 g). B6 × B8 showed significantly least pupal weight of 0.84g.

Under thermal treatment, FC2 exhibited highest reduction (17.57%) and B6 × B8 showed least reduction (6.25%) over control in pupal weight. Present findings were justified by Jayashree *et al.* (2020a) who reported that, B1 × B4 exhibited significantly highest pupal weight of 1.25 g, followed by B1 × B8 (1.21 g). Upon fungal exposure, FC2 exhibited highest reduction (20.13%) and B2 × B4 showed least reduction (15.18%) in pupal weight over the control. The present findings are also supported by Jayashree *et al.* (2020a) who reported highest pupal weight in B1 × B8 (1.18g) followed by B1 × B4 (1.04g).

Shell Ratio (%)

Significant differences in shell ratio were observed among various foundation crosses under control, high temperature treatment and muscardine inoculation (Table 3).

Under high temperature conditions of 36 ± 1°C and 85 ± 5 per cent relative humidity, B1 × B2 and B1 ×

B4, were statistically on par with each other and recorded significantly highest shell ratio of 21.55 and 21.00 per cent, respectively. B6 × B8 recorded significantly least shell ratio (17.90%). Upon *B. bassiana* inoculation, B1 × B4 exhibited significantly highest shell ratio of 20.65 per cent, followed by B1 × B2 (19.24%). Notably, B6 × B8 showed significantly least shell ratio of 17.06 per cent.

B1 × B4 showed least reduction of 1.15 per cent and FC2 recorded highest reduction of 12.68 per cent in shell ratio over control upon exposure to high temperature. In a similar study, Among the hybrids, the higher shell ratio was recorded in B8 × B1 (20.49%), followed by B8 × B6 (20.35%) under high temperature of 36 ± 1ºC (Jayashree *et al*., 2020a). Upon muscardine inoculation, B6 × B8 recorded least reduction of 5.27 per cent over control and FC1 showed highest reduction of 15.63 per cent over the control in shell ratio. Jayashree *et al*. (2020a) recorded significantly highest shell ratio in B1 × B8 (17.83%), followed by B1 × B6 (17.23%) under muscardine inoculation.

Pupation Rate (%)

Pupation rate exhibited significant variations among all the foundation crosses under thermal treatment and muscardine inoculation (Table 3).

Under elevated temperature conditions of 36 ± 1°C and 85 ± 5 per cent relative humidity, B6 × B8 exhibited significantly highest pupation rate of 98.00 percent, followed by B1 × B2 and B1 × B4 which were statistically on par recorded pupation rate of 96.33 and 95.33 per cent pupation rate, respectively. Whereas, FC2 showed significantly least pupation rate of 82.00 per cent. Upon inoculation with *B. bassiana*, foundation crosses B1 × B4 and B1 × B2, were statistically on par and showed highest pupation rate of 50.00 and 44.33 per cent, respectively. FC1 exhibited significantly least pupation rate of 20.67 per cent.

Under high temperature treatment, FC2 exhibited highest reduction of 18.00 per cent, while B6 × B8 showed least reduction (1.00%) in pupation rate over control. Present findings were supported by Jayashree *et al*. (2020b) wherein cent per cent pupation rate was observed among all the breeds and hybrids except B4 (96.67%), B4 × B6 (96.67%) and B6 × B8 (93.38%) hybrids at high temperature of 36 ± 1 ^{0}C for 6 h / day from 2nd day to 7th day of fifth instar. Under muscardine inoculation, FC1 showed highest reduction of 79.33 per cent, while B1 × B4 exhibited least reduction of 50.00 per cent in pupation rate over control. Presence of saturated fatty acids *viz*., capric acid and caprylic acid in the pupal cuticle were found to show antifungal activity against *B. bassiana* infection (Koidsumi, 1957 and Chandrasekharan & Nataraju, 2008). This could be a possible reason to show increased per cent pupation in B1 × B4 and B1 × B2 if *B. bassiana* infection tends to affect in later periods of fifth instar. According to Jayashree *et al*. (2020b) maximum pupation rate was noticed in B1 × B8 (90.00%), followed by B4 × B6 (89.67%).

Cumulative Survival Index (%)

ERR and pupation rate being of paramount important in deciding the survivability of silkworm breeds and hybrids, the cumulative survival index (CSI) was calculated utilizing those values for each hybrid under both high temperature treatment and *B. bassiana* inoculation (Table 3).

Under elevated temperature conditions of 36 ± 1°C and 85 ± 5 per cent relative humidity, significantly highest CSI was recorded in B6 × B8 (98.00%), followed by B1 × B2 and B1 × B4, which were statistically on par and recorded 96.33 and 93.45 per cent CSI, respectively. FC2 exhibited significantly lowest CSI (78.22%). Upon *B. bassiana* inoculation, both B1 × B4 and B1 × B2 were statistically on par with each other showing significantly highest CSI of 32.61 and 28.85 per cent, respectively. While FC1 exhibited lowest CSI (11.01%).

Under thermal treatment, FC2 showed a notable reduction of 21.78 per cent, whereas B6 × B8 recorded least reduction of 1.00 per cent in CSI over the control. In a similar study, Sahana *et al*. (2021) reported that CSR2 recorded highest CSI of 91.11 per cent followed by B4 (90.67%), B6 and APS12 (90.00% each) under high temperature treatment. Upon muscardine inoculation, FC1 showed a pronounced reduction of 88.99 per cent while B1 × B4 exhibited least reduction (67.39%) in CSI over control. Sahana *et al*. (2021) reported that B1 showed highest CSI of 77.33 per cent, followed by B2 (72.80%), B8 (60.15%) and B4 (49.33%) breeds in *B. bassiana* inoculated batch, wherein during the present study, hybrids derived from B1, B2, B4 and B8 have shown better CSI.

The thermotolerant bivoltine silkworm foundation crosses which show CSI above the mean CSI *i.e.,* 90.76 per cent under high temperature treatment and 20.26 per cent under *B. bassiana* inoculation were considered to perform better over other foundation crosses. In case of both treatments, B1 × B4 and B1 × B2 consistently showed least reduction in CSI over control indicating their superior performance in enduring thermal stress and muscardine inoculation.

Average Filament Length (m)

Filament length showed significant differences among all the foundation crosses under control, thermal treatment and muscardine inoculation (Fig. 1).

Under high temperature conditions of 36 ± 1°C and 85 ± 5 per cent relative humidity, foundation crosses B1 × B2 and B1 × B4 were statistically on par with each other exhibited significantly longest filament length of 1156.63 and 1134.89 m, respectively. FC1 showed significantly shortest filament length of 974.37. Upon *B. bassiana* inoculation, B1 × B4 exhibited significantly longest filament length of 724.39 m followed by B1 × B2 with 699.23 m. Notably, B6 × B8 showed significantly shortest filament length of 534.38 m.

FC1 showed highest reduction of 17.04 per cent while B1 × B2 showed least reduction of 14.60 per cent in filament length over the control under thermal treatment. Comparable findings were also reported under high temperature treatment (36±1^{0}C) by Jayashree *et al*. (2020a) who indicated that, among the hybrids, longest filament length was recorded in B1 × B4 (928.51 m), followed by B1 × B8 (900.31 m). Under muscardine inoculation, B2 × B4 showed highest reduction of 51.65 per cent while B1 × B4 showed least reduction of 46.16 per cent in filament length over the control. Present findings correspond to the previous findings of Jayashree *et al*. (2020a) who observed that filament length significantly decreased from 1015.50 m in control conditions to 768.94 m under *B. bassiana* inoculation. The reason for producing longer filament even under stress conditions might be attributed to specific genetic makeup, responsive heat related genes and elevated protein synthesis in certain hybrids.

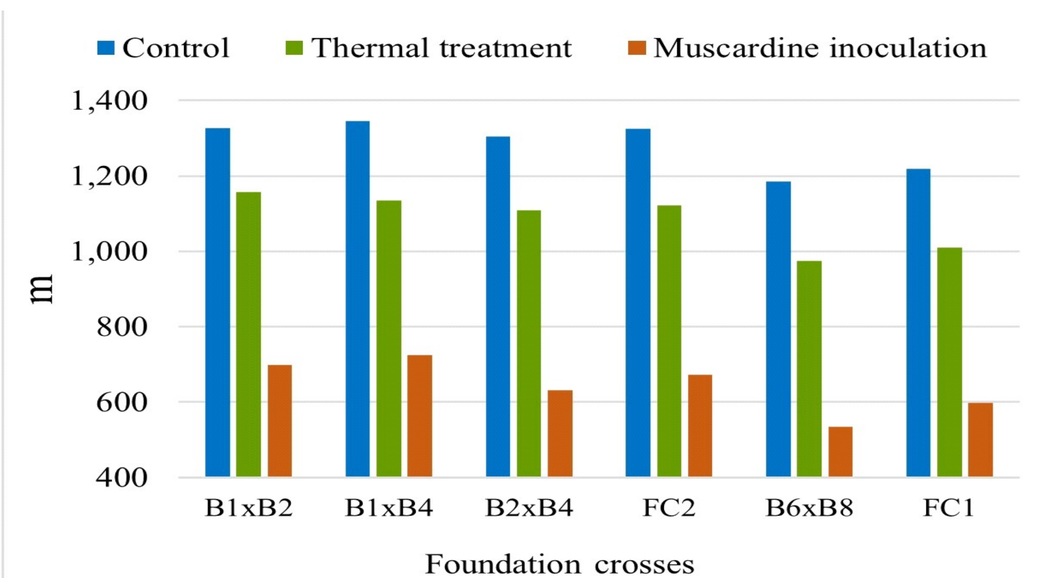

Fig. 1 : Filament length in thermotolerant bivoltine silkworm foundation crosses under thermal and muscardine treatments

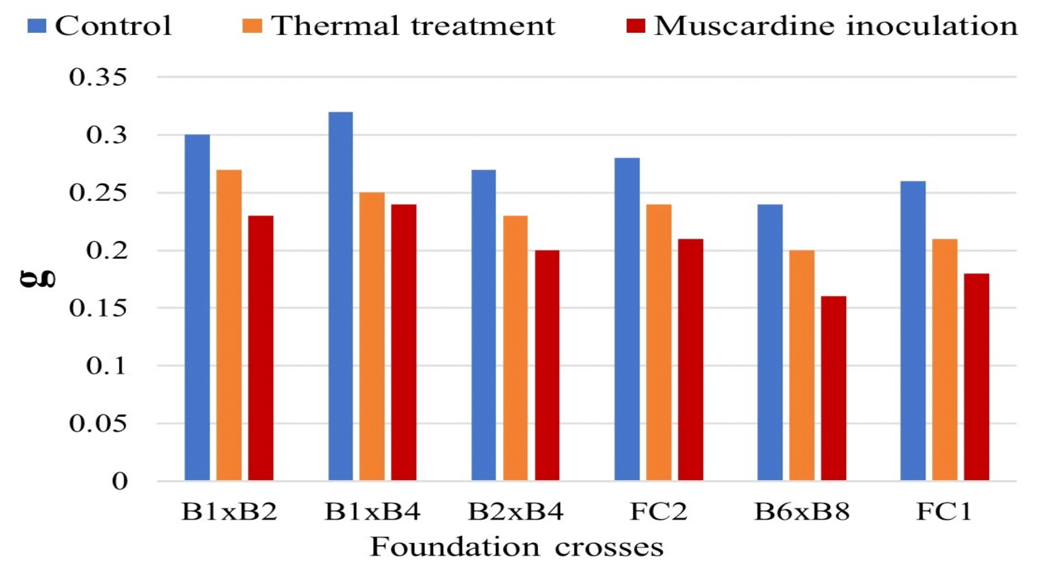

Fig. 2: Filament weight in thermotolerant bivoltine silkworm foundation crosses under thermal and muscardine treatments

Filament Weight (g)

Filament weight exhibited statistically significant variations among all the foundation crosses under control, thermal treatment and muscardine inoculation (Fig. 2).

Upon exposure to thermal treatment of 36 ± 1°C and 85 ± 5 per cent relative humidity, B1 × B2 showed significantly maximum filament weight of 0.27 g, followed by B1 × B4 and FC2 with 0.25 g and 0.24 g, respectively. B6 × B8 exhibited significantly lowest filament weight of 0.20 g. Upon inoculation with *B. bassiana*, B1 × B4 recorded significantly highest filament weight of 0.24 g, followed by B1 × B2 (0.23g). Notably, B6 × B8 hybrid produced significantly lowest filament weight of 0.16 g.

Under thermal treatment, B1 × B4 exhibited highest reduction (21.88%) while B1 × B2 showed least reduction of 10.00 per cent in filament weight over the control. Similar findings were reported by Jayashree *et al*. (2020a) among hybrids, highest filament weight was recorded in B1 × B4 (0.27 g) and B1 × B8 (0.25 g). FC2 exhibited highest reduction of 33.33 per cent, while B1 × B4 showed least reduction of 17.86 per cent in filament weight over the control under muscardine inoculation. Similar observations were reported by Jayashree *et al*. (2020a) who showed that, B1 × B8 (0.18 g) exhibited significantly highest filament weight, followed by B1 × B4 (0.16 g).

Cocoon Denier

Significant variations were observed in cocoon denier across all foundation crosses under control, thermal treatment and muscardine inoculation (Fig. 3).

Under high temperature conditions of 36 ± 1°C and 85 ± 5 per cent relative humidity, B1 × B2 recorded significantly highest cocoon denier of 2.10, followed by B1 × B4 (2.06). B6 × B8 significantly recorded lowest cocoon denier of 1.84. Upon inoculation with *B. bassiana*, FC2 exhibited significantly highest denier of 3.07, followed by B2 × B4 (2.91). Notably, B1 × B2 displayed significantly least denier of 2.59, followed by B1 × B4 which were statistically on par with each other recording a denier of 2.71 and 2.72, respectively.

Under high temperature treatment, FC2 showed highest deviation of 18.72 per cent, while B1 × B2 exhibited least deviation of 5.41 per cent in cocoon denier over the control. In the context of high temperature treatment (36 ± 1°C) across various silkworm breeds, denier value ranged from 1.9 to 3.1

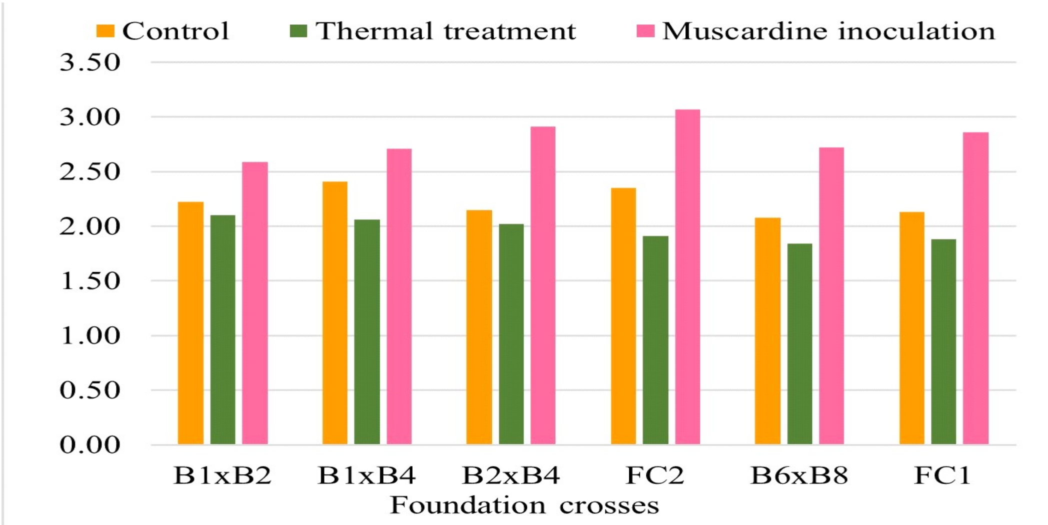

Fig. 3: Cocoon denier in thermotolerant bivoltine silkworm foundation crosses under thermal and muscardine treatments

(Kumari *et al*., 2011), whereas in the present study, it ranged from 1.84 to 2.10. Comparable outcomes were also obtained by Sahana *et al*. (2022), who reported denier values of 2.69 for B1 breed and 2.60 for B4 breed. B2 × B4 showed highest deviation of 35.35 per cent while B1 × B4 exhibited least deviation of 12.45 per cent over the control in denier. In a similar study, Sahana *et al*. (2021) noted significant elevation in denier of B2 breed (2.74), followed by the CSR4 breed (2.54). Furthermore, Chandrakala *et al*. (2022) recorded highest denier value of 3.18 in the CSR4 breed, followed by F2 of (B8 × CSR4) (2.85).

The purpose of sericulture is to produce qualitatively and quantitatively superior cocoons which can be achieved through rearing of bivoltine silkworms. The present findings revealed that, the foundation cross B1 × B2 performed better under thermal treatment as it exhibited least larval mortality, maximum ERR, cocoon yield by number and weight, larval weight, single cocoon, shell and pupal weights, shell ratio, average filament length, weight and medium denier. In *B. bassiana* inoculated batch, foundation cross B1 × B4 performed better with least larval mortality, maximum fifth instar larval weight, ERR, cocoon yield by number and weight, pupation rate, cumulative survival index, single cocoon, shell and pupal weights, shell ratio, filament length and weight and medium cocoon denier. Thus, two potential foundation crosses, B1×B4 and B1×B2 would be the potent sources with genetic plasticity to buffer against the dual stress conditions and can be used as parental material for developing new double cross hybrids.

Acknowledgement: The authors are thankful to Central Sericultural Research and Training Institute, Mysore for timely supply of thermotolerant bivoltine silkworm eggs to conduct the experiments.

References

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

Chandrakala, Manjunath Gowda, Narayanaswamy, K. C. and Amarnatha, N., 2022, *Per se* performance of six generation crosses of muscardine resistant thermotolerant bivoltine silkworm breeds of silkworm, *Bombyx mori* L. provides a lead for genetic analysis of their resistance to muscardine disease. *Pharma. Innov.,* **11** (11) : 1869 - 1874.

Chandrasekharan, K. and Nataraju, B., 2008, Studies on white muscardine disease of mulberry silkworm in India. *Indian J. Seric*., **47** (2) : 136 - 154.

Dandin, S. B. and Giridhar, K., 2014, *Handbook of Sericulture Technologies*. CSB Publications, pp. : 427.

DUNCAN, F., 1955, Multiple range test and multiple 'F' test. *Biometrics*, **11** : 1 - 42.

JANAKIRAMAN, A. T., 1961, Diseases affecting the Indian silkworm races. *Indian J. Seric*., **13** : 91 - 101.

JAYASHREE, 2019, Studies on the response of thermotolerant bivoltine hybrids of *Bombyx mori* L. to *Beauveria bassiana* (Bals.-Criv.) Vuill. Infection. *M.Sc. (Agri.) Thesis*, Univ. Agric. Sci., Bangalore, India, pp. : 40 - 116.

JAYASHREE, MANJUNATH GOWDA, NARAYANASWAMY, K. C. AND NARAYANA REDDY, R., 2020a, Response of 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. Studies*, **7** (5) : 1 - 8.

JAYASHREE, MANJUNATH GOWDA, NARAYANASWAMY, K. C. AND NARAYANA REDDY, R., 2020b, 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. Agric. Sci.,* **54** (2) : 67 - 76.

KEERTHANA, A., 2018, Studies on response of thermotolerant bivoltine silkworm breeds to white muscardine infection. *M.Sc. (Agri.) Thesis*, Univ. Agric. Sci., Bangalore, India, pp. : 33 - 78.

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

KEERTHANA, A., MANJUNATH GOWDA., NARAYANASWAMY, K. C. AND AMARANATHA, N., 2020, Some thermotolerant bivoltine silkworm breeds tolerate white muscardine disease 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.

KUMARI, S. S., SUBBARAO, S. V., SUNIL MISHRA AND MURTHY, U. S., 2011, Screening strains of the mulberry silkworm, *Bombyx mori* L. for thermotolerance. *J. Insect Sci*., **11** (6) : 1 - 14.

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.

MANJUNATHA, S. R., NARAYANASWAMY, K. C., MANJUNATH GOWDA, CHANDRASHEKHAR, S., PRASANNA KUMAR, M. K. AND BENHERLAL, P. S., 2023, Studies on the effect of white muscardine infection on growth and yield performance of bivoltine silkworm, *Bombyx mori* breeds. *Mysore J. Agric. Sci*., **57 (**2) : 212 - 219.

NASEEMA BEGUM, A., REKHA, M., BASAVARAJA, H. K. AND AHSAN, M. M., 2003, Evolution and identification of thermo-tolerant hybrids in the silkworm, *Bombyx mori* L. *Int. J. Indust. Entomol*., **6** (2) : 171 - 178.

PANDEY, P. AND TRIPATHI, S. P., 2006, Effect of ecological factors on larval duration of silkworm (*Bombyx mori* L.). *J. Ecophysiol. Occup. Health*, **6** (3) : 3 - 5.

PILLAI, V. S. AND KRISHNASWAMI, S., 1980, Studies on the effect of high temperature during later development stages of silkworm. *Indian J. Seric*., **26 :** 63 - 71.

SAHANA, K. P., GOWDA, M., NARAYANASWAMY, K. C. AND CHANDRASHEKHAR, S., 2022, Evaluation of thermotolerant bivoltine silkworm breeds through cocoon yield and filament characteristics against *Beauveria bassiana* (Bals-Criv.) Vuill. inoculation. *Int. J. Environ. Clim. Change*, **12** (12) : 1371 - 1378.

SAHANA, K. P., MANJUNATH GOWDA, NARAYANASWAMY, K. C. AND CHANDRASHEKHAR, S., 2021, 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.,* **55** (3) : 59 - 68.

SREEDHAR, S. R. AND REDDY, K. R., 2017, Assessment of diseases in *Bombyx mori* silkworm – A survey. *Science Direct*, **26** (6) : 331 - 334.

SREEJITH, 2019, Biochemical changes in thermotolerant bivoltine breeds during high temperature treatment and muscardine disease. *M.Sc. (Agri.) Thesis*, University of Agricultural Sciences, Bangalore, India, pp. : 96.

SUNDARRAJ, N., NAGARAJU, S., VENKATARAMU, M. N. AND JAGANNATH, M. K., 1972, *Designs and Analysis of Field Experiments*. University of Agricultural Sciences, Bangalore, pp. : 424.

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