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Per-se Performance of Six Generations of Crosses between Muscardine Resistant Thermotolerant Bivoltine Breeds and Muscardine Susceptible Productive Bivoltine Breed of Silkworm, *Bombyx mori* L.

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Abstract

A study was conducted to understand the genetics of muscardine disease resistance in thermotolerant bivoltine silkworm breeds by analysing the parents, F_1 and their segregating generation *viz*., F_2, BC1 and BC2, to identify suitable bivoltine hybrids. Three muscardine resistant thermotolerant bivoltine breeds B1, B4 and B8 were crossed with a productive but muscardine susceptible bivoltine breed CSR_4 to raise six generations, *viz*., P1, P2, F_1, F_2, BC1 and BC2. The silkworms of all the generations were inoculated with 9.04×10^4 spores / ml of *Beauveria bassiana* @ 0.5 ml per silkworm to know their performance under fungal stress. The results revealed that the thermotolerant bivoltine silkworm breed B4 and its crosses performed significantly better than the crosses of B1 and B8 for six parameters studied, *viz*., larval duration (days), fifth instar larval weight (g), larval mortality (%), effective rate of rearing (%), cocoon yield by number (per 10,000 worms) and weight (kg/10,000 worms). The genetics of muscardine resistance needs to be studied by generation mean analysis.

Keywords: Bivoltine, *Bombyx mori*, Six generations, Muscardine, Thermotolerance

The silkworm, *Bombyx mori* L. is economically important sericigenous insect. However, it is highly sensitive to varying abiotic conditions and susceptible to different diseases. Silkworm being a poikilothermic insect, abiotic factor, temperature plays a major role on its growth and cocoon productivity (Benchmin and Jolly, 1986). While among the diseases, white muscardine caused by *Beauveria bassiana*, is common in India, predominantly during rainy and winter seasons, which is favoured by humidity and temperature for its multiplication and spread (Janakiraman, 1961).

To improve the cocoon yield and silk quality, introduction of productive bivoltine breeds from temperate countries to India is envisaged (Dandin *et al.,* 2006). For the success of the introduced bivoltine breeds, it is necessary to have stability in cocoon crop under high temperature environment of tropics with improved disease tolerence. Lack of high temperature and high humidity tolerant bivoltine breeds are the major constraints as the economically important qualitative and quantitative traits are affected by rearing them under such conditions. To overcome such problem CSR&TI, Mysore (Suresh Kumar *et al*., 2003) and APSSRDI, Hindupur (Lakshmi *et al.,* 2011) developed thermotolerant bivoltine silkworm breeds adoptable to high temperature conditions prevailing in summer. However, their resistance to diseases is not evaluated and therefore, studies on muscardine disease resistance in thermotolerant bivoltine silkworm breeds was initiated at the Department of Sericulture, UAS, Bangalore.

Studies so far conducted on muscardine disease resistance in thermotolerant bivoltine breeds revealed that, B1, B4, B6 and B8 breeds from CSRTI, Mysore had relatively higher tolerance to white muscardine disease among ten breeds evaluated (Keerthana, 2018). 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). These results indicated that there is a possibility of evolving bivoltine silkworm breeds with multiple/ dual tolerance *viz*., to both high temperature and fungal infection. In continuation, an attempt is made to understand the genetics of muscardine disease resistance by analysing the segregating generation of promising muscardine resistant thermotolerant bivoltine breeds to further proceed towards evolving suitable bivoltine hybrids or double hybrids with multiple resistance.

Material and Methods

The experiment was conducted during 2020-22, at the Department of Sericulture, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bengaluru. The muscardine resistant thermotolerant bivoltine breeds B1, B4 and B8 identified from the previous studies were crossed with a muscardine susceptible bivoltine breed CSR_4 to raise F_1 and F_2 generations. Also, BC1 and BC2 generations were obtained by crossing F_1 with tolerant parent and susceptible parents, respectively. All these six generations *viz*., P1, P2, F_1, F_2, BC1 and BC2 were reared once. Accordingly, B1×CSR_4, B4×CSR_4 and B8×CSR_4 hybrids were prepared and their subsequent generations were raised as mentioned above. The characteristic features of the parents utilized in the study are provided in Table 1.

Table 1

Characteristicfeatures of the bivoltine breeds used in the experiment

Genotypes	Breed traits	Resistance to muscardine
B1	Plain larva spinning oval shaped cocoon	Thermotolerant and resistance to muscardine infection
B4	Plain larva spinning oval shaped cocoon	
B8	Marked larva spinning peanut cocoon	
CSR_4	Plain larva spinning peanut cocoon	Productive but susceptible to muscardine muscardine infection

Rearing of Six Generation of Silkworm Breeds

To develop six generation of the selected parents, three rearings were conducted, first by rearing the parents, next by rearing the parents and hybrids and finally obtained all the generation of crosses in the third rearing. The six generation of silkworm crosses were reared at once and divided into two batches, one was inoculated with muscardine fungus and other batch was maintained as control. Treatments were done to segregating batches in such a way that half the brood was inoculated with muscardine disease and the other half of the brood was raised under normal conditions and the worms in each batch were grouped into three replications randomly. For non-segregating generations, the treatment was imposed for thirty worms each in three replications. Inoculation with fungus load of 9.04×10^4 spores / ml @ 0.5 ml per silkworm was done immediately after the fourth moult, before resuming the feed in the V instar.

Two rearings of six generations with treatments as indicated above was done, once during February-April 2021 and again during December 2021 to February 2022. Pooled mean data of the two rearing on larval duration, larval weight, larval mortality, ERR, cocoon yield by number (per 10,000 worms) and weight (kg/ 10,000 worms) were recorded. The data so collected was analysed using a completely randomised design (Sundarraj *et al.,* 1972).

Results and Discussion

Fifth Instar Larval Duration (days)

Significant differences were observed for fifth instar larval duration among the six generations of crosses between muscardine resistant thermotolerant bivoltine breeds and muscardine susceptible productive bivoltine breed, both under normal condition and muscardine inoculation (Table 2).

TABLE 2

Fifth instar larval duration (days) and larval weight (g) as affected by muscardine inoculation among six generations of crosses between muscardine resistant thermotolerant bivoltine breeds and muscardine susceptible productive bivoltine breed

Breeds and generations		Larval duration (days)		Larval weight (g)	
		Control	Muscardine inoculation	Control	Muscardine inoculation
Parents	B1	7.99 [ab]	8.65 [abc]	3.78 [bcd]	2.73 [d]
	B4	7.82 [abc]	8.15 [def]	3.67 [cde]	2.77 [d]
	B8	8.03 [a]	8.43 [bcd]	2.68 [ij]	2.14 [g]
	CSR4	8.10 [a]	8.96 [a]	2.91 [i]	1.94 [gh]
F_1	B1 × CSR4	7.35 [cde]	7.79 [f]	4.12 [a]	3.19 [ab]
	B4 × CSR4	7.39 [cde]	7.96 [ef]	3.98 [ab]	3.30 [ab]
	B8 × CSR4	7.05 [e]	7.76 [f]	3.22 [h]	2.49 [ef]
F_2	B1 × CSR4	8.11 [a]	8.71 [abc]	3.65 [cdef]	2.85 [cd]
	B4 × CSR4	7.47 [bcde]	8.72 [abc]	3.48 [efg]	2.92 [cd]
	B8 ×CSR4	7.75 [abc]	8.42 [cd]	2.86 [i]	2.42 [f]
BC1	(B1 × CSR4) × B1	7.60 [abcd]	8.48 [bcd]	3.80 [bc]	3.30 [ab]
	(B4 ×CSR4) × B4	7.80 [abc]	8.67 [abc]	3.54 [defg]	3.32 [a]
	(B8 × CSR4)×B8	7.72 [abcd]	8.34 [cde]	3.34 [gh]	3.07 [bc]
BC2	(B1 × CSR4) × CSR4	7.64 [abcd]	8.46 [bcd]	3.53 [efg]	2.70 [de]
	(B4 × CSR4) × CSR4	7.70 [abcd]	8.83 [ab]	3.41 [fgh]	2.90 [cd]
	(B8 × CSR4) × CSR4	7.19 [de]	7.98 [ef]	2.58 [j]	1.84 [h]
F test		**	**	**	**
S.Em±		0.192	0.141	0.087	0.079
CD @5%		0.554	0.407	0.251	0.227
CV (%)		4.343	2.919	4.419	4.971

Figures with same superscript are statistically on par *-Significant @5%; ** - Significant @1%

Among the parents in the batch without any fungal treatment, shorter larval duration as desired was observed in B4 (7.82 days) and among F_1, shorter larval duration of 7.05 days was recorded in B8×CSR_4. In segregating generations, among F_2, shorter larval duration of 7.47 days was observed in B4×CSR_4 cross, in first back crosses (BC1) (F_1 crossed with their respective muscardine resistant parents, B1, B4 and B8) shorter larval duration of 7.60 days was recorded in (B1×CSR_4)×B1 and in second back crosses (BC2) (F_1 crossed with susceptible parent CSR4) shorter larval duration of 7.19 days was recorded in (B8×CSR4)×CSR4.

Under *B. bassiana* infection, larval duration among the parents was minimum in B4 breed (8.15 days) and among F_1, shorter larval duration of 7.76 days was recorded in B8×CSR4 hybrid. Among F_2, shorter larval duration of 8.42 days was observed in B8×CSR4 cross, among BC1, the larval duration was minimum in (B8×CSR4)×B8 (8.34 days) and among BC2, shorter larval duration of 7.98 days was observed in (B8×CSR4)×CSR4.

Among all the generations, shorter larval duration under normal condition was observed in F_1 of B8×CSR4, followed by BC2 of (B8×CSR4)×CSR4 and longer larval duration was recorded in F_2 of (B1×CSR4). Similarly, under muscardine inoculation, shorter larval duration was observed in F_1 of B8×CSR4, followed by F_1 (B1×CSR4) and longer larval duration was recorded in CSR4 breed.

In a similar study, four thermotolerant silkworm breeds (B1, B4, B6 and B8) and their F_1 hybrids were inoculated with *B. bassiana,* wherein B6 (10.58 days) breed, B6 x B1 and B6 x B8 (10.50 days each) hybrids showed prolonged larval duration compared to control (Jayashree *et al.,* 2020b). In fungus inoculated batch, the fifth instar larval duration was extended and the extent of prolongation was higher in susceptible breed *i.e.,* CSR4 (8.96 days) and was minimum in F_1 hybrids *i.e.,* B8×CSR4 (7.76 days) and B1×CSR4 (7.79 days). The prolongation of larval duration is due to reduced metabolic activity in the infected silkworms (Janakiraman *et al.,* 1961). Results of the current study are also in conformity with other findings (Manjunath Gowda *et al.,* 2011; Keerthana *et al.,* 2019 and Sahana *et al.,* 2021). Least prolongation of larval duration is desirable while evolving muscardine resistant breeds.

Fifth Instar Larval weight (g)

Fifth instar larval weight among the six generations of crosses between muscardine resistant thermotolerant bivoltine breeds and muscardine susceptible productive bivoltine breed showed significant differences, under both normal condition and muscardine inoculation condition (Table 2).

In the batch without any fungal treatment, among the parents, maximum larval weight of 3.78 g was observed in B1 and in B1×CSR4 (4.12 g) among F_1. Maximum larval weight of 3.65 g was recorded in F_2 generation of B1×CSR4 cross. Among BC1, highest larval weight of 3.80 g was recorded in (B1×CSR4)×B1and in BC2 generation maximum larval weight of 3.53 g was recorded in (B1×CSR4)×CSR4.

In muscardine treated batch (Table 2), larval weight among the parents was significantly higher in muscardine resistant thermotolerant bivoltine breed B4 (2.77 g) and among the F_1, B4×CSR4 recorded highest larval weight (3.30 g). In segregating generations, among the F_2, maximum larval weight of 2.92 g was recorded in B4×CSR4 cross, in (B4×CSR4)×B4 among BC1 with maximum larval weight of 3.32 g and in (B4×CSR4)×CSR4 among BC2 with a larval weight of 2.90 g.

Among all the generations, maximum larval weight under normal condition was observed in F_1 of (B1×CSR4), followed by F_1 of (B4×CSR4) and minimum larval weight was recorded in BC2 (B8×CSR4)×B8. Similarly, maximum larval weight under muscardine inoculation was recorded in BC1 of (B4×CSR4)×B4, followed by F_1 of (B4×CSR4) and minimum larval weight was recorded in BC2 of (B8×CSR4)×CSR4.

Decrease in body weight in *B. bassiana* infected silkworms can be 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/10 larvae, 20.78 g/10 larvae and 20.50 g/10 larvae, respectively) under *B. bassiana* inoculation (Keerthana *et al*., 2020) which supports the present findings. Further, the larval weight was significantly reduced under muscardine inoculation. Among the parents, the tolerant breed B4 lost its body weight from 3.67 g (control) to 2.77 g (muscasrdine treated) and among hybrids B4×CSR4 lost its body weight from 3.98 g (control) to 3.30 g (muscasrdine treated), which were the least reduction in larval body weight. Results of the current study are in conformity with the earlier findings of Jayashree *et al.* (2020) under muscardine inoculation to thermotolerant bivoltine breeds and their hybrids.

Larval Mortality (%)

Significant differences were observed for larval mortality among the six generations of crosses between muscardine resistant thermotolerant bivoltine breeds and muscardine susceptible productive bivoltine breed, under both normal condition and muscardine inoculation (Table 3).

TABLE 3

Larval mortality (%) and effective rate of rearing (%) as affected by muscardine inoculation among six generations of crosses between muscardine resistant thermotolerant bivoltine breeds and muscardine susceptible productive bivoltine breed

Breeds and generations		Larval mortality (%) Control	Larval mortality (%) Muscardine inoculation	ERR (%) Control	ERR (%) Muscardine inoculation
Parents	B1	0.15 [a] (1.072)	56.48 [d] (48.718)	99.85	43.52 [de] (41.246)
	B4	0.18 [a] (1.087)	46.29 [f] (42.851)	99.82	53.71 [c] (47.115)
	B8	0.34 [a] (1.151)	60.33 [cd] (50.95)	99.66	39.67 [ef] (39.014)
	CSR4	0.29 [a] (1.136)	69.83 [a] (56.669)	99.71	30.17 [h] (33.294)
F1	B1 × CSR4	0.00 [a] (1.00)	30.80 [g] (33.687)	100.00	69.20 [ab] (56.281)
	B4 × CSR4	0.00 [a] (1.002)	27.75 [h] (31.647)	100.00	72.25 [a] (58.321)
	B8 × CSR4	0.02 [a] (1.012)	45.43 [f] (42.363)	99.98	54.57 [c] (47.604)
F2	B1 × CSR4	0.06 [a] (1.031)	67.38 [a] (55.263)	99.94	32.62 [h] (34.7)
	B4 × CSR4	0.11 [a] (1.055)	67.15 [a] (55.044)	99.89	32.86 [h] (34.922)
	B8 × CSR4	0.13 [a] (1.063)	67.54 [a] (55.252)	99.88	32.46 [h] (34.718)
BC1	(B1 × CSR4) × B1	0.00 [a] (1.002)	62.85 [bc] (52.43)	99.99	37.15 [fg] (37.535)
	(B4 ×CSR4) × B4	0.03 [a] (1.015)	32.47 [g] (34.534)	99.97	67.53 [b] (55.434)
	(B8 × CSR4)× B8	0.06 [a] (1.026)	66.33 [a] (54.519)	99.95	33.67 [gh] (35.449)
BC2	(B1 × CSR4) × CSR4	0.03 [a] (1.013)	55.15 [e] (47.954)	99.98	44.85 [d] (42.014)
	(B4 × CSR4) × CSR4	0.06 [a] (1.028)	54.85 [e] (47.766)	99.94	45.15 [d] (42.201)
	(B8× CSR4) × CSR4	0.11 [a] (1.052)	55.22 [e] (47.979)	99.89	44.79 [d] (41.99)
F test		*	**	NS	**
S.Em±		0.029	1.802	0.068	1.802
CD @5%		0.084	5.215	-	5.214
CV (%)		4.826	6.592	0.117	7.324

#- Figures in parentheses are square root transformed values ; $-Figures in parentheses are angular transformed values ; Figures with same superscript are statistically on par ; *-Significant @5%; ** - Significant @1%; NS-Non-significant

In the batch without any fungal treatment, significantly least larval mortality of 0.15 per cent was recorded in B1 breed among the parents, while no larval mortality was observed in B1×CSR4 and B4×CSR4 hybrids among F1. In segregating generations, among F2, least larval mortality of 0.06 per cent was recorded in B1×CSR4 cross, while among BC1, no larval mortality was recorded in (B1×CSR4)×B1 cross and among BC2, least larval mortality of 0.03 per cent was recorded in (B1×CSR4)×CSR4.

Under *B. bassiana* infection, larval mortality among the parents was significantly least in muscardine resistant thermotolerant bivoltine breed B4 (46.29%) and among the F1, B4×CSR4 recorded least larval mortality of 27.75 per cent. Among the F2, minimum larval mortality of 67.15 per cent was recorded in B4×CSR4 cross, among BC1,in (B4×CSR4)×B4 crosswith a least larval mortality of 32.47 per cent and among BC2, minimum larval mortality of 54.85 per cent was recorded in (B4×CSR4)×CSR4 cross.

Among all the generations, no larval mortality under normal condition was observed in B1×CSR4 (F_1), B4×CSR4(F_1) and BC1 (B1×CSR4)×B1, while least larval mortality was recorded in the hybrid B8×CSR4 and maximum larval mortality was recorded in B8 breed. Similarly, under muscardine inoculation least larval mortality was observed in F_1 of B4×CSR4, followed by F_1 of B8×CSR4 and maximum larval mortality was recorded in CSR4 breed.

Though all the stages of silkworm are susceptible to the muscardine disease, fifth instar larvae were the most susceptible as they show high larval mortality (Venkataramana Reddy, 1978). According to Sreejith (2019), when eight thermotolerant bivoltine silkworm breeds *viz.,* B1, B2, B3, B4, B5, B6, B7 and B8 were inoculated with *B. bassiana*, maximum larval mortality was recorded in 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%). The thermotolerant bivoltine breeds and their crosses used in this study also exhibited variation in the larval mortality due to fungal infection. B4 and its crosses showed minimum larval mortality when infected with fungal pathogen.

Effective Rate of Rearing (%)

Effective rate of rearing (ERR) among the six generations of crosses between muscardine resistant thermotolerant bivoltine breeds and muscardine susceptible productive bivoltine breed showed non-significant differences under normal condition and significant differences under muscardine inoculation (Table 3).

In un-inoculated batches, 100 per cent ERR was observed in F_1 hybrids of B1×CSR4 and B4×CSR4 though it showed non-significant difference. Minimum ERR was recorded in B8 (99.66%) and CSR4 (99.71%) breeds.

In muscardine treated batch, ERR was significantly highest in the thermotolerant bivoltine breed B4 among the parents with 53.71 per cent. Among the F1, B4×CSR4 hybrid recorded highest ERR (72.25%). Among the F2, B4×CSR4 cross recorded highest ERR of 32.86 per cent, among BC1,(B4×CSR4)×B4 recorded maximum ERR of 66.53 per cent and among BC2, (B4×CSR4)×CSR4 recorded highest ERR of 45.15 per cent.

Previously, when eight races of silkworms *viz.,* Pure Mysore, Hosa Mysore II, C. Nichi, HS6, NN6D, NB4D2, 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 %) breed (Keerthana *et al.,* 2020). So also, the breed B4 performed better with respect to ERR under *B. bassiana* inoculation in the present study. From the present results, it is clear that the performance of hybrids was superior with respect to ERR and that the F_1 hybrids B4×CSR4 and B1×CSR4 recorded higher ERR. Results of the current study are also in conformity with the earlier findings of Jayashree *et al.* (2020) and Sahana *et al.* (2021), who reported the better performance of hybrids and B4 and B1 breeds, respectively, under *B. bassiana* infections.

Cocoon Yield by Number (per 10,000 worms)

Significant differences were observed for cocoon yield by number (per 10,000 worms) among the six generations of crosses between muscardine resistant thermotolerant bivoltine breeds and muscardine susceptible productive bivoltine breed, under both normal and muscardine inoculation condition (Table 4).

In non-inoculated batches, among the parents, B1 breed recorded highest cocoon yield by number (9,985.17 / 10,000 worms) and among F_1, highest cocoon yield by number (10,000 cocoons /10,000 worms) was recorded in the hybrid B1×CSR4. In segregating generations, among F_2, maximum cocoon yield by number of 9,993.83 / 10,000 worms was recorded in B1×CSR4 cross. Among BC1, highest cocoon yield by number was recorded in (B1×CSR4)×B1(9,999.67/10,000 worms) and among BC2, maximum cocoon yield by number was recorded in (B1×CSR4)×CSR4(9,997.50/10,000 worms).

In muscardine inoculated batch, among the parents, the thermotolerant bivoltine breed B4 recorded significantly highest cocoon yield by number (6,175.50 /10,000 worms) and among the F_1, B4×CSR4 hybrid recorded highest cocoon yield by number (7,278.00/

TABLE 4

Cocoon yield by number (per 10,000 worms) and weight (kg/10,000 worms) as affected by muscardine inuculation among six generations of crosses between muscardine resistant thermotolerant bivoltine breeds and muscardine susceptible productive bivoltine breed

Breeds and generations		Cocoon yield by number (per 10,000 worms)		Cocoon yield by weight (kg/10,000 worms)	
		Control	Muscardine inoculation	Control	Muscardine inoculation
Parents	B1	9985.17 [abc]	5766.67 [cd]	19.03 [bc]	8.82d [ef]
	B4	9981.67 [abc]	6175.50 [bc]	18.57 [cd]	9.97 [cde]
	B8	9965.83 [c]	4554.67 [e]	15.35 [f]	8.38d [ef]
	CSR4	9970.50 [bc]	2915.00 [gh]	17.80 [de]	5.07 [g]
F1	B1 × CSR4	10000.00 [a]	6886.17 [ab]	20.10 [a]	12.27 [abc]
	B4 × CSR4	9999.83 [a]	7278.00 [a]	19.79 [ab]	14.33 [a]
	B8 × CSR4	9997.50 [a]	6616.67 [abc]	14.00 [g]	8.89 [def]
F2	B1 × CSR4	9993.83 [a]	4833.15 [de]	18.98 [bc]	11.05 [bcd]
	B4 × CSR4	9988.67 [ab]	5804.44 [cd]	17.82 [de]	11.37 [bcd]
	B8 × CSR4	9987.33 [ab]	3933.33 [efg]	10.75 [i]	6.88 [fg]
BC1	(B1 × CSR4) × B1	9999.67 [a]	4069.53 [ef]	18.73 [c]	9.43 [cdef]
	(B4 × CSR4) × B4	9997.00 [a]	4343.20 [e]	17.55 [e]	13.43 [ab]
	(B8 × CSR4) × B8	9994.33 [a]	3211.00 [fgh]	15.1 [f]	9.50 [cdef]
BC2	(B1 × CSR4) × CSR4	9997.50 [a]	2893.60 [gh]	19.03 [bc]	10.43 [cde]
	(B4 × CSR4) × CSR4	9994.17 [a]	3215.70 [fgh]	17.30 [e]	11.10 [bcd]
	(B8 × CSR4) × CSR4	9989.17 [ab]	2252.49 [h]	11.78 [h]	7.50 [efg]
F test		*	**	**	**
S.Em±		6.715	362.936	0.2668	0.8908
CD @ 5 %		19.343	1045.494	0.7687	2.5662
CV (%)		0.116	13.456	2.722	15.583

Figures with same superscript are statistically on par ; *-Significant @5%; ** - Significant @1%

10,000 worms). In segregating generations, among the F_2, maximum cocoon yield by number was recorded in B4×CSR4 (5,804.44/10,000 worms), whereas, among BC1, maximum cocoon yield by number was recorded in (B4×CSR4)×B4(4,343.20/10,000 worms) and among BC2, in (B4×CSR4)×CSR4 (3,215.70/ 10,000 worms).

Among the generations, highest cocoon yield (by number) under normal condition was observed in F_1 of B1×CSR4, followed by F_1 of B4×CSR4 and least cocoon yield (by number) was recorded in B8 breed. Similarly, highest cocoon yield (by number) under muscardine inoculation was observed in F_1 of B4×CSR4, followed by F_1 of B1×CSR4 and least cocoon yield (by number)S was recorded in BC2 of (B8×CSR4)×CSR4.

Inoculation of eight races of silkworm with nine conidial concentrations (10^1 - 10^9 spores/ml) of *B. bassiana* has resulted in no cocoons at the highest concentrations and 48 to 78 per cent in the case of the batches with the lower concentration (Raghavaiah and Jayaramaiah, 1990). The breeds, B4 (9,066.7 cocoons /10,000 silkworms) and B1 (8,033.33 cocoons / 10,000 silkworms), recorded highest cocoon yield (by number) under *B. bassiana* inoculation (Sahana *et al.*, 2021). Keerthana *et al.* (2020) 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. Similar trend was observed in the present study and among the parent, B4 (6,175.50 / 10,000 worms) and among hybrid, B4×CSR4 (7,278.00 / 10,000 worms) recorded significantly highest cocoon yield by number. Results of the current study are also in conformity with the earlier findings of Jayashree *et al.* (2020a) under *Beauveria bassiana* infections, who also reported better performance of certain B-series hybrids.

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

Cocoon yield by weight among the six generations of crosses between muscardine resistant thermotolerant bivoltine breeds and muscardine susceptible productive bivoltine breed showed significant differences, both under normal condition and muscardine inoculation (Table 4).

In the batch without any fungal treatment, among the parents, B1 recorded significantly highest cocoon yield by weight of 19.03 kg/10,000 worms and among F_1, highest cocoon yield by weight of 20.10 kg/10,000 worms was observed in the hybrid, B1×CSR4. In segregating generations, among F_2 maximum cocoon yield by weight of 18.98 kg/10,000 worms was recorded in B1×CSR4 cross, among BC1, highest cocoon yield of 18.73 kg/10,000 wormswas recorded in (B1×CSR4)×B1 cross and among BC2, maximum cocoon yield of 19.03 kg/10,000 worms was recorded in (B1×CSR4)×CSR4 cross.

Under *Beauveria bassiana* infection, cocoon yield by weight was significantly different among the generations (Table 4). Among the parents, the thermotolerant bivoltine breed B4 recorded highest cocoon yield by weight (9.97 kg/10,000 worms). Among the F_1, B4×CSR4 hybrid recorded highest cocoon yield of 14.33 kg/10,000 worms, among the F_2, maximum cocoon yield of 11.37 kg/10,000 worms was recorded in B4×CSR4 cross, among BC1, maximum cocoon yield by weight of 13.43 kg/10,000 worms was recorded in (B4×CSR4)×B4 and among BC2, highest cocoon yield of 11.10 kg/10,000 worms was recorded in (B4×CSR4)×CSR4.

Among all the six generations, highest cocoon yield by weight under normal condition was observed in B1×CSR4F_1 hybrid, followed by B4×CSR4 F_1 hybrid and least cocoon yield by weight was recorded in F_2 of B8×CSR4 cross, Similarly, highest cocoon yield by weight under Muscardine inoculation was recorded in F_1 of B4×CSR4, followed by BC1 of (B4×CSR4)×B4 cross and least cocoon yield was recorded in CSR4 breed.

When four thermo tolerant bivoltine breeds *viz.,* B1, B4, B6, B8 and their hybrids, were inoculated with *B. bassiana*, significantly highest cocoon yield by weight was recorded in B1 (867.00 g/1000 worms) among parents and in B1×B8 (960.47 g/1000 worms) among hybrids (Jayashree, 2019). These observations are in conformity with the present findings, that inoculation of fungal spore to the thermotolerant bivoltine breeds and generations showed significantly highest cocoon yield by weight in B4 among parents and in B4×CSR4 among hybrids. The thermotolerant bivoltine silkworm breeds B4, B6 and B8 recorded highest cocoon yield by weight when Keerthana *et al.* (2019) inoculated them with *B. bassiana* spores, which might be due to their ability to spin good cocoons even under infected condition. According to Sreejith (2019), among the eight thermotolerant bivoltine breeds (B1 to B8), significantly highest cocoon yield by weight under *B. bassiana* infection was recorded in B4, followed by B1.

Since effective rate of rearing indicates the survival ability in the larval stage, reduction in ERR due to muscardine infection over control and deviation in ERR among muscardine infected batches over the mean ERR due to infection was calculated and presented in a bi-plot (Fig. 1). The breeds and their crosses which occupy the extreme right corner of the plot, have least per cent reduction in ERR over control and also show maximum positive deviation from mean ERR under muscardine infection, indicating their resistance to muscardine disease. Accordingly, among the crosses F_1 of B4×CSR4 followed by F_1 of B1×CSR4 shows

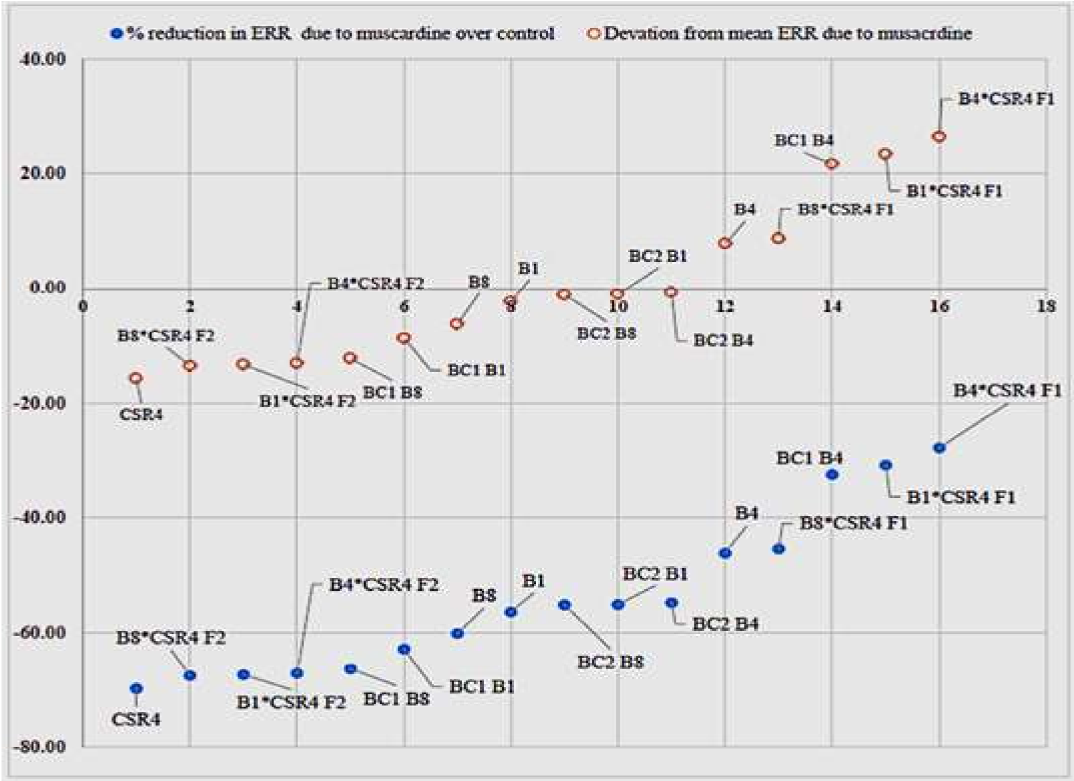

Fig. 1 : *Per-se* performance of parents and crosses for ERR. Y-axis represents % reduction in ERR due to muscardine over control, and deviation from mean ERR due to muscardine. X-axis represents the base line at zero per cent reduction as well zero deviation in ERR. The breeds or crosses above X-axis have positive deviation from mean ERR due to muscardine and hence show higher relative resistance.

better resistance to muscardine inoculation. Similarly, among parents B4 shows better resistance to muscardine infection. The breeds or crosses which occupy extreme left of the bi-plot indicate their susceptibility to muscardine fungal infection.

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