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Performance of Thermotolerant Bivoltine Silkworm Breeds for Larval Growth and Cocoon Yield Parameters under *Beauveria bassiana* Infection

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Abstract

An experiment to study the performance of thermotolerant bivoltine silkworm breeds under *Beauveria bassiana* infection for larval growth and cocoon yield was conducted at the Department of Sericulture, UAS, GKVK, Bengaluru. Ten thermotolerant silkworm breeds *viz*., B1, B2, B3, B4, B5, B6, B7, B8, APS12 and APS45 along with CSR_2 as control were used in the experiment. The fifth instar silkworms were topically inoculated with different dilutions of the fungal spore suspension of *Beaveria bassiana viz.,* stock (1.45×10^5), 10^{-1} to 10^{-5}. The results revealed that the thermotolerant bivoltine silkworm breed B4 performed significantly better than any breed at 10^{-3} and 10^{-4} fungal spore dilution for the parameters studied, *viz*., ERR, fifth instar larval weight, cocoon yield by number, cocoon yield by weight. However, at 10^{-5} fungal spore dilutions, B8 performed better, though B6 and CSR_2 performed variedly for larval characters.

Keywords: Silkworm, *Bombyx*, Bivoltine, Thermotolerance, *Beauveria bassiana*

The success of the sericulture industry depends on several variables that include both biotic and abiotic factors. Among the biotic factors pathogenic microorganisms like fungi, protozoa, bacteria, viruses and rickettsia cause infectious diseases in silkworm. Fungi constitutes the major group and cause mycosis or muscardine. In India, 10-40 per cent of loss has been accounted for white muscardine of the total loss due to diseases (Chandrasekharan and Nataraju, 2008). Among the abiotic factors, temperature plays a major role on growth and productivity of silkworm and silk yield is adversely affected by high temperature condition prevailing during summer in tropical countries like in India. Rearing bivoltine silkworm breeds / hybrids is the way to produce internationally competitive quality silk. However, these breeds are susceptible to fluctuating environmental conditions, poor quality mulberry leaves and unhygienic rearing conditions and hence, evolving bivoltine silkworm breeds which can tolerate high temperature and disease incidence becomes imperative. Under these context, CSR&TI, Mysore and APSSRDI, Hindupur have evolved thermo tolerant bivoltine silkworm breeds adoptable to temperature fluctuations and high temperature conditions prevailing during summer. Thus such breeds being suitable for summer rearing, their tolerance to white muscardine caused by *Beauveria bassiana* (Bals) Vuill. which occurs during winter when fluctuations in temperature in more than in summer is unknown. Hence, to look into the possibilities of multiple / dual stress tolerance among thermotolerant bivoltine silkworms, it was envisaged to study their tolerance level to white muscardine disease using LC_{50} value and a few growth and yield parameters.

Material and Methods

The performance of thermotolerant bivoltine silkworm breeds under *Beauveria bassiana* infection for larval growth and cocoon yield was studied at the Department of Sericulture, UAS, GKVK, Bengaluru during 2017-2018. Ten thermotolerant bivoltine silkworm breeds (Eight *viz*., B1, B2, B3, B4, B5, B6, B7 and B8 from CSRTI, Mysore and two breeds *viz*., APS12 and APS45 from APSSRDI, Hindupur) along with CSR_2 as control were utilized for conducting the present study. The characteristic feature of these breeds in depicted in Table 1. Rearing of each breed was conducted up to fourth instar by following standard rearing practices, by feeding on V1 mulberry leaves (Dandin *et al*., 2001). Newly ecdysed fifth instar larvae

TABLE 1

Larval and cocoon features of the thermotolerant breeds used in the experiments

Breeds	Breed characters
B1	Plain larva spinning oval shaped cocoon
B2	Plain larva spinning oval shaped cocoon
B3	Plain larva spinning oval shaped cocoon
B4	Plain larva spinning oval shaped cocoon
B5	Marked larva spinning peanut shaped cocoon
B6	Marked larva spinning peanut shaped cocoon
B7	Marked larva spinning peanut shaped cocoon
B8	Marked larva spinning peanut shaped cocoon
APS12	Plain larva spinning peanut shaped cocoon
APS45	Plain larva spinning peanut shaped cocoon
CSR_2 (Control)	Plain larva spinning oval shaped cocoon

(50 worms per replication in three replications each) were topically inoculated with different dilutions of the fungal spore suspension *i.e.*, stock (1.45×10^5), 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} at the rate of 0.5 ml per worm by spraying with an automizer (Venkataramana Reddy, 1978). High relative humidity of 95 ± 5 per cent and a temperature of 25 ± 1ºC were maintained in the rearing room. Control batch was also maintained by spraying with distilled water. White muscardine incidence was recorded for ten days post inoculation. Ripe silkworms were shifted to the mountages for spinning at ambient temperature of 25 ± 1˚C and 65 ± 5 per cent RH.

Observations on LC50, LT50 and the silkworm rearing and cocoon parameters *viz.,* ERR, fifth instar larval weight, cocoon yield by number and cocoon yield by weight were recorded. The data obtained were analysed using completely randomized design (Sundarraj *et al*., 1972). The per cent data was analysed after transformation by using the formula $\sin^{-1}\sqrt{p/100}$. The zero values in the data obtained when treated with different spore concentrations was analysed after $\sqrt{x+1}$ transformation, to normalize the distribution. The mean values of the experiments were compared by using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

LC_{50}

Ten thermotolerant bivotine silkworm breeds *viz.,* B1, B2, B3, B4, B5, B6, B7, B8, APS12 and APS45 and one commercial bivotine silkworm breed *viz.,* CSR2 were employed for the study (Table 2). These breeds when treated immediately after fourth moult with different dilutions of *Beauveria bassiana* spores *i.e.,* stock, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} spores per ml, B4 breed showed highest LC_{50} value (68,625.71 spores/ml), followed by B1 (67,648.73 spores/ml) and B8 (66,428.85 spores/ml). Whereas, B2, B3, APS12 and APS45 breeds showed lowest LC_{50} value (63,250.71 spores/ml). The LC_{50} value of thermotolerant bivoltine silkworm breeds revealed that, the B4 breed being relatively more tolerant to *B. bassiana* infection. The lethal concentration of the fungus, *B. bassiana* required for 50 per cent mortality was maximum in C-nichi during both fourth (7.71×10^2 spores / ml) and fifth (4.73×10^2 spores / ml) instars, while the same was minimum in NB_{18} (1.66×10^2 and 0.65×10^2 spores / ml during fourth and fifth instars, respectively) indicating their varied degree of susceptibility to the infection. The results further confirmed that the fifth instar larvae of all the races were highly susceptible to *B. bassiana* compared to fourth instar larva. Earlier findings have shown that the median lethal concentration (LC_{50}) of *A. flavus* spores increased with increase in instars. In the present study it was also observed that, the thermotolerant bivoltine breeds possessed varied degree of tolerance to *B. bassiana* infection and that thermotolerant breeds like B4, B1 and B8 could be tolerant to the fungal infection as well.

LT_{50}

Time taken for 50 per cent mortality (LT_{50}) among ten thermotolerant silkworm breeds *viz.,* B1, B2, B3, B4, B5, B6, B7, B8, APS12 and APS45 along with CSR_2 as control breed inoculated by *B. bassiana* at different doses are presented in Table 3. Among different doses, 10^{-1}, 10^{-3} and 10^{-4} dilutions showed significant differences for LT_{50} value. At 10^{-1} dilution the B4 breed showed significantly highest LT_{50} value (5.56 days), followed by B5 (5.26 days) and CSR_2

TABLE 2

LC_{50} value for *B. bassiana* inoculation among thermotolerant bivoltine silkworm breeds

Breeds	LC_{50} (spores / ml)	Upper Limit (spores / ml)	Lower Limit (spores / ml)	Regression equation
B1	67,648.73	71,566.77	63,945.18	y=1.3201x-0.7010
B2	63,250.17	68,028.02	58,807.87	y=1.3613x-0.6059
B3	63,250.17	68,028.02	58,807.87	y=1.3613x-0.6059
B4	68,625.71	72,368.92	65,076.09	y=1.3098x-0.7248
B5	64,430.32	68,965.67	60,193.21	y=1.3051x-0.6297
B6	65,554.35	69,866.83	61,508.04	y=1.3407x-0.6534
B7	66,096.49	70,304.28	62,140.54	y=1.3355x-0.6653
B8	66,428.85	70,607.38	62,497.60	y=1.3298x-0.6671
APS12	63,250.17	68,028.02	58,807.87	y=1.3613x-0.6059
APS45	63,250.17	68,028.28	58,807.87	y=1.3613x-0.6059
CSR_2(Control)	65,058.05	69,504.26	60,896.25	y=1.3247x-0.6373

TABLE 3

LT_{50} of thermotolerant bivoltine silkworm breeds inoculated by different doses of *B. bassiana* spores

Breeds	LT_{50} (days)					
	Stock	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
B1	4.37 ^{ab}	4.73 ^{c}	5.79 ^{b}	5.75 ^{bc}	5.84 ^{ab}	6.94 ^{c}
B2	3.74 ^{a}	4.47 ^{bc}	5.29 ^{ab}	5.50 ^{a}	5.67 ^{ab}	5.36 ^{ab}
B3	3.45 ^{a}	3.53 ^{a}	4.60 ^{a}	4.84 ^{a}	5.53 ^{ab}	5.44 ^{abc}
B4	5.30 ^{b}	5.56 ^{e}	5.79 ^{b}	6.63 ^{d}	6.41 ^{b}	5.59 ^{bc}
B5	3.73 ^{a}	5.26 ^{de}	5.21 ^{ab}	6.24 ^{cd}	4.84 ^{a}	6.58 ^{bc}
B6	4.06 ^{a}	4.73 ^{c}	5.80 ^{b}	6.17 ^{cd}	5.59 ^{ab}	6.18 ^{bc}
B7	3.91 ^{a}	4.98 ^{cde}	5.85 ^{b}	6.18 ^{cd}	6.08 ^{b}	5.49 ^{abc}
B8	4.15 ^{a}	4.97 ^{cd}	5.31 ^{ab}	5.72 ^{bc}	6.21 ^{b}	6.77 ^{bc}
APS12	4.03 ^{a}	3.78 ^{a}	4.72 ^{a}	5.70 ^{bc}	4.93 ^{a}	5.52 ^{abc}
APS45	3.35 ^{a}	3.62 ^{a}	4.55 ^{a}	4.49 ^{a}	4.71 ^{a}	4.51 ^{a}
CSR_2(control)	3.95 ^{a}	5.14 ^{cde}	5.03 ^{a}	5.18 ^{a}	6.48 ^{b}	6.48 ^{bc}
F-test	NS	*	NS	*	*	NS
SEm±	-	0.26	-	0.37	0.39	-
CD at 5%	-	0.77	-	1.10	1.14	-
CV (%)	17.54	9.94	11.29	11.45	11.84	14.84

*- Significant at 5%; NS-Non significant; $-Figures in parentheses are transformed values and with same superscript are on par; Stock-1.45×10^5 spores/ml.

Note: *B.bassiana* infection was done immediately after fourth moult. Each treatment comprised of three replications with 50 silkworms each.

(5.14 days), while the least LT_{50} was recorded in B3 (3.53 days), followed by APS45 (3.62 days). Similarly, at 10^{-3} dilution, the breed B4 showed significantly highest LT_{50} (6.63 days), followed by B5 (6.24 days) and significantly least LT_{50} value was observed in APS45 (4.49 days). At 10^{-4} dilution, the CSR_2 breed recorded significantly highest LT_{50} value (6.48 days), followed by B4 (6.41 days) and B8 (6.21 days), while the lowest LT_{50} was recorded in APS45, B5 and APS12 (4.71 days, 4.84 days and 4.93 days, respectively). Although, non-significant differences for LT_{50} at stock, 10^{-2} and 10^{-5} fungal spore dilutions were observed, at stock the breed B4 scored highest LT_{50} value (5.30 days), followed by B1 (4.37 days) and B8 (4.15 days) and least LT_{50} was recorded in breed APS45 (3.35 days), followed by B3 (3.45 days). At 10^{-2} dilution, the highest LT_{50} of 5.85 days was observed in breed B7, followed by B6 (5.80 days) while the least LT_{50} was recorded in APS45 (4.55 days), followed by B3 (4.60 days). Thermotolerant breed B1 scored highest LT_{50} value (6.94 days), followed by B8 (6.77 days) at 10^{-5} spore dilution. However, lowest LT_{50} value was recorded in breed B2 (5.36 days), followed by APS45 (4.51 days). The hydrid PM×KA when treated with different dilution of *Beauveria bassiana i.e.*, 10^{-9} to 10^{-2} showed highest LT_{50} (9.94 days) at 10^{-2} spore dilution and lowest LT_{50} (4.95 days) at 10^{-9} spore dilution. It was found that LT_{50} increases with decrease in spore concentrations used to inoculate different larval instars. Thus LT_{50} showed indirect relationship with concentration of spore suspension as it increased with decrease in spore concentration. In the present study, similar trend was observed wherein, the thermotolerant bivoltine silkworm breeds B4, B8, B7 and B5 showed higher LT_{50} values at all spore dilutions and that the LT_{50} value increased with decrease in spore dilutions, indicating that they could be better tolerant to fungal infection.

Effective rate of rearing (%):

The effective rate of rearing based on fifth instar initial larval number showed significant differences among the thermotolerant bivoltine silkworm breeds treated with different doses of *B. bassiana* (Table 4). At stock, 10^{-1}, 10^{-2} dilutions of fungal spores, no survivors were observed and hence ERR was not recorded. However, at the dilution of 10^{-3} survivors were observed in B1, B4, B5, B6, B7, B8, and CSR_2 breeds only and at 10^{-4} and 10^{-5} spore dilutions survivors were observed in all the breeds. At 10^{-3} dilution, significantly highest ERR was recorded in breed B4 (12.67 %), followed by B1 (10.00 %) and B8 (5.33 %), whereas, significantly least ERR was observed in CSR_2 (2.67 %), followed by both B6 and B7 (4.67 %). At 10^{-4} spore dilutions significantly highest ERR was observed in breed B4 (14.67 %), followed by both B6 and B8 (14.00 %) and significantly lowest ERR was observed in APS12 (1.33 %), followed by B3 (2.67 %). Similarly, at 10^{-5} dilution the breed B8 showed significantly highest ERR (28.00 %), followed by both B6 and B7 (22.00 %) and B2 (20.00 %). Significantly lowest ERR was recorded in APS45 (6.66%) followed by breed B3 (10.00 %). In control batches *i.e.*, without fungal inoculation cent per cent ERR based on fifth instar larval population was recorded in all breeds.

In earlier studies when eight silkworm races were inoculated with nine conidial concentrations (10^1-10^9 spores/ml) of *B. bassiana*, no cocoons were formed at the two highest concentrations (10^9-10^8), as compared with 96.67 per cent-100 per cent cocoons formed in the batches with no treatment (Control batch) and 48-78 per cent in the case of the batches with the lowest concentration. From the present results it was clearly noticed that, B4 and B8 breeds showed higher ERR at different fungal dilutions indicating it could be tolerant to the fungal infection and APS45 scored lowest ERR in all the dilutions, which indicates its susceptibility to muscardine.

Fifth instar larval weight (g / 10 worms)

Significant differences were observed for fifth instar larval weight of thermotolerant bivoltine silkworm breeds infected with different dilutions of *B. bassiana* (Table 4). Because of the presence of high spore concentrations at stock, 10^{-1} and 10^{-2} dilutions of *B. bassiana*, resulted in cent per cent mortality and hence, larval weight could not be recorded. At 10^{-3} spore dilution thermotolerant bivoltine silkworm breed B4 recorded significantly highest larval weight (19.23 g),

TABLE 4

ERR (%) and fifth instar larval weight (g/10 worms) of thermotolerant bivoltine silkworm breeds infected by different doses of *Beauveria bassiana* spores

Breeds	ERR (%)$							Fifth instar larval weight (g/10 worms)$						
	Stock	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Control	Stock	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Control
B1	0	0	0	10 ^{d} (3.24)	9.00 ^{c} (3.02)	14.00 ^{bc}	100	0	0	0	18.34 ^{a} (4.34)	18.99 ^{ab} (4.41)	19.45 ^{ab}	41.22 ^{abc}
B2	0	0	0	0 ^{a} (0.70)	3.33 ^{b} (1.94)	20.00 ^{de}	100	0	0	0	18.15 ^{a} (4.31)	18.94 ^{ab} (4.40)	20.60 ^{bc}	41.40 ^{abc}
B3	0	0	0	0 ^{a} (0.70)	2.67 ^{ab} (1.76)	10.00 ^{ab}	100	0	0	0	18.20 ^{a} (4.32)	18.26 ^{a} (4.33)	18.70 ^{a}	38.68 ^{a}
B4	0	0	0	12.67 ^{d} (3.61)	14.67 ^{c} (3.86)	17.33 ^{cd}	100	0	0	0	19.23 (4.44)	20.54 ^{c} (4.58)	20.46 ^{abc}	43.43 ^{cd}
B5	0	0	0	2.00 ^{b} (1.46)	10.00 ^{c} (3.04)	14.66 ^{bcd}	100	0	0	0	17.52 ^{a} (4.24)	17.94 ^{a} (4.29)	20.25 ^{abc}	41.15 ^{abc}
B6	0	0	0	4.67 ^{c} (2.26)	14.00 ^{c} (3.80)	22.00 ^{e}	100	0	0	0	17.92 ^{a} (4.29)	20.01 ^{bc} (4.52)	21.97 ^{cd}	40.25 ^{ab}
B7	0	0	0	4.67 ^{c} (2.26)	11.33 ^{c} (3.41)	22.00 ^{e}	100	0	0	0	18.09 ^{a} (4.31)	18.36 ^{a} (4.34)	21.14 ^{bcd}	42.17 ^{bc}
B8	0	0	0	5.33 ^{c} (2.31)	14.00 ^{c} (3.78)	28.00 ^{f}	100	0	0	0	18.29 ^{a} (4.33)	18.08 ^{a} (4.31)	21.12 ^{bcd}	41.33 ^{abc}
APS12	0	0	0	0 ^{a} (0.70)	1.33 ^{ab} (1.17)	12.00 ^{ab}	100	0	0	0	19.13 ^{a} (4.43)	18.33 ^{a} (4.33)	21.40 ^{bcd}	41.14 ^{abc}
APS45	0	0	0	0 ^{a} (0.70)	0 ^{a} (0.70)	6.66 ^{a}	100	0	0	0	18.30 ^{a} (4.33)	18.78 ^{a} (4.39)	21.11 ^{bcd}	40.07 ^{ab}
CSR_2(control)	0	0	0	2.67 ^{bc} (1.76)	10.33 ^{c} (3.25)	10.66 ^{ab}	100	0	0	0	19.11 ^{a} (4.42)	20.78 ^{c} (4.61)	22.57 ^{d}	45.60 ^{cd}
F-test	NA	NA	NA	*	*	*	NS	NA	NA	NA	*	*	*	*
SEm±	-	-	-	0.22	0.36	1.95	-	-	-	-	0.02	0.04	0.61	1.05
CD at 5%	-	-	-	0.64	1.07	5.73	-	-	-	-	0.074	0.13	1.81	3.08
CV (%)	-	-	-	21.35	23.52	20.97	-	-	-	-	1.01	1.76	5.15	4.39

*- Significant at 5%; NS-Non significant; $-Figures in parentheses are transformed values and with same superscript are on par; Stock- 1.45×10^{5} spores/ml.

Note: *B.bassiana* infection was done immediately after fourth moult. Each treatment comprised of three replications with 50 silkworms each.

followed by APS12 and CSR_2 (19.13 g and 19.11g, respectively) while, lowest larval weight was recorded in B5 (17.52g), followed by B6 (17.92g). At 10^{-4} fungal spore dilution, significantly highest larval weight of 20.78 g was recorded in CSR_2 breed, followed by B4 (20.54 g) and B6 (20.01 g) and significantly lowest larval weight was reported in APS12 (18.33 g), followed by B8 and B5 (18.08 g and 17.94 g respectively). At 10^{-5} spore dilution, non-thermotolerant bivoltine silkworm breed CSR_2 exhibited significantly highest larval weight of 22.57 g, followed by thermotolerant bivoltine breed B6 (21.97 g) and B4 (20.46g). Significantly least larval weight was recorded in B3 breed followed by B5 (18.70 g and 20.25 g, respectively). In control batch, significantly maximum larval weight of 45.60 g was produced in breed CSR_2, followed by B4 and B7 (43.43 g and 42.17 g, respectively) and significantly lowest larval weight was recorded in B3 (38.68 g), followed by APS45 (40.07 g).

The muscardine infected larva is known to loose its body weight from third day onwards due to cessation of feeding. Reduction in the grown up larval weight in fifth instar of *B. mori*, infected with *B. Bassiana* which may be due to the consequence of fungal infection that led to decrease in food consumption, digestion, relative consumption rate and efficiency of conversion of ingested food. Rajitha and Savithri (2015) reported that silkworm hybrid PM × CSR_2 treated with sub-lethal concentration of *B. bassiana* conidial suspension (2.15×10^6 spores / ml), exhibited significant reduction in matured larval weight (2.08 g) compared to control (2.58 g). In the present findings maximum larval weight at different dilutions was recorded in B4, followed by B6 and APS12 compared to other breeds, which might be due to greater immune resistant to fight and inhibit the growth and multiplication of pathogen spores. All the breeds showed reduced fifth instar larval weight under fungal infection compared to non-inoculated control batches.

Cocoon yield by number (No. / 1000 worms)

Cocoon yield by number showed significant differences among the thermotolerant bivoltine silkworm breeds inoculated with different spore dilutions of *B. bassiana* (Table 5). In all the breeds evaluated, no survivors were observed at stock, 10^{-1} and 10^{-2} fungal spore dilutions and hence cocoon yield by number could not be recorded. However, survivors were observed in all the breeds except in B2, B3, APS12 and APS45 at 10^{-3} dilution and except APS45 at 10^{-4}. At 10^{-5} survivors were observed in all the breeds. At 10^{-3} dilution, the breed B4 recorded significantly highest cocoon yield by number (133.33 / 1000 worms), followed by B1 (106.67 /1000 worms) and both B7 and B8 (66.67 /1000 worms). However, significantly lowest cocoon yield by number was observed in both B5 and CSR_2 breeds (26.67 /1000 worms) followed by B6 (53.33 /1000 worms). At 10^{-4} dilution, significantly highest cocoon yield by number (173.33 /1000 worms) was recorded in B4 breed, followed by both B6 and B8 (153.33/1000 worms), while significantly lowest cocoon yield by number was recorded in APS12 (13.33 /1000 worms), followed by B3 breed (60.00 /1000 worms). At 10^{-5} spore dilution the breed B8 reported significantly maximum cocoon yield (286.66 /1000 worms), followed by both B6 and B7 (220.00 /1000 worms). Significantly lowest cocoon yield by number was observed in APS45 breed (66.66 No/1000 worms), followed by B3 (100.00 /1000 worms). In non-inoculated control batches 1000 cocoons per 1000 worms were recovered in all the breeds. In the present study, cocoon formation was significantly superior in non-inoculated batches compared to different spore doses. As the dosage increased, cocoon formation decreased considerably and no cocoon formation occurred at stock, 10^{-1} and 10^{-2} dilutions of *B. bassiana* spores. In the earlier studies, the breed NB_4D_2 was treated with different dilutions of fungal spore concentrations (10^9 to 10^1), highest percentage of cocoon (65.33%) formation was observed at 10^1 spore concentration and no cocoons were formed at 10^9 and 10^8 spore concentrations. The cocoon formation significantly decreased with increase in spore concentration. Nataraju (2008) could not recover any cocoons at two highest fungal spore concentrations, as compared with 96.67 per cent to 100 per cent cocoons formed in the batches with no treatment and 48-78 per cent in the case of the batches with the lowest fungal spore concentration. They also found that among the bivoltine breeds, NB_4D_2 was the least susceptible and NB18 and NB7, the most

TABLE 5

Cocoon yield by number per 1000 worms and cocoon yield by weight (g/1000 worms) of thermotolerant bivoltine silkworm breeds infected by different doses of *Beauveria bassiana* spores.

Breeds	Cocoon yield by number per 1000 worms							Cocoon yield by weight (g/1000 worms)						
	Stock	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Control	Stock	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Control
B1	0	0	0	106.67 ^{d} (10.34)	126.67 ^{c} (11.22)	140.00 ^{bc}	1000	0	0	0	124.6 ^{d} (11.17)	152.33 ^{def} (12.31)	160.53 ^{bc}	1753.33 ^{abc}
B2	0	0	0	0 ^{a} (0.70)	66.67 ^{b} (8.17)	206.66 ^{d}	1000	0	0	0	0 ^{a} (0.70)	79.26 ^{bc} (8.89)	226.67 ^{cde}	1760.67 ^{abc}
B3	0	0	0	0 ^{a} (0.70)	60.00 ^{b} (7.70)	100.00 ^{ab}	1000	0	0	0	0 ^{a} (0.70)	75.46 ^{b} (8.60)	107.47 ^{ab}	1700.00 ^{ab}
B4	0	0	0	133.33 ^{d} (11.47)	173.33 ^{cd} (12.33)	173.33 ^{cd}	1000	0	0	0	162.66 ^{d} (12.68)	200.33 ^{f} (14.13)	217.33 ^{cde}	1876.67 ^{d}
B5	0	0	0	26.67 ^{b} (5.14)	113.33 ^{c} (10.65)	146.66 ^{bc}	1000	0	0	0	30.93 ^{b} (5.53)	115.4 ^{cd} (10.75)	168.40 ^{bcd}	1699.33 ^{ab}
B6	0	0	0	53.33 ^{c} (7.30)	153.33 ^{d} (13.14)	220.00 ^{d}	1000	0	0	0	56.66 ^{c} (7.53)	185.73 ^{ef} (13.52)	235.00 ^{def}	1853.00 ^{abc}
B7	0	0	0	66.67 ^{c} (8.17)	133.33 ^{cd} (11.51)	220.00 ^{d}	1000	0	0	0	62.00 ^{c} (7.89)	141.06 ^{de} (11.86)	245.73 ^{ef}	1689.67 ^{a}
B8	0	0	0	66.67 ^{c} (8.05)	153.33 ^{cd} (12.36)	286.66 ^{e}	1000	0	0	0	70.00 ^{c} (8.30)	166.06 ^{ef} (12.89)	306.13 ^{f}	1826.00 ^{abc}
APS12	0	0	0	0 ^{a} (0.70)	13.33 ^{a} (2.59)	120.00 ^{abc}	1000	0	0	0	0 ^{a} (0.70)	5.73 ^{a} (0.70)	117.67 ^{ab}	1836.67 ^{cd}
APS45	0	0	0	0 ^{a} (0.70)	0 ^{a} (0.70)	66.66 ^{a}	1000	0	0	0	0 ^{a} (0.70)	0 ^{a} (0.70)	66.13 ^{a}	1836.67 ^{cd}
CSR_2(control)	0	0	0	26.67 ^{b} (5.14)	100.00 ^{b} (9.99)	106.66 ^{ab}	1000	0	0	0	25.93 ^{b} (5.07)	93.00 ^{bc} (9.62)	115.33 ^{ab}	1920.00 ^{d}
F-test	NA	NA	NA	*	*	*	NS	NA	NA	NA	*	*	*	*
SEm±	-	-	-	0.55	0.84	18.85	-	-	-	-	0.52	3.64	64.79	45.50
CD at 5%	-	-	-	1.62	2.46	55.20	-	-	-	-	1.59	2.10	72.72	133.21
CV (%)	-	-	-	18.11	15.99	20.03	-	-	-	-	17.00	13.14	24.02	4.41

*- Significant at 5%; NS-Non significant; $-Figures in parentheses are transformed values and with same superscript are on par; Stock- 1.45×10^5 spores/ml.

Note: *B. bassiana* infection was done immediately after fourth moult. Each treatment comprised of three replications with 50 silkworms each susceptible. Similar trend was found in cocoon formation in C-nichi breed treated with different dilutions of fungal spores. In the present study, thermotolerant bivoltine silkworm breeds, B4, B1 and B8 formed significantly highest number of cocoon per 1000 worms, at different spore dilutions and could yield relatively more number of cocoons even under fungal stress, indicating that they may be relatively tolerant to *B. bassiana* infection.

Cocoon yield by weight (g / 1000 worms)

Significant difference was noticed for cocoon yield by weight when thermotolerant bivoltine silkworm breeds were treated with different spore dilutions of *B. bassiana* (Table 5). The fungal spore dilutions at stock, 10^{-1} and 10^{-2} showed cent per cent mortality and no cocoon yield could be recorded. However, at 10^{-3} dilution of *B. bassiana* spore, the breed B4 recorded highest cocoon yield by weight (162.66 g/ 1000 worms), followed by B1 (124.60 g/1000 worms) and B8 (70 g/1000 worms). Significantly least cocoon yield by weight of 25.93 g/1000 worms was recorded in CSR_2, followed by B5 (25.93 g/1000 worms). At 10^{-4} spore dilution significantly highest cocoon yield was recorded in breed B4 (200.33 g/1000 worms), followed by B6 and B8 (185.73 and 166.06 g/1000 worms, respectively). Significantly lowest cocoon yield by weight was reported in breed APS12 (5.73 g/1000 worms) followed by B3 (75.46 g/1000 worms). APS45 breed showed 100 per cent mortality even at 10^{-4} and hence no cocoon yield by weight was recorded. At 10^{-5} fungal dilution breed B8 recorded significantly highest cocoon yield by weight of 306.13 g/1000 worms, followed by B7 and B6 (245.73 and 235.00 g/ 1000 worms, respectively). However, lowest cocoon yield by weight was reported in breed APS45 (66.13 g/1000 worms), followed byB3 (107.47 g/1000 worms). In non-inoculated control batch, breed CSR_2 reported significantly highest cocoon yield by weight (1920.00 g/1000 worms), followed by B4 (1876.67 g/1000 worms) and B6 (1853.00 g/1000 worms) while, lowest cocoon yield by weight was observed in B3 breed (1700.00 g/1000 worms), followed by B7 (1689.67 g/ 1000 worms). The thermotolerant bivoltine silkworm breeds B4, B6 and B8 recorded highest cocoon yield by weight at different dilutions of *B. bassiana* spores, which might be due to their ability of the breeds to spin few good cocoons even under infected condition. Literature is silent on the cocoon yield among silkworm breeds infected with *B. bassiana* fungus. However it could be understood that yielding higher cocoons would result in yielding higher weight of cocoons.

Therefore, it could be inferred that thermotolerant bivoltine silkworm breeds *viz.,* B4, B8, B6, and B1 also show relatively better tolerance to *B. bassiana* infection indicating that there could be possibility of dual tolerance for high temperature and muscardine infection. Further they can be explored for genetic analysis to determine possible association of tolerance to high temperature fluctuations and muscardine infections.

REFERENCES

CHANDRASEKHARAN, K. AND NATARAJU, B., 2008, Studies on white muscardine disease of mulberry silkworm *Bombyx mori* (L.) in India. *Indian J. Seric.*, **47** (2) : 136 - 154.

DANDIN, S. B., JAYASWAL, J. AND GIRIDHAR, K., 2001, *Handbook of Sericulture Technologies*. Central Silk Board, Bangalore, pp 287.

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

NATARAJU, B., 2008, Influence of systemic fungicide on the hematology of silkworm *Bombyx mori* (L.) infected with *Beauveria bassiana*. *Int. J. Indust. Entomol.,* **6** (1) : 11 - 14.

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.

RAMESH BABU, M., LAKSHMI, H., PRASAD, J. SEETHARAMULU, J., CHANDRASHEKARAIAH AND GOEL, A. K., 2005, Evaluation and selection of potential bivoltine parents for silkworm (*Bombyx mori* L.) breeding. *Indian J. Seric*., **44** (1) : 82 - 91.

SUNDARRAJ, N., NAGARAJU, S., VENKATARAMU, M. N. AND JAGANNATH, M. K., 1972, *Designs and Analysis of Field Experiments*. Univ. of Agric. Sci., Bangalore, India, pp. 424.

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