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Some thermotolerant bivoltine silkworm breeds tolerate white muscardine diseases caused by *beauveria bassiana* (bals.-criv) vuill. infection

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Abstract
To look into the possibilities of dual tolerance for both abiotic and biotic stresses in silkworms, thermotolerant bivoltine silkworm breeds were infected with *Beauveria bassiana* and their growth response was studied. Ten thermotolerant bivoltine silkworm breeds of which eight breeds *viz*., B1, B2, B3, B4, B5, B6, B7 and B8 from CSRTI, Mysore and two breeds *viz*., APS12 and APS45from APSSRDI, Hindupur along with CSR_2 as control were inoculated with *Beauveria bassiana* spores. The B4 breed showed highest LC_{50}value (68,625.71 spores / ml) indicating to be more tolerant to fungal infection than others. The quantitative traits in B4 thermotolerant bivoltine silkworm breed under both muscardine inoculation and high temperature treatment revealed significantly highest ERR, fifth instar larval weight, cocoon yield by number, cocoon yield by weight, single cocoon weight, shell weight, filament length and filament weight. However, shell ratio was higher in B4 under muscardine infection but in B3 under high temperature treatment. Thepupal weight showed non-significant difference in all the breeds under muscardine infection but showed significant difference under high temperature treatment. The Correlation between the performance of the breeds under thermal treatment and muscardine infection revealed significant positive correlation with respect to fifth instar larval weight (0.80), shell weight (0.58), filament length (0.79) and filament weight (0.83). Under both muscardine infection and high temperature treatment the regression analysis revealed that the cocoon yield by number per 1000 worms (2.35 and 3.02, respectively) and single cocoon weight (1823.32 and 4771.60, respectively) had a positive contribution to cocoon yield under both the conditions.

Keywords: Rearing performance, Thermotolerant bivoltine, Muscardine, High temperature, ERR, Cocoon yield parameters

Introduction
In India, silkworm rearing is being practiced since time immemorial. The silkworm, *Bombyx mori* L. is delicate, sensitive and completely domesticated insect and classic model organisms for Lepidoptera. The success of cocoon production depends on disease management. Exploitation of the resistant/tolerance of silkworm breeds towards different diseases causing pathogens is a better option for managing the crop loss. White muscardine caused by *Beauveria bassiana* (Bals.) Vulli. is one of the most devastating silkworm disease. The fungal disease is common during winter and rainy seasons.In India 10-40 percent of loss has been accounted for white muscardine out of total loss due to diseases (Janakiraman, 1961; Chandrasekharan and Nataraju, 2008) [8, 5]. The climatic condition in the tropics is congenialfor the occurrence and easy spread of fungal diseases. The spread of muscardine is due to high humidity and low temperature (Samson *et al*., 1990) [20]. Like the pathogenic stress, the abiotic stress, particularly temperature plays a major role on growth and productivity of silkworm. Silkworms have adapted to a temperature of 25 ±1ºC and humidity 75±5 per cent. Any increase or decrease in temperature and humidity causes susceptibility in silkwormand silk yield is adversely affected particularly by high temperature condition prevailing during summer in tropical conditions.Generally multivoltine silkworm strains are more tolerant to high temperature but produce inferior quality silk. On the other hand, bivoltine silkworms produce high quality silk, but are less tolerant. Attempts to use bivoltine

silkworm breeds throughout the sericulture belt of India resulted in serious crop losses, especially in the hot and humid climatic condition (Chandrakanth *et al*., 2015) [3]. Many important qualitative characters such as viability and cocoon traits sharply decline when temperature exceeds 28°C (Basavaraja and Sudhakara Rao, 2009) [1]. Under this context CSR&TI, Mysore (Suresh Kumar *et al*., 2002) [32] and APSSRDI, Hindupur (Ramesh Babu *et al*., 2005) [22], have evolved thermotolerant bivoltine silkworm breeds adoptable to high temperature and low humidity prevailing during summer. Thus such breeds being suitable for summer rearing are inherently tolerant to bacterial and viral disease to a greater extent. Their tolerance to white muscardine caused by *Beauveria bassiana* (Bals) vuill., is unknown. Hence, to look into the possibilities of multiple/ dual tolerance for both abiotic and biotic stresses among thermotolerant bivoltine silkworms, a study on their tolerance *vis-à-vis* susceptibility to white muscardine disease caused by a fungal pathogen was envisaged.

Material and Methods

The Comparative performance of thermotolerant bivoltine silkworm breeds under both *Beauveria bassiana* infection and high temperature treatments was studied at the Department of Sericulture, UAS, GKVK, Bengaluru. Eight thermotolerant bivoltine silkworm breeds *viz*., B1, B2, B3, B4, B5, B6, B7 and B8 from CSRTI, Mysore and two breeds *viz*., APS12 and APS45 were procured from APSSRDI, Hindupur along with CSR_2 as control. To determine LC_{50} value newly ecdysed fifth instar larvae (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} to 10^{-5} at the rate of 0.5 ml per worm by spraying with an automizer (Venkataramana Reddy, 1978) [34]. In the subsequent rearing, one set of all the eleven breeds (50 worms per replication in three replications, each) were topically inoculatedon the first day of fifth instar with the dose equivalent to LC_{50} for most tolerant breeddetermined in the first assay and their performance was assessed. Similarly, another set of all the eleven breeds (50 worms per replication in three replications each) were treated with high temperature of 36 ± 1°C and 85 ± 5% RHfor five days from third day to seventh day of fifth instar, for a duration of 6 hours daily (10.00 to 16.00 hours) by keeping in a BOD incubator.

Rearing of each breed was conducted by following standard rearing practices, on V1 mulberry leaves. Ripe silkworms were shifted to the mountages for spinning at ambient temperature of 25 ± 1°C and 65 ± 5% RH (Dandin *et al*., 2001) [6]. Observations on the silkworm rearing and cocoon parameters *viz.*, ERR, fifth instar larval weight, cocoon yield by number and cocoon yield by weight, single cocoon weight, pupal weight, shell weight, shell ratio, filament length and filament weight were recorded.

LC 50 was determined by the method given by (Reed and Muench, 1932) [23]. The data on silkworm growth and cocoon parameters were analyzed using completely randomized design (Sundarraj *et al*., 1972) [30]. The per cent data was analyzed after transformation by using the formula $\sin^{-1}\sqrt{p/100}$. The mean values of the experiments were compared by using Duncan's Multiple Range Test (DMRT) (Duncan, 1955) [7]. The Correlation between the performance of the breeds under thermal treatment and muscardine infection and regression analysis for contribution of different traits towards cocoon yield under thermal treatment and muscardine infection was done by the procedures given by Snedecor and Cochran (1989) [28].

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*., CSR_2 were employed for the study (Fig. 1). These breeds when treated immediately after fourth moult with different dilutions of *Beauveria bassiana* spores *i.e*., stock, 10^{-1} to 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. It has been earlier observed that 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 (4.73×10^2spores / 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 (Raghavaiah and Jayaramaiah, 1990) [19]. Similar variation among thermotolerant bivoltine silkworm breeds was observed in the present study, which can be used for identifying those breeds with dual tolerance to both high temperature and fungal infection.

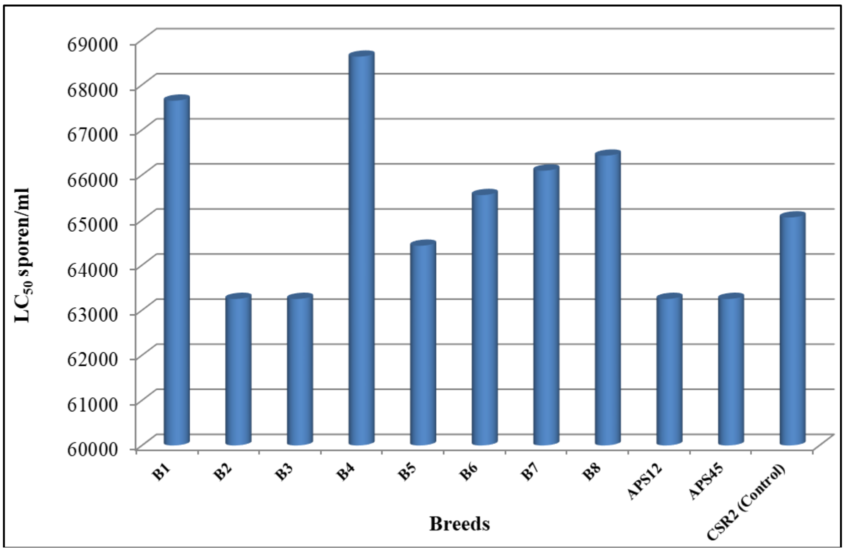

Fig 1: LC_{50} for *B. bassiana* inoculation among thermotolerant bivoltine silkworm breeds.

Effective Rate of Rearing (%)

The ERR showed significant difference among the thermotolerant silkworm breeds treated with *B. bassiana* spores at the dose equivalent to LC_{50} (68,625.71 spores/ml). Significantly highest ERR was recorded in B4 (54.67%) breed, followed by B8 (42.67%) and B1 (40.00). Significantly lowest ERR was observed in both B2 and B3 breeds (2.67%), followed by APS12 (3.33 %) (Table 1).High temperature treatment of thermotolerant bivoltine silkworm breeds showed significant effect on ERR. Maximum ERR was recorded in B4 (84.67 %), followed by APS45 (81.33%), B8 (76.67%) and APS12 (74.00%). Significantly lowest ERR was reported in CSR_2 (31.33%), followed by B1 (42.67 %) and B3 (45.33 %) as presented (Table 1). Venkataramana Reddy (1978) [34] found that when eight races of silkworms were inoculated with nine conidial concentrations (10^1-10^9 spores/ml) of *B. bassiana*, the survival which influences ERR varied over spore concentrations and between breeds. Similarly, the thermotolerant bivoltine silkworm breeds did show such variations in survival and hence in ERR due to fungal infection. Of which, B4, B8 and B1 showed highest ERR when challenged with the fungal pathogen at LC_{50} of B4. CSR_{18} and CSR_{19}thermotolerantbivoltine silkworm breeds evolved by utilizing Japanese bivoltine hybrid B201×BCS12 by exposing to high temperature of 36±1°C and 85±5 per cent RH showed better pupation rate (92.30 per cent and 92.00 per cent, respectively) than control breeds KA and NB_4D_2 (76.60 per cent and 88.20 per cent, respectively) (Suresh Kumar *et al*., 2002) [32]. Productive bivoltine breed CSR_2showed significantly low values for ERR, which may be due to itssensitivity to high temperature, as also supported by earlier findings of Shen (1986) [25]. The thermotolerant bivoltine silkworm breeds B4, B1 and B8 also showed significantly high value for ERR when treated with high temperature.

Fifth instar larval weight (g/10 worms)

Significant difference was observed for fifth instar larval weight of thermotolerant bivoltine silkworm breeds when treated with LC_{50} (68,625.71 spores/ml) of B4 for *B. bassiana* infection and high temperature (Table 1). Under fungal stress the thermotolerant breed B4 showed significantly highest larval weight (21.35 g / 10 worms), followed by B2 (20.78 g / 10 worms) and B1 (20.50 g / 10 worms) and minimum larval weight was recorded in B3 (18.73 g / 10 worms), which was followed by APS45 (19.18 g / 10 worms) and CSR_2 (19.34 g / 10 worms) (Table 1). At high temperature treatment the larval weight varied significantly and the breed B1 and B4 recorded highest larval weight (38.57 g / 10 worms), followed by B5 (36.73 g / 10 worms) and B2 (36.38 g / 10 worms). Significantly lowest larval weight was recorded in CSR_2 (30.34 g / 10 worms), followed by B3 (34.51 g / 10 worms). The muscardine causes loss of body weight in infected silkworms from third day onwards due to cessation of feeding (Venkataramana Reddy, 1978) [34]. As a consequence of fungal infection, decrease in food consumption, digestion, relative consumption rate and efficiency of conversion of ingested food are observed (Cai, 1989) [2]. Rajitha and Savithri (2015) [21] reported that sub lethal concentration of *B. bassiana* resulted in significant reduction of fifth instar larval weight compared to control. In the present findings, B4, B2 and B1 breeds showed higher fifth instar larval weight under fugal pathogen stress.Screening of different silkworm breeds for thermotolerance at 36±1°C has revealed that fifth instar larval weight ranged from 35 g to 40.4 g (Kumari *et al*., 2011) [11]. Thermotolerant silkworm breeds HTO_5 and HTP_5 yielded larval weight of 37 g to 40.28 g / 10 worms, respectively (Lakshmi *et al*., 2011) [12]. Rearing of CSR_2 at 36±1°C resulted in larval weight of 32.73 g / 10 worms, while the larvae of CSR_{17}weighed 31.93 g / 10 worms (Chandrakanth *et al*., 2015) [3]. Similarly in the present study the fifth instar larval weight ranged from 0.34 to 38.57 g / 10 worms under high temperature stress and the breeds B1, B4 and B5 produced highest larval weight. Pillai and Krishnaswamy (1980) [18] reported that, the low survival rate in the silkworms exposed to high temperature in fifth instar is attributed to low feeding activity of the silkworm resulting in the physiological imbalance and poor health of the larvae.Under these circumstances, B1 and B4 thermotolerant breeds performed better with respect to fifth instar larval weight both under fungal infection and high temperature exposure.

Cocoon yield by number (No. / 1000 worms)

Thermotolerant bivoltine silkworm breeds when treated with *B. bassiana* spore at LC_{50} of B4 breed and high temparature, showed significant differences for cocoon yield by number per 1000 worms (Table 1). Under fungal stress, significantly highest value was noticed in breed B4 (620 /1000 worms), followed by B1 (500.00 /1000 worms) and B8 (486.67 /1000 worms) and significantly least cocoon yield by number per 1000 worms was recorded in breed B3 (53.33 /1000 worms) followed by B2 (60.00 /1000 worms) and APS12 (66.67/1000 worms). At high temperature treatment significantly highest cocoon yield by number was noticed in breed APS45 (880.00 /1000 worms), followed by APS12 (853.33 No/1000 worms) and B4 (846.67 /1000 worms). Significantly lowest value was observed in CSR_2 breed (440.00 /1000 worms), followed by B2 (460.00 /1000 worms) and B1 (473.33 /1000 worms) (Table 1). When the breed NB_4D_2 was treated with different dilutions of fungal spore concentrations (10^9 to10^1), the cocoon formation significantly decreased with increase in spore concentration and highest percentage of cocoon formation was at 10^1 spore concentration and no cocoon formation was observed at 10^9 and 10^8 spore concentrations. (Raghavaiah and Jayaramaiah, 1990) [19]. Chandrasekharan and Nataraju (1998) [4] reported that among bivoltine races NB_4D_2 was the least susceptible to muscardine infection and that NB_{18} and NB_7 breeds the most susceptible. Venkataramana Reddy (1978) [34] also recorded higher cocooning per cent in KA, followed by NB_2 and NB_{18}, at different dilutions of fungal spores. In the present study B4, B1 and B8 could yield more cocoons per 1000 worms under fungal infection.Screening of twenty silkworm breeds for thermotolerance at 36±1°C revealed that the cocoon yield by number per 10,000 larvae ranged from 2191 to 7190 (Kumari *et al*., 2011) [11] and in the present study the cocoon yield by number ranged from 440.00 to 880.00 per 1000 worms. In earlier studies APS12 thermotolerant bivoltine silkworm breed recorded 9200cocoons per 10,000 larvae at 32±1°C (Lakshmi and Chandrashekaraiah, 2006) [12]. In the present study it resulted in 853.33 cocoons per 1000 larvae whichwas nearing to the earlier findings. Overall APS45 thermotolerant silkworm breed showed significantly highest cocoon yield by number followed by APS12 and B4, when reared at high temperature 36±1°C condition. Thus among the thermotolerant bivoltine silkworm breeds studied, B4 alone could yield higher number of cocoons per 1000 worms under both muscardine infection and high temperature treatment.

Cocoon yield by weight (g / 1000 worms)

Effect of *B. bassiana* infection on cocoon yield by weight showed significant difference and the highest value for cocoon yield by weight wasobservedin breed B4 (710.93 g/1000 worms), followed by B1 (532.67 g/1000 worms) and B8 (525.20 g/1000 worms) and significantly lowest value was noticed in breed B3 (50.67 g/1000 worms), followed by B2 (55.07 g/1000 worms) and APS12 (62.87g/1000 worms) (Table 1). The cocoon yield by weight was significantly maximum when fifth instar silkworms were treated with high temperature in the breed APS45 (1409.80 g/1000 worms), followed by B4 (1387.33 g/1000 worms) and B8 (1370.33 g/1000 worms). Significantly least cocoon yield by weight was recorded in CSR_2 (420.53 g/1000 worms), followed by breed B2 (708.40 g/1000 worms) and B1 (712.87 g/1000 worms) (Table 1). Though the literature is silent on the cocoon yield among thermotolerant silkworm breeds infected with *B. bassiana* fungus, it was observed that B4, B1 and B8 thermotolerant bivoltine silkworms yield higher cocoon yield by weight in the present study. Earlier studies on effect of high temperature treatment on CSR_{18} and CSR_{19} thermotolerant silkworm breed yielded 823 g / 1000 worms and 793 g / 1000 worms, respectively at 36±1°C and relative humidity 85±5% (Suresh Kumar *et al*., 2002 & 2003) [32, 33]. Other thermotolerant breeds *viz*., SR_1 and SR_4 could produce cocoon yield with 1353 g / 1000 worms and 1381 g /1000 worms at 36±1°C and 85±5% (Sudhakara Rao *et al*., 2006) [29]. CSR_{46} and CSR_{47} produced of 935 g /1000 larvae and 761 g / 1000 larvae, respectively at 36±1°C (Suresh Kumar *et al*., 2006) [31]. APS12 thermotolerant breed yielded 1490 g of cocoon per 1000 larvae at 32±1°C (Lakshmi and Chandrashekaraiah, 2006) [12]. HTO_5 and HTP_5 thermotolerant silkworm breed could produce cocoon yield of 1538 g / 1000 worms and 1557 g / 1000 worms at 32±1°C (Lakshmi *et al*., 2011) [12]. While CSR_2 breed could produce 158 g /1000 worms and CSR_{17} could produce 396 g /1000 worms (Chandrakanth *et al*., 2015) [3]. All these results corroborate with the present findings and that APS45, B4 and B8 breeds performed better and resulted in higher cocoon yield by weight, when reared under high temperature of 36±1°C. Thus, it was observed that B4 and B8 could perform better than other breeds for cocoon yield by weight under both muscardine infection and high temperature treatment.

Table 1: Growth and yield parameters of thermotolerant bivoltine silkworm breeds as affected by *B. bassiana*i noculation and high temperature treatment

Breeds	ERR$ (%)		Fifth instar larval weight (g/10 worms)		Cocoon yield by number per 1000 worms		Cocoon yield by weight (g/1000 worms)	
	B. bassiana infection	High temp. treatment	*B. bassiana* infection	High temp. treatment	*B. bassiana* infection	High temp. treatment	*B. bassiana* infection	High temp. treatment
B1	40.00^{de} (39.14)	42.67^{ab} (40.77)	20.50^{bc}	38.57^{d}	500.00^{de}	473.33^{ab}	532.67^{de}	712.87^{b}
B2	2.67^{a} (9.26)	45.33^{b} (42.31)	20.78^{bc}	36.38^{c}	60.00^{a}	460.00^{a}	55.07^{a}	708.40^{b}
B3	2.67^{a} (9.26)	59.33^{c} (50.39)	18.73^{a}	34.51^{b}	53.33^{a}	646.67^{bc}	50.67^{a}	945.33^{c}
B4	54.67^{f} (47.77)	84.67^{f} (67.06)	21.35^{c}	38.57^{d}	620.00^{e}	866.67^{f}	710.93^{e}	1387.33^{d}
B5	26.00^{cd} (30.57)	69.33^{cd} (56.64)	19.66^{ab}	36.73^{cd}	333.33^{cd}	733.33^{bc}	333.67^{bc}	1100.20^{c}
B6	28.67^{cde} (32.28)	61.33^{cd} (51.61)	19.46^{ab}	35.70^{bc}	333.33^{cd}	653.33^{bc}	348.33^{bcd}	1014.33^{c}
B7	25.33^{cd} (29.79)	60.67^{cd} (51.27)	19.79^{ab}	35.73^{bc}	333.33^{cd}	646.67^{bc}	342.33^{bc}	1003.40^{c}
B8	42.67^{ef} (40.65)	76.67^{ef} (61.17)	19.69^{ab}	36.18^{bc}	486.67^{de}	846.67^{df}	525.20^{cde}	1370.33^{d}
APS12	3.33^{ab} (10.14)	74.00^{def} (59.81)	20.03^{abc}	35.22^{bc}	66.67^{ab}	853.33^{df}	62.87^{a}	1346.80^{d}
APS45	4.67^{ab} (12.41)	81.33^{ef} (64.43)	19.18^{ab}	35.16^{bc}	93.33^{ab}	880.00^{f}	87.00^{a}	1409.80^{d}
CSR_2(Control)	17.33^{c} (24.46)	31.33^{a} (34.00)	19.34^{ab}	30.34^{a}	233.33^{bc}	440.00^{a}	220.20^{ab}	420.53^{a}
F-test	*	*	*	*	*	*	*	*
SEm±	3.18	2.72	0.50	0.63	57.90	42.30	64.26	60.16
CD at 5%	9.33	7.98	1.47	1.86	169.52	123.85	188.48	176.24
CV (%)	21.24	8.97	4.38	3.09	35.43	10.74	37.45	10.02

*- Significant at 5%; NS-Non significant; figures with same superscript are statistically on par; $-Figures in parentheses are sin^{-1} transformed values.
Note- *B. bassiana* inoculation at 68,625.71 spores/ml @ 0.5 ml per worm; High temperature treatment @ 36±1°C for 6 hrs/day from 3rd to 7th day of fifth instar.

Single cocoon weight (g)

Significant difference was observed for single cocoon weight among thermotolerant bivoltine breeds treated with fungal spore dilution (68,625.71 spores/ml). Significantly highest cocoon weight was recorded in breed B4 (1.16 g), followed by B1 (1.06 g) and B8 (1.05 g). Significantly lowest cocoon weight was observed in breed B2 (0.90 g), followed by APS45 (0.93 g) and B3, APS12 and CSR_2 (0.94 g, each) (Table 2). High temperature treatment at 36±1°C and 85±5% caused significant differences among thermotolerant breeds for cocoon weight. Significantly maximum cocoon weight was recorded in B8 thermotolerant breed (1.62 g), followed by both B4 and APS45 (1.60 g), wherein, the least cocoon weight was reported in CSR_2 (0.95 g), followed by B3 (1.46 g) and B1 breed (1.50 g) (Table 2). Raghavaiah (1986) reported that NB_7 formed cocoons with maximum weight (1.027 g) compared to NB_{18} (0.940 g) when infected with muscardine. Also, Rajitha and Savithri, (2015) [21] reported significant reduction in cocoon weight in PM×CSR_2 when infected with *B. bassiana* spore on first day of fifth instar.

Accordingly, B4, B1 and B8 thermotolerant breeds showed better single cocoon weight than other breeds. Screening of different silkworms genotypes for thermotolerance at 31°C has revealed that the cocoon weight among 44 silkworm hybrids ranged from 1.474 to 1.686 g (Naseema Begum *et al*., 2003) [15] and in the present study it ranged from 0.95 g (CSR_2) to 1.62 (B8) when treatment with much higher temperature of 36±1°C. The thermotolerant breeds CSR_{46} and CSR_{47} yielded 1.48 g and 1.34 g, respectively when reared at 36±1°C (Suresh Kumar *et al*., 2006) [31], APS12 breed of silkworm yielded 1.591 g of cocoon weight at 32±1°C (Lakshmi and Chandrashekaraiah, 2006) [12] and HTO_5 and HTP_5 produced cocoon with 1.698 g and 1.718 g when exposed to 32±1°C (Lakshmi *et al*., 2011) [12]. Rearing performance of CSR_2at 36±1°C revealed that it could produce cocoons with 1.308 g of weight, while CSR_{17} could produce cocoons with 1.417 g (Chandrakanth *et al*., 2015) [3]. In the present study rearing of eleven silkworm breeds showed varied performance for cocoon weight when reared at 36±1°C, among which B8 could produce higher cocoon weight followed by B4 and APS45. Earlier results have shown that APS12 yielded 1.59 g of cocoon weight which was as similar to present study (1.58 g). Thus, under both muscardine infection and high temperature treatment B4 and B8 thermotolerant breeds performed better than other breeds evaluated.

Pupal weight (g)

The thermotolerant bivoltine silkworm breeds when treated with fungal infection at the dose equivalent to LC_{50} (68,625.71 spores/ml) showed non-significant difference for pupal weight. However, maximum pupal weight was noticed in breed B4 (0.92 g), followed by B1 B6, B7 and B8 (0.87 g each) and minimum pupal weight was recorded in B2 breed (0.77 g), followed by APS45 (0.79 g) and B3, CSR_2 and APS12 (0.80 g each) (Table 2). The pupal weight in high temperature treated thermotolerant bivoltine silkworm breeds showed significant difference (Table 2). Significantly highest pupal weight was observed in B8 breed (1.31 g) followed by APS45 (1.28 g), APS12 (1.27 g) and B4 (1.26 g). Significantly lowest pupal weight was recorded in CSR_2 breed (0.79 g) followed by B3 (1.15 g). The literature on effect of muscardine infection on pupal weight in different silkworm breeds is limited. However, Rajitha and Savithri (2015) [21] reported reduction of pupal weightin silkworm hybrid PM ×CSR_2 treated with sub lethal concentration of *B. bassiana* conidial suspension. In the present study, B4, B1, B6, B7 and B8 breeds produced pupae with higher weight than other breeds when infected with *B. bassiana* spores. Under the influence of higher temperature *i.e*., 36±1°C and 85±5% relative humidity CSR_{18} and CSR_{19} breed had recorded pupal weight of 1.15 g and 1.08 g, respectively (Suresh Kumar *et al*., 2002) [32]. Screening the performance of silkworm hybrids at 31°C has revealed that the pupal weight ranged from 1.15 g to 1.34 g (Naseema Begum *et al*., 2003) [15]. Thermotolerant bivoltine silkworm breeds SR_1 and SR_4 breeds recorded pupal weight of 1.17 g and 1.22 g, respectively (Sudhakara Rao *et al*., 2006) [29] and CSR_{46} and CSR_{47} produced pupal weight of 1.15 g and 1.07 g, respectively at 36±1°C (Suresh kumar *et al*., 2006) [31]. APS12 silkworm breed yielded pupal weight of 1.24 g at 32±1°C and low humidity 50±5% relative humidity (Lakshmi and Chandrashekaraiah, 2006) [12]. Thermotolerant silkworm breed HTO_5 and HTP_5 yielded 1.33 g and 1.35 g of pupal weight when exposed to 32±1°C during fifth instar (Lakshmi *et al*., 2011) [12]. CSR_2 produce pupal weight 1.06 g while CSR_{17} could produce 1.15 g of pupal weight at 36±1°C (Chandrakanth *et al*, 2015) [3]. In the present study rearing of eleven silkworm breeds showed varied performance for pupal weight when reared at 36±1°C, among which B8 could produce higher pupal weight followed by APS45, APS12 and B4. Earlier results have shown that the breed APS12 yield 1.24 g of pupal weight, which was slightly less than the pupal weight recorded in the present study (1.27 g). Thus the two thermotolerant bivoltine silkworm breed *viz*., B8 and B4 could produce relatively higher pupal weight under both fungal infection and high temperature treatment.

Shell weight (g)

Among the eleven bivoltine silkworm breeds, thermotolerant bivoltine silkworm breed B4 showed significantly highest shell weight of 0.24 g, when treated with fungal spore at the dose of 68,625.71 spores/ml, followed by B1 (0.19 g). Significantly lowest shell weightwas recorded in CSR_2 (0.13g), followed byboth B2 and B3 (0.14 g each) (Table 2).Among the different thermotolerant bivoltine breeds treated with high temperature, significantlyhighest shell weight was recorded in B4 breed (0.34 g) followed by B3, B6 and APS45 (0.32 g each) and significantly lowest shell weight was recorded in CSR_2 (0.15 g). Shell weight for other breeds ranged from 0.29 g in B1 breed to 0.32 g in APS45 breed (Table 2). In earlier studies, Venkataramana Reddy (1978) [34] reported that, NB_7 larvae spun the cocoons with maximum shell weight compared to NB_4D_2, KA and NB_{18}bivoltine breeds, when infected with different doses of *B. bassiana* spore during fifth instar. Similarly, in the present study, B4 and B1 breeds showed higher shell weight among the thermotolerant bivoltine silkworm breeds. Different thermotolerant silkworm breeds respond variedly to high temperature treatment with respect to shell weight. CSR_{18} and CSR_{19}thermotolerantbivoltine silkworm breeds produced shell weight of 0.30 g and 0.23 g, when reared at 36±1°C and 85±5% relative humidity (Suresh Kumar *et al*., 2003) [33]. Generation wise performance of CSR_{18} and CSR_{19} revealed 0.28 g and 0.26 g shell weight, respectively at high temperature of 36±1°C (Suresh Kumar *et al*., 2002) [32]. SR_1 and SR_4 recorded 0.36 g and 0.35 g of shell weight when exposed to 36±1°C, RH 50±5% (Sudhakara Rao *et al*., 2006) [29], CSR_{46} and CSR_{47} showed shell weight of 0.32 g and 0.26 g at 36±1°C (Suresh kumar *et al*., 2006) [31], APS12 breed produced shell weight of 0.39g at 32±1°C (Lakshmi and Chandrashekaraiah, 2006) [12] and HTO_5 and HTP_5 produce shell weight 0.35 g and 0.36, respectively, at 32±1°C (Lakshmi *et al*., 2011) [12]. The shell weight among 25 silkworm breeds screened at high temperature ranged from 0.23 g to 0.30 g (Kumari *et al*., 2011) [11]. In the present study shell weight ranged from 0.15g in CSR_2 to 0.34g in B4, when fifth instar silkworms were exposed to 36±1°C and significantly higher shell weight was recorded in B4, B3, B6 and APS45.Thus B4 breed could produce better shell weight both under muscardine infection and high temperature treatment.

Shell ratio (%)

Different thermotolerant silkworm breeds showed significant differences for shell ratio when inoculated with *B. bassiana* fungal spores at LC_{50} of B4 and treated at high temperature (Table 2). Fungal inoculation revealed that the breed B4 recorded significantly highest shell percentage (20.71 %), followed by B1 (17.85 %) and B8 (16.64 %) and CSR_2 (14.44%) scored significantly lowest shell ratio followed by B2 (15.37 %) and B3 (15.39 %). At high temperature, the breed B3 recorded significantly highest shell ratio of 21.83 per cent followed by B4 (21.80 %) and B6 (20.75%). Significantly lowest shell ratio was recorded in CSR_2 (16.43 %), followed by B2 (19.06 %) and APS12 (19.64 %). In earlier studies, NB_4D_2 produced highest shell ratio compared to ND_7, KA and NB_{18} (Venkataramana Reddy, 1978) [34] and PM×CSR_2 breed showed reduction in shell ratio under *B. bassiana* infection compared to control (Rajitha and Savithri, 2015) [21]. In the present study B4 and B1 could produce higher shell ratio than other breeds under *B. bassiana* infection.Several studies on effect of high temperature on shell ratio reveal that the breed CSR_{18} and CSR_{19} yield shell per cent of 21.1 per cent and 20.7 per cent, respectively when treated with high temperature of 36±1°C (Suresh Kumar *et al*., 2002) [32]. CSR_{18} and CSR_{19} recorded shell ratio of 20.3 per cent and 19.5% respectively when reared at 36±1°C, (Suresh Kumar *et al*., 2003) [33]. Screening of 44 hybrids of silkworms for thermotolerance at 32±1°C revealed that shell ratio ranged from 21.36 per cent to 20.57 per cent (Naseema Begum *et al*., 2003) [15]. SR_1 and SR_4 showed shell ratio of 22.81per cent and 22.26per cent (Sudhakara Rao *et al*., 2006) [29] and CSR_{46} and CSR_{47} produced shell ratio of 21.8per cent and 21.5per cent, respectively at high temperature of 36±1°C (Suresh Kumar *et al*., 2006) [31]. Thermotolerant silkworm breed APS12 produce shell ratio of 21.09per cent at 32±1°C (Lakshmi and Chandrashekaraiah, 2006) [12]. HTO_5 and HTP_5 exhibited shell ratio of 21.3per cent and 22.3per cent respectively when exposed at 32±1°C (Lakshmi *et al*., 2011) [12]. Screening 25 silkworm genotypes at high temperature of 36±1°C has revealed that the shell ratio ranged from 16.38per cent to 21.53per cent (Kumari *et al*., 2011) [11]. Rearing twenty silkworm breeds at 36±1°C revealed that CSR_2 could produce shell ratio of 18.24per cent while the CSR_{17} could produce shell ratio of 18.25per cent (Chandrakanth *et al*., 2015) [3]. In the present study the shell weight ranged from 16.43 per cent (CSR_2) to 21.83 per cent (B3) among eleven breeds exposed to 36±1°C during fifth instar. The thermotolerant bivoltine silkworm breeds B3, B4 and B6 produced higher shell ratio.Thus, B4 breed alone showed better performance for shell ratio under both muscardine infection and high temperature treatment.

Filament length (m)

The data on filament length in thermotolerant bivoltine silkworm breeds reared under fungal and thermal stress is presented in Table 2. Significant difference was observed for filament length among the thermotolerant bivoltine silkworm breeds treated with the fungal dose of 68,625.71 spores/ml. Significantly longest filament length was observed in B4 breed (695.47 m), followed by B8 (657.47 m) and B7 (629.33 m) and significantly lowest filament length was noticed in B2 breed (480.00 m). Among the other breeds, filament length ranged from 571.33 m (APS12) to 608.27 m (B6). Effect of high temperature treatment on filament lengthfrom single cocoon of thermotolerant breeds showed significant differences among the breeds. The breed B4 exhibited longest filament length (887.67 m) followed by APS45 (871.53 m) and B8 (867.40 m).Wherein the lowest filament length was produced by CSR_2 (671.53 m), followed by B2 (708.27 m) and B3 (745.47 m). The literature on the effect of muscardine infection on filament length of different silkworm breeds is limited. Rajitha and Savithri (2015) [21] compared the performance ofPM×CSR_2 under muscardine infection and normal rearing and recorded filament length of 478.90 m in infected and 671.6 m in healthy silkworms. In the present findings, B4, B8 and B7 produced higher filament length under muscardine infection. Under high temperature treatment of different thermotolerant bivoltine silkworm breeds it has been observed that at 36±1°C, CSR_{18} and CSR_{19} produced filament length of 1112 m and 964 m, respectively (Suresh Kumar *et al*., 2003) [33], SR_1 recorded filament length of 854m and SR_4 recorded 811 m (Sudhakara Rao *et al*., 2006) [29] and CSR_{46} and CSR_{47} yielded filament length of 1200m and 1005m, respectively (Suresh Kumar *et al*., 2006) [31]. The filament length ranged from 686 m to 1012 m among 44 silkworm breeds when screened at high temperature of 32±1°C (Naseema Begum *et al.*, 2003) [15]. At 32±1°C the thermotolerant bivoltine silkworm breed APS12 could produce filament length of 922m at 32±1°C (Lakshmi and Chandrashekaraiah, 2006) [12] and HTO_5 and HTP_5 could yield filament length of 934 m and 936 m, respectively (Lakshmi *et al*., 2011) [12]. Kumari *et al*., 2011, reported filament length ranging from 623 m to 1022 m among 25 silkworm genotypes when screened at high temperature of 36±1°C. In the present study the rearing of thermotolerant silkworm breeds at higher temperature of 36±1°C showed varied performance for filament length, among which B4 and APS45 could produce high filament length. Comparing the performance under both fungal infection and high temperature treatment, B4 and B8 thermotolerant bivoltine breeds could produce longer filament length in both the conditions.

Filament weight *(g)*

Filament weight of the thermotolerant bivoltine silkworm breeds treated with LC_{50} fungal spore dosage of B4 at 68,625.71 spores / ml is presented in table 2. Significantly highest filament weight was recorded in B4 breed (0.18 g), followed by B8 (0.16 g). Significantly least filament weight was observed in CSR2 breed (0.12 g), followed by B2 and B3 (0.13 g, each) and B5 and B7 (0.14 g). Under high temperature treatment, significantly highest filament weight (Table 2) was recorded in B4 thermotolerant bivoltine breed (0.23 g), followed by B8 (0.22 g) and APS45 (0.21 g). However, lowest filament weight was shown by CSR_2 breed (0.16 g), followed byB2 (0.17 g) and B3 and B5 (0.18 g, each). The literature on the effect of muscardine infection and high temperature treatment in silkworms on filament weight is rather limited. However, it has been observed in silkworm hybrid PM ×CSR_2 treated with sub lethal concentration of *B. bassiana* conidia, filament weight was significantly reduced compared to control batch (Rajitha and Savithri, 2015) [21]. Also high temperature treatment at 36±1°C of different silkworm breeds resulted in filament weight ranging from 0.28g to 0.26g (Kumari *et al*., 2011) [11], while in the present study it ranged from 0.16 g in CSR_2 to 0.23 g in B4. Overall, under muscardine infection the breeds B4 and B8 produced higher filament weight and under high temperature treatment the breeds B4, B8 and APS45, indicating that B4 and B8 breeds could yield better filament weight under both the stresses.

Table 2: Cocoon and filament parameters of thermotolerant bivoltine silkworm breeds as affected by *B. bassiana* inoculation and high temperature treatment

Breeds	**Single cocoon weight (g)**		**Pupal weight (g)**		**Shell weight (g)**		**Shell ratio (%)**		**Filament length (m)**		**Filament weight (g)**	
	***B. bassiana* infection**	**High temp. treatment**	***B. bassiana* infection**	**High temp. treatment**	***B. bassiana* infection**	**High temp. treatment**	***B. bassiana* infection**	**High temp. treatment**	***B. bassiana* infection**	**High temp. treatment**	***B. bassiana* infection**	**High temp. treatment**
B1	1.06^{c}	$1.50b^{c}$	0.87^{cde}	1.21^{bc}	0.19^{e}	0.29^{bc}	17.85^{c}	19.70^{b}	587.47^{bc}	828.47^{de}	0.15^{b}	0.20^{d}
B2	0.90^{a}	1.54^{bcd}	0.77^{a}	1.25^{bc}	0.14^{a}	0.29^{bc}	15.37^{ab}	19.06^{b}	480.00^{a}	708.27^{ab}	0.13^{ab}	0.17^{b}
B3	0.94^{ab}	1.46^{b}	0.80^{ab}	1.15^{b}	0.14^{ab}	0.32^{cd}	15.39^{ab}	21.83^{c}	573.50^{b}	745.47^{bc}	0.13^{ab}	0.18^{b}
B4	1.16^{d}	1.60^{cd}	0.92^{e}	1.26^{c}	0.24^{f}	0.34^{e}	20.71^{d}	21.80^{bc}	695.47^{e}	887.67^{e}	0.18^{e}	0.23^{g}
B5	1.01^{b}	1.51^{bcd}	0.85^{bcd}	1.22^{bc}	0.16^{bc}	0.29^{bc}	16.08^{ab}	19.75^{b}	584.33^{bc}	770.00^{bc}	0.14^{bc}	0.18^{c}
B6	1.04^{c}	1.55^{bcd}	0.87^{cde}	1.24^{bc}	0.17^{de}	0.32^{cd}	16.23^{bc}	20.75^{bc}	608.27^{bcd}	809.33^{cd}	0.15^{c}	0.20^{d}
B7	1.03^{c}	1.55^{bcd}	0.87^{cde}	1.25^{bc}	0.16^{bc}	0.31^{bc}	16.23^{bc}	20.15^{bc}	629.33^{cd}	805.00^{cd}	0.14^{bc}	0.19^{d}
B8	1.05^{c}	1.62^{d}	0.87^{cde}	1.31^{c}	0.17^{de}	0.31^{cd}	16.64^{bc}	19.90^{b}	657.47^{de}	867.40^{de}	0.16^{d}	0.22^{f}
APS12	0.94^{ab}	1.58^{cd}	0.80^{ab}	1.27^{c}	0.16^{bc}	0.30^{bcd}	15.49^{ab}	19.64^{b}	571.33^{b}	808.07^{cd}	0.15^{c}	0.20^{d}
APS45	0.93^{ab}	1.60^{cd}	0.79^{ab}	1.28^{c}	0.15^{ab}	0.32^{de}	16.03^{ab}	20.55^{bc}	577.83^{bc}	871.53^{de}	0.15^{c}	0.21^{fg}
CSR_2(Control)	0.94^{ab}	0.95^{a}	0.80^{abc}	0.79^{d}	0.13^{a}	0.15^{a}	14.44^{a}	16.43^{a}	618.38^{bcd}	671.53^{a}	0.12^{ab}	0.16^{a}
F-test	*	*	NS	*	*	*	*	*	*	*	*	*
SEm±	0.032	0.44	-	0.03	0.008	0.011	0.58	0.5981	18.49	24.28	0.004	0.006
CD at 5%	0.09	0.12	-	0.11	0.02	0.03	1.71	1.75	54.13	71.08	0.018	0.018
CV (%)	5.63	5.11	5.55	5.61	8.62	6.48	6.17	5.18	5.35	5.27	4.4	5.53

*- significant at 5%; NS-non significant; figures with same superscript are statistically on par; $-figures in parentheses are sin^{-1} transformed values.
Note- *B.bassiana* inoculationat68, 625.71 spores/ml @ 0.5 ml per worm; High temperature treatment @ 36±1°C for 6 hrs/day from 3rd to 7th day of fifth instar.

Correlation between thermal treatment and muscardine infection for larval and cocoon parameters

Correlation co-efficient were estimated from the average estimates of ten variables in two conditions *i.e*., thermal treatment and muscardine infection for larval and cocoon parameters presented in table 3.

It was observed that the parameters *viz*., fifth instar larval weight (0.80), shell weight (0.58), filament length (0.79) and filament weight (0.83) showed significant positive correlation between thermal treatment and muscardine infection. Other parameters *viz.*, ERR (0.20), cocoon yield by number per 1000 worms (0.10), cocoon yield by weight in g/1000 worms (0.15), single cocoon weight (0.27), pupal weight (0.09) and shell percentage (0.43) showed non-significant correlation between thermal treatment and muscardine infection.

Kumar *et al*. (2012) [9] have analysed the correlation of various characters of multivoltine breeds between different environmental conditions. Accordingly they have found that larval weight, cocoon yield by weight, single cocoon weight, shell weight and filament length had shown significantly positive correlation between the environment, *viz*., high temperature with high humidity and high temperature with low humidity. Further, Singh *et al*. (2011) have observed positive correlation between cocoon weight with larval weight, shell weight, filament length, pupal weight and denier. Similarly, shell weight had positive correlation with cocoon weight, pupal weight and filament length, but observed positive correlation between fecundity and robustness and between shell ratio and pupal weight. In the present study, significantly positive correlation was observed between thermal treatment and muscardine infection for larval weight, shell weight, filament length and filament weight, which could be indicators of robust performance of thermotolerant breeds under both high temperature condition and muscardine infection. This has been supported by the observation of Kumaresen *et al*. (2012) [10], who observed such positive correlation between different environments for most of the economic parameters. In addition, positive correlation of shell weight with pupal weight and filament length from earlier studies, indicate that though the thermal treatment and muscardine infection showed no correlation for cocoon weight and pupal weight in the present study, selection for shell weight under any of these condition could improve cocoon weight and pupal weight in the other condition as well. Corroborating with the present observation earlier studies have documented that correlation between different pairs of quantitative characters revealed high significant ($P < 0.01$) positive values between cocoon weight and pupal weight ($r = +0.994$), cocoon weight and shell weight ($r = +0.614$), pupal weight and shell weight ($r = +0.527$), while significant ($P < 0.01$) negative values between pupal weight and cocoon shell ratio ($r = –0.827$). This indicates that selection by cocoon weight would lead to increase of pupal weight and shell weight but selection by pupal weight would lead to decrease of cocoon shell ratio (Singh, 1994) [26]. Pal and Moorthy (2011) [17] demonstrated that both the length of the larva body and the length of cocoon had a positive correlation with cocoon shell weight. Anticipated cocoon weight had a significant and positive correlation with cocoon shell weight and also, cocoon shell weight had a significant and positive correlation with cocoon weight and cocoon length. According to Ohi *et al*. (1970) [16] who worked out multiple correlation between the yield components, particularly cocoon shell weight which is positively correlated with cocoon filament length, indicated that the increase or decrease in filament length is dependent on the increase or decrease of the thickness of the filament and cocoon shell weight. Studies have thus shown that direct selection for one trait is correlated with other quantitative traits. Some have positive correlations and others have been found to have negative correlations. Mirhosseini *et al*. (2010) [14] observed that, a high and positive genetic correlation exists between cocoon weight and cocoon shell weight. These two important economic traits in which selection was on cocoon weight caused the increase of cocoon shell weight. Hence, in the present study though no relationship existed between muscardine infection and thermal treatment for cocoon weight, selection for shell weight which showed significant positive correlation with cocoon weight, may also improve cocoon weight. Thus, it may be concluded that at least a few of the studied thermotolerant bivoltine silkworm have a tendency of exhibiting tolerance to muscardine disease. Hence, those breeds which have such association leads to be identified for further breed improvement. Thus indicating an association in tolerance to high temperature and muscardine infection in the breeds B4, B8 and APS45.

Table 3: Correlation co-efficient between muscardine infection and thermal treatment for larval and cocoon parameters in thermotolerant bivoltine silkworms

Parameter	Co-efficient of correlation
ERR	0.20^{NS}
Fifth instar larval weight	0.80*
Cocoon yield by number	0.10^{NS}
Cocoon yield by weight	0.15^{NS}
Single cocoon weight	0.27^{NS}
Pupal weight	0.09^{NS}
Shell weight	0.58*
Shell percentage	0.43^{NS}
Filament length	0.79*
Filament weight	0.83*

NS: Non-significant,*: Significant at 5%.

Regression equations for cocoon yield under muscardine infection and thermal treatment

Under muscardine infection the regression model revealed that, the cocoon yield by number per 1000 worms (2.34), single cocoon weight (1823.92) and filament weight (2968.67) showed positive impact on cocoon yield. Remaining parameters *viz*., ERR (-18.39), fifth instar larval weight (-77.50), pupal weight (-1146.42), shell weight (-1338.29), shell percentage (-25.70) and filament length (-0.74) had negative impact on cocoon yield (Table 4).

Under thermal treatment condition, the co-efficient of regression for fifth instar larval weight (611.44), cocoon yield by number per 1000 worms (3.02), single cocoon weight (4771.86), shell percentage (265.45) and filament length (2.87) had a positive impact on cocoon yield, indicating that the increase of cocoon yield parameter dependent on the increase in the values of these parameters. Whereas, the ERR (-16.58), shell weight (-11638.70), filament weight (-15861.60) had a negative impact on cocoon yield while, the pupal weight had no impact on cocoon yield (Table 4).

Since, this is the first instance on the studies of muscardine infection in thermotolerant breeds, no supporting literature is available to compare the present findings. However positive contribution of cocoon yield by number and single cocoon weight to cocoon yield by weight, under both thermal treatment and muscardine infections indicate that these traits have direct bearing on yield performance of thermotolerant breeds under both high temperature and muscardine conditions. Thus, it may be inferred that, the breeds may possess ability to tolerant both fungal infection and high temperature treatment. However, identify those breeds with such ability holds key for further breed improvement.

Table 4: Regression equations for cocoon yield under muscardine infection and thermal treatment in thermotolerant bivotine silkworms

Condition	Regression model
Muscardine infection	$Y = -98.90 - 18.39 (X_1) - 77.50 (X_2) + 2.34 (X_3) + 1823.92 (X_4) - 1146.42 (X_5) - 1338.29 (X_6) - 25.70 (X_7) - 0.74 (X_8) + 2968.67 (X_9)$
Thermal treatment	$Y = -10562.80 - 16.58(X_1) + 611.44 (X_2) + 3.02 (X_3) + 4771.86 (X_4) + 0 (X_5) - 11638.70 (X_6) + 265.45 (X_7) + 2.87 (X_8) - 15861.60 (X_9)$

Y = Cocoon yield by weight (g/1000 worms), X_1 = ERR, X_2= Fifth instar larval weight, X_3 = Cocoon yield by number per 1000 worms, X_4 = Single cocoon weight, X_5 = Pupal weight, X_6 = Shell weight, X_7 = Shell percentage, X_8 = Filament length and X_9 = Filament weight.

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