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Studies on the Effect of White Muscardine Infection on Growth and Yield Performance of Bivoltine Silkworm, *Bombyx mori* Breeds

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Authors Contribution

S. R. Manjunatha, K. C. Narayanaswamy & Manjunath Gowda : Conceptualization, designing & conduct of experiment; S. Chandrashekar : Contribution of experimental materials; M. K. Prasanna Kumar & P. S. Benherlal : Execution of field and lab experiments

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Abstract

To know the possibilities of tolerance for both abiotic and biotic stresses in silkworm, *Bombyx mori*, an investigation was conducted to assess the growth and yield performance of thermotolerant bivoltine silkworm breeds inoculated with *Beauveria bassiana.* Three muscardine resistant thermotolerant bivoltine breeds *viz*., B1, B4, B8 and muscardine susceptible but productive breed CSR_4 were selected and topically inoculated with the fungal spore suspension of *Beauveria bassiana* (6.86×10^4 spores /ml). The results revealed that the thermotolerant bivoltine silkworm breed B1 appeared to be more tolerant to fungal infection followed by B4 breed. The quantitative traits in B1 thermotolerant bivoltine silkworm breed under muscardine inoculation treatment revealed significantly longer larval duration (7.44 days) and highest fifth instar larval weight (37.28 g), pupation rate (68.12%) and cocoon yield by weight (14,731.75/10,000 worms), cocoon weight (1.49 g), pupal weight (1.16 g) and shell weight (0.31 g), as compared to other breeds such as B4 and B8. While least larval mortality (40.75%) and highest effective rate of rearing (59.25%), cocoon yield by number (5,925 per 10,000 worms) and shell ratio (22.64%) were recorded in B4 breed.

Keywords : Thermotolerance, Bivoltine, *Bombyx mori* L, *Beauveria bassiana*

India occupies the second position in global silk production next only to China. Sericulture in India is being practiced predominantly in tropical regions and to limited extent in temperate region. The existing tropical situation in the country provides scope for the exploitation of multivoltine breeds / hybrids as these breeds show the inherent capacity to perform well under varied and fluctuating environmental conditions. But the quality of multivoltine silk is low compared to the international standards. To meet this standard it is necessary to shift to bivoltine sericulture which assures the production of quantitatively and qualitatively superior cocoons (Sahana *et al.,* 2021).

The silkworm, *Bombyx mori* L. is delicate, sensitive and completely domesticated insect and classic model organism for lepidoptera. The success of cocoon production depends on disease management. Exploitation of the resistant of silkworm breeds towards different diseases causing pathogens is a better option for managing the crop loss. Diseases in silkworm are fairly common in occurrence inflicting serious losses. Four major categories of disease *viz.,* the microsporidian, viral, bacterial and fungal diseases, which are popularly known as pebrine, grasserie, flacherie and muscardine, respectively, cause damage to silkworms in different seasons. Among these diseases white muscardine caused by *Beauveria bassiana* (Bals.) Vuill. is one of the most devastating silkworm disease common during winter and rainy seasons. In India 10-40 per cent of the total

loss due to disease has been accounted for white muscardine (Janakiraman, 1961; Chandrasekharan and Nataraju, 1998). The climatic condition in the tropics is congenial for the occurrence and easy spread of fungal diseases and spreads easily under high humidity and low temperature conditions (Samson *et al*., 1990).

It is a well-established fact that, unlike multivoltine silkworms, bivoltines are more vulnerable to different stresses under tropical condition as these bivoltines have originated from temperate region. It is, therefore, imperative to evolve bivoltine silkworm breeds which can give stable yields under both abiotic and biotic stress conditions (Sahana *et al.,* 2021). Keeping this in view, ten thermotolerant bivoltine silkworm breeds *viz.,* B1, B2, B3, B4, B5, B6, B8, APS12 and APS45 under muscardine infection revealed that, B4 breed performed better with respect to cocoon weight, pupal weight, shell weight, shell ratio, filament length and filament weight, followed by B1 and B8 breeds (Keerthana *et al.,* 2019). In order to asses these thermotolerant silkworm breeds for their physiological responses under muscardine infection, a preliminary assay was done in the present study.

Material and Methods

The layings of three thermotolerant bivoltine silkworm breeds*, i.e.,* B1, B4 and B8 identified as muscardine resistant from the previous studies (Keerthana, 2018; Sreejith Vakayil, 2019; Jayashree, 2019 and Sahana, 2021) and susceptible bivoltine silkworm breed $CSR_{4,}$ were utilized for the present study. The characteristic features of these breeds are given in Table 1.

Larval and cocoon parameters of the thermotolerant bivoltine silkworm breeds used in the experiment.

Rearing of Silkworm Breeds

Silkworm rearing was conducted during July 2020-April 21 at the Department of Sericulture, UAS, GKVK, Bengaluru. Silkworms were reared in following the standard rearing practices (Dandin

Table 1

Effect of *B. bassiana* infection on growth and survival of thermo tolerant bivoltine silkworm breeds

Breeds	Fifth instar larval Duration (days)		Fifth instar larval weight (g/10 larvae)		Efeective rate of rearing (%)		Pupation rate (%)	
	Control	*B. bassiana* infected	Control	*B. bassiana* infected	Control	*B. bassiana* infected	Control	*B. bassiana* infected
B1	7.29	7.44[b] (+2.06)	39.17	37.28[a] (-4.82)	100	58[a] (-42)	100	68.12[a] (-31.88)
B4	7.21	7.27[b] (+0.83)	38.38	33.79[b] (-11.95)	100	59.25[b] (-40.75)	100	63.75[b] (-36.25)
B8	7.33	7.41[b] (+1.09)	29.66	22.94[d] (-22.65)	100	44.25[c] (-55.75)	100	51.87[c] (-48.13)
CSR4	8.39	8.47[a] (+0.95)	31.12	27.29[c] (-12.30)	100	18.38[d] (-81.62)	100	26.87[d] (-73.13)
F TEST	**	**	**	**	NA	**	NA	**
SEm±	0.08	0.05	0.09	0.25	-	0.04	-	0.65
CD at 5%	0.26	0.13	0.29	0.79	-	1.11	-	2
CV (%)	2.23	1.17	0.54	1.69	-	1.16	-	2.47

Note: Positive and negative figures in the parenthesis indicate per cent increase (+) or decrease (-) over ; control, respectively; ** - Significant @1%; NA – Not analysed

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Genotypes	Breed traits
B1	Plain larva spinning oval shaped cocoon
B4	Plain larva spinning oval shaped cocoon
B8	Marked larva spinning peanut cocoon
CSR_4	Plain larva spinning oval shaped cocoon

and Giridhar, 2014) on V-1 mulberry leaves till spinning. Newly ecdysed fifth instar silkworms (50 silkworms per replication in four replication each) were topically inoculated with *B. bassiana* spore suspension with 6.86 x 10^4 spores per ml at the rate of 0.5 ml per silkworm (Keerthana *et al*., 2019). Simultaneously, a control batch of all the four breeds was also maintained. Three such rearings was conducted and pooled mean data on fifth larval duration, larval weight, larval mortality, ERR, cocoon yield by number and by weight, cocoon weight, pupal weight, shell weight, pupation rate and shell ratio 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 among the breeds utilized under *B. bassiana* inoculation with respect to larval duration. The fifth instar larval duration was determined from first day of fifth instar till spinning both under normal condition and muscardine inoculation (Table 1).

Among the thermotolerant bivoltine breeds, under normal condition the shorter larval duration was observed in B4 (7.21 days), followed by B1 (7.29 days) and longer larval duration was recorded in B8 (7.33 days) and CSR_4 (8.39) breeds. In *B. bassiana* inoculated batch, CSR_4 breed showed maximum larval duration of 8.47 days, followed by B1 and B8 (7.44 and 7.41 days) and the minimum in B4 breed (7.27 days). In the pathogen inoculated batch, higher increase in larval duration was observed in B1 breed (2.06%) and B8 (1.09%) breeds. While least was seen in B4 (0.85%) breed (Table 2). In a similar study, four thermotolerant silkworm breeds (B1, B4, B6 and B8) and their hybrids were inoculated with different dilutions (10^{-2}, 10^{-4}, 10^{-6}, 10^{-8}) of *B. bassiana* where

Table 2

Cocoon yield in thermo tolerant bivoltine silkworm breeds subjected to *B. bassiana*

Breeds	Cocoon yield per 10,000 larvae			
	By number (No.)		By weight (g)	
	Control	*B. bassiana* infected	Control	*B. bassiana* infected
B1	10000	5,800	19,349.12	14731.75
B4	10000	5,925	18,620.25	12551.00
B8	10000	4,425	13,292.50	11518.25
CSR4	10000	1,837	16,115.12	9681.80
F TEST	NA	**	**	**
SEm±	-	36.08	33.79	53.36
CD at 5%	-	111.197	104.116	164.45
CV (%)	-	0.160	0.40	0.881

Note: ** - Significant @1% ; NA- Not analysed

in B6 (10.58 days) breed, B6 x B1 and B6 x B8 (10.50 days each) hybrids showed prolonged larval duration over all the dilutions compared to control (Jayashree *et al*., 2020b). In fungus inoculated batch the fifth instar larval duration was extended and the extent of prolongation was higher in B1 breed. (7.44 days). Results of the current study with respect to prolongation of larval duration are in conformity with the findings of Manjunath Gowda *et al.* (2011) under *Bm*NPV infection and Keerthana *et al.* (2019) and Sahana *et al.* (2021) under muscardine infection. The prolongation of larval duration is due to reduced metabolic activity in the infected silkworms (Janakiraman *et al.,* 1961). Least prolongation of larval duration is desirable which evolving muscardine resistance breeds and hence B4 breed is suitable.

Fifth Instar Larval Weight (g)

Fifth instar larval weight was affected significantly among all the thermotolerant bivoltine silkworm breeds due to *B. bassiana* inoculation (Table 2). In the normal batch, among thermotolerant bivoltine silkworm breeds the maximum fifth instar larval weight of 39.17 g/10 larva was observed in B1, followed by B4 (38.38 g/10 larva) and minimum larval weight was recorded in B8 (29.66 g/10 larva) followed by CSR4 (31.12/10 larva g). In muscardine treated batch (Table 1) the larval weight was significantly higher in muscardine resistant thermo tolerant bivoltine breed B1 (37.28 g10 larva), followed by B4 (33.79 g/10 larva) and lowest larval weight was observed in B8 (22.94 g/10 larva), followed by CSR_4 (27.29 g/10 larva) breeds. Decrease in body weight in *B. bassiana* infected silkworms is due to cessation of feeding, decrease in food consumption, digestion, relative consumption rate and efficiency of conversion of ingested food (Venkataramana Reddy, 1978 and Cai, 1989). In earlier studies B4, B2 and B1 exhibited highest larval weight (21.35 g/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 breeds, the thermotolerant breed B1 lost its body weight from 42.27 g (control) to 35.39 g (muscasrdine treated) which was the least reduction in larval body weight (Jayashree *et al.,* 2020b). Results of the current study are in conformity with the earlier findings in thermotolerant bivoltine breeds under muscardine inoculation.

Larval Mortality (%)

Significant differences were observed for larval mortality among the four thermotolerant bivoltine breeds under muscardine inoculation (Fig.1). In the batch without any fungal treatment, all the four thermotolerant bivoltine breeds had no mortality. Under *B. bassiana* infection, larval mortality among thermotolerant bivoltine breeds was significantly least in breed B4 (40.75%), followed by B1 (42.00%), B8 (55.75%) and maximum larval mortality was observed in CSR_4 (81.63%) (Fig.1).

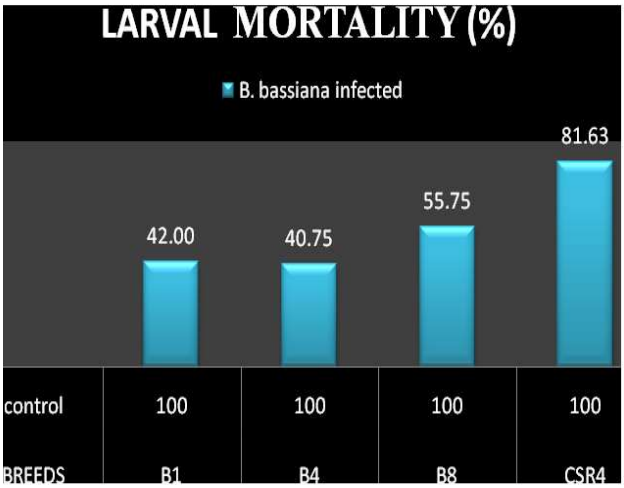

*Fig*1. Larval mortality (%) of the thermotolerant bivoltine silkworm breeds inoculated by *B. basssiana* (6.86×10^4 spores/ ml @ 0.5 ml per/silkworm)

According to Sreejith vakayil (2019), 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%) under *B. bassiana* inoculation. The thermotolerant bivoltine breeds used in this study also showed variation in the larval mortality due to fungal infection. B4 breed showed minimum larval mortality when infected with fungal pathogen.

Effective Rate of Rearing (%)

Effective rate of rearing (ERR) among the 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 1).

In muscardine treated batch, ERR was significantly different among the breeds (Table 1). Among the thermotolerant bivoltine silkworm breed B4 recorded highest ERR of 59.25 per cent, followed by B1 (58.00%) and lowest ERR was observed in B8 (44.25%) and CSR_4 (18.38%) breeds. Previously, when eight races of silkworms *viz.,* Pure Mysore, Hosa Mysore II, C-Nichi, HS_6, NN_6D, NB_4D_2, KA, J_{122} 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. Results of the current study are also in conformity with the earlier findings of Jayashree *et al.* (2020a) and Sahana *et al.* (2021), who reported better performance of B4 & B1 breeds, respectively, under *B. bassiana* infection.

Pupation Rate (%)

There was no significant difference among the breeds in control batch for pupation rate (Table 1). The thermotolerant bivoltine silkworm breeds inoculated with fungal spores of *B. bassiana* showed significant differences in pupation rate (Table 1). Among the treated batch the maximum pupation rate was recorded in the breed B1 (68.12%) followed by B4 (63.75%) and B8 (51.87%). Minimum pupation rate was observed in breed CSR4 (26.87%). The literature pertaining to the effect of muscardine infection in silkworms on pupation rate is rather limited. However, Chandrashekaran *et al.,* (2006) have observed reduced pupation rate in silkworms infected with *Bm*NPV and the reduction was lesser at lower dose of inoculation of viral inoculation and comparatively higher at the higher dose. Pupation rate was significantly affected by the *Bm*NPV infection in different silkworm genotypes. Multivoltine breeds, Pure Mysoe, Nistari and C-Nichi and diapausing breed Diazo showed comparatively higher pupation rate than bivoltine breeds CSR_2 and CSR_4 at all doses of *Bm*NPV infection (Manjunath Gowda, 2011).

Cocoon Yield by Number (No. / 10,000 worms)

Thermotolerant bivoltine silkworm breeds when treated with *B. bassiana* showed significant differences for cocoon yield by number per 10,000 worms (Table 2). Under fungal stress, significantly highest number was recorded in breed B4 (5,925/ 10,000 worms), followed by B1 (5,800/10,000 worms) and B8 (4,425/10,000 worms) and significantly least cocoon yield by number per 10,000 worms was recorded in breed CSR4 (1,837).

Chandrasekharan and Nataraju (1998) 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) 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 B1 and B4 could yield more cocoons per 10,000 worms under fungal infection.

When eight races of silkworm were inoculated with nine conidial concentrations (10^1 -10^9 spores/ml) of *B. bassiana*, no cocoons were spun at the two highest concentrations, as compared with 96.67 to 100 per cent cocoons formed in the batches with no treatment and 48 to 78 per cent in the case of the batches with the lowest 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 (500/1,000 worms) under *B. bassiana* inoculation. Results of the

TABLE 3

Effect of *B. bassiana* infection on cocoon parameters of thermo tolerant bivoltine silkworm breeds

Breeds	cocoon weight (g)		Shell weight (g)		Pupal weight (g)		Shell ratio (%)	
	Control	*B. bassiana* infected	Control	*B. bassiana* infected	Control	*B. bassiana* infected	Control	*B. bassiana* infected
B1	1.94[a]	1.49[a]	0.45a	0.31[a]	1.48[a]	1.16[a]	23.78[a]	20.97[a]
		(-38.65)		(-31.11)		(-21.62)		(-11.81)
B4	1.91[a]	1.27[b]	0.47[a]	0.28[b]	1.41[a]	0.97[b]	24.97[a]	22.64[a]
		(-33.50)		(-40.42)		(-31.20)		(-9.33)
B8	1.36[c]	1.15[c]	0.24[c]	0.20[c]	1.09b	0.90[b]	17.35[b]	17.83[b]
		(-15.44)		(-16.66)		(-17.43)		(+2.76)
CSR4	1.69[b]	1.10[c]	0.38[b]	0.22[c]	1.31[a]	0.71[c]	24.24[a]	22.01[a]
		(-34.91)		(-42.10)		(-45.80)		(-9.19)
F TEST	*	**	**	**	**	**	**	**
SEm±	0.05	0.02	0.009	0.004	0.05	0.03	1.23	0.78
CD at 5%	0.16	0.07	0.03	0.014	0.14	0.108	3.77	2.43
CV (%)	6.05	3.68	5.18	3.66	7.06	7.49	10.85	7.56

Note: Positive and negative figures in the parenthesis indicate per cent increase (+) or decrease (-) over control, respectively;
*-Significant @ 5%; ** - Significant @1%

current study are also in conformity with the earlier findings of Jayashree *et al.* (2020) under *Beauveria bassiana* infection.

Cocoon Yield by Weight (g / 10,000 Worms)

Effect of *B. bassiana* infection on cocoon yield by weight showed significant difference and the highest value for cocoon yield by weight was observed in breed B1 (14731.75 g/10,000 worms), followed by B4 (12,551.00 g/10000 worms) and B8 (11,518.00 g/ 1000 worms) and significantly lowest value was noticed in the breed CSR_4 (9681.80 g/10000 worms) (Table 3).

When four thermo tolerant bivoltine breeds *viz.,* B1, B4, B6, B8 and their hybrids *viz.*, B1× B4, B1× B6, B1× B8, B4× B1, B4× B6, B4× B8, B6× B1, B6× B4, B6× B8, B8× B1, B8× B4 and B8× B6, 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). The thermotolerant bivoltine silkworm breeds B4, B6 and B8 recorded highest cocoon yield by weight when inoculated with *B. bassiana* spores, which might be due to their ability to spin good cocoons even under infected condition (Keerthana *et al*., 2019). According to Sreejith Vakayil (2019), among the eight thermotolerant bivoltine breeds (B1 to B8), significantly highest cocoon yield by weight was recorded in B4 (1007.50 g / 1000 worms), followed by B1 (971.00 g / 1000 worms) / 1000 worms) under *B. bassiana* infection, which is in alternation with the present study.

Cocoon Weight (g)

Significant differences were observed among the thermotolerant bivoltine silkworm breeds for single cocoon weight in *B. bassiana* inoculated and control batches. In *B. bassiana* inoculated batches, maximum cocoon weight of 1.49 g was recorded in B1 followed by B4 (1.27 g) and B8 (1.15 g) breeds and it was minimum in CSR4 (1.10 g) breed. The decrease in cocoon weight over control by 15.44 per cent, 38.65

per cent, 34.91 per cent and 33.50 per cent were observed in B8, B1, CSR4 and B4 respectively (Table 2). Suggesting B8 to be productive under infection. Raghavaiah and Jayaramaiah, (1990) reported that NB7 formed cocoons with maximum weight (1.027 g) compared to NB_{18} (0.940 g) when infected with muscardine. Also, Rajitha and Savithri, (2014) reported significant reduction in cocoon weight in PM×CSR_2 when infected with *B. bassiana* spore on first day of fifth instar which was similar to the present study.

Shell Weight (g)

The breeds showed significantly different cocoon shell weights when they were subjected to *B. bassiana* inoculation. In *B. bassiana* inoculated batch, significantly highest cocoon shell weight was recorded in B1 (0.31 g) followed by B4 (0.28 g) and CSR4 breeds (0.22 g). The breed B8 recorded the lowest cocoon shell weight of 0.20 g. (Table 3). In earlier studies NB7 silkworm spun cocoons with maximum shell weight compared to NB4D2, KA and NB18 bivoltine silkworm breeds when they were infected with different doses of *B. bassiana* spore during fifth instar (Venkataramana Reddy, 1978). Topical application of *B. bassiana* spores to ten thermotolerant silkworm breeds recorded the highest cocoon shell weight in B4 (0.24 g) and B1 (0.19 g) breeds (Keerthana *et al.,* 2020) these findings are corroborating with that observed in the present study.

Pupal Weight (g)

Pupal weight was significantly affected among the *B. bassiana* inoculated thermotolerant bivoltine silkworm breeds. In *B. bassiana* treated batch, B1 breed exhibited significantly maximum pupal weight of 1.16 g, followed by B4 (0.97 g) and B8 (0.90 g) breeds. Pupal weight was significantly lowest in CSR_4 (0.71 g) breed. The B4 breed recorded highest (31.20%) reduction in pupal weight over control and it was lowest in B8 breed (17.43%) (Table 3). Reduction in pupal weight in cross breed (PM x CSR_2) silkworm was observed when treated with sub-lethal concentration of *B. bassiana* conidial suspension (Rajitha and Savithri, 2014). The present findings were also supported by results of Keerthana *et al.* (2020), wherein B1 breed recorded maximum pupal weight of 0.92 g followed by B4, B6,B7 and B8 (0.87 g each) under muscardine infection.

Shell Ratio (%)

Cocoon shell ratio was significantly affected due to *B. bassiana* inoculation among the thermotolerant silkworm breeds. In *B. bassiana* inoculated batch, B4 breed exhibited significantly highest cocoon shell percentage of 22.64, followed by CSR4 and B1 breeds (22.01 and 20.97%, respectively). Silkworm breed B8 exhibited significantly lowest cocoon shell ratio of 17.83 per cent. Cocoon shell ratio was highly affected in B1 breed as it recorded 11.81 per cent decrease over control while, it increased in B8 (2.76%) breed and least reduction in CSR4 breed (9.19%) (Table 3). In earlier studies, NB_4D_2 produced highest shell ratio compared to NB_7, KA and NB_{18} (Venkataramana Reddy, 1978) and cross breed, PM x CSR_2 recorded lesser cocoon shell ratio of 12.80 per cent under *B. bassiana* infection compared to control (16.43%) (Seema *et al.,* 2019).

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