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# Thermo-tolerance characterization and bioassay of selected entomopathogenic fungal (EPF) isolates on larva of *Spodoptera litura* Fab.

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**Abstract**
Entomopathogenic fungi *viz. Metarizhium anisoplae* and *Beauveria bassiana* are well characterized in respect to pathogenicity to several insects and have been used for biological control of agriculture pests worldwide. Despite their potential, several factors like low resistance to elevated temperature have hindered widespread adoption of fungi as part of biological control regimes. In this study, five isolates of *B. bassiana* (Bb 01, Bb 02, Bb 03, Bb 04 and Bb 05) and two isolates of *M. anisoplae* (Ma 01 and Ma 02) were evaluated for thermotolerance capacity and bioefficacy against third instar of *Spodoptera litura*. Among the isolates studied, Bb 04 recorded the higher temperature tolerance up to 34°C with mean spore germination of 35.00 percent and mean dry mycelial weight of 443.38 mg. Further, in the bioassay studies, Bb 04 caused significantly (@ P<0.01%) higher percent mortality of *S. litura* larva at $1\times10^8$ spores $ml^{-1}$ on fifth day (80.00 %) with $LT_{50}$ value of 4.08 days and $LC_{50}$ value of $2.07\times10^4$ conidia $ml^{-1}$ at five days. Investigation results indicated that BB 04 is a suitable EPF isolate for management of *S. litura* where higher temperature prevails.

**Keywords:** *Beauveria bassiana,* bioassay, *Metarizhium anisoplae, Spodoptera litura,* Thermo-tolerance

## INTRODUCTION

Over reliance on broad spectrum pesticides has been severely condemned in different parts of the world after International Conference on Chemicals Management (Tscharntke *et al*., 2005). Since then, an alternative eco-friendly strategy for the management of noxious insect pests has been explored to trim down the harmful effects of chemical insecticides on humanity. Studies on biodiversity in agro-ecosystems and the delivery of ecosystem services to agricultural products have usually ignored the contribution of entomopathogens in the regulation of pest population. In recent years, biological control based on crop protection with the inundative release of natural enemies and entomopathogens *viz.,* fungi, bacteria, virus *etc.,* are recognized as a valuable tool in pest management (Anand and Tiwary, 2009). In inundative applications of microbial control agents, combination treatment with two entomopathogens offers an attractive biorational strategy. If the two entomopathogens complement each other, or act synergistically, a beneficial effect can be obtained. Rao *et al*., 2006).

Entomopathogenic fungi are parasites of insects and kills or seriously disables them. An attractive feature of these fungi is that infectivity is by contact and the action is through penetration (Nadeau *et al.,* 1996). Entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana* are well characterized in respect to pathogenicity to several insects and have been used for biological control of agriculture pests worldwide (Sandhu *et al.,* 2012).

Despite their potential, several factors have hindered widespread adoption of fungi as part of biological control regimes. In particular, efficacy against certain insects is impeded by the relatively low resistance to elevated temperatures (Rangel *et al*., 2008). The optimal temperatures for the development of pathogenicity in most entomopathogenic fungi are 25-28$^{o}$C. Most previous studies on temperature tolerance of entomopathogenic fungi involved selection of isolates with higher tolerance to extreme environmental temperatures in the field (Rangel *et al*., 2005). Therefore, the current investigation was formulated to evaluate thermo-tolerance capacity and bioassay potential of selected entontomopathogenic fungal isolates on third instar of *Spodoptera litura.*

## MATERIALS AND METHODS

**Isolates collection:** Seven EPF isolates were collected from different Agricultural Colleges of Karnataka, of which five were *B. bassiana* isolates and two were *M. anisopliae* (Table 1). This was a part of doctoral study conducted in Department of Agricultural Entomology, University of Agricultural Sciences, Raichur (Karnataka) during 2014-15 and 2015-16.

**Morphological characterization:** EPF isolates were cultured on Potato Dextrose Agar (PDA) media in petriplates and incubated at 25$^{o}$C in the dark for two weeks for mycelial growth and spore production. Then, spores of each EPF isolates were harvested using a loop and placed the spores in one ml of sterile distilled water containing tween 80 (0.01%). The concentration of spore suspension was determined by haemocytometer and further it was adjusted to $1\times10^{6}$ conidia/ml under microscope at 40X magnification. Subsequently, morphological characters of EPF *viz.,* colony colour, shape, conidia diameter and conidia colour of each EPF were recorded separately (Isra' Omar Ahmad, 2011).

**Thermo-tolerance characterization:** Temperature tolerance capacity of each EPF isolates was determined through spore germination test and dry mycelial weight at temperatures of 25, 28, 30, 32 and 34$^{o}$C in incubators.

**Spore germination test:** Spores were collected from two week old culture using a loop and transferred to a glass vial containing 10 ml of sterile distilled water and 0.01 percent Tween 80. Concentration of spore suspensions of each fungal isolates was adjusted to $10^{6}$ spore ml$^{-1}$. A volume of 100 µl of spore suspension was inoculated and spread over a thin layer of PDA medium in separate petriplates (3 ml medium per 90 mm petriplate). After the inoculation, petriplates were sealed with parafilm and incubated at temperatures of 25, 28, 30, 32 and 34$^{o}$C for 24 hrs. After the 24 hrs of incubation, a total number of 100 spores were chosen at random from different fields of the petriplate to count the number of germinated spores under compound microscope at the magnification of 40X. (A spore was considered germinated, if the germ tube length reached at least the length of the conidium). The spore counting was performed in four replicates for each isolate and for each temperature.

**Dry mycelial weight:** Mycelial disc of five mm size was cut from seven days old actively grown fungal culture and inoculated separately into conical flasks containing Potato Dextrose Broth (Each 50ml conical flask containing 30 ml broth). For each isolate at each temperature, four replicates were maintained. Flasks were incubated at temperatures of 25, 28, 30, 32 and 34$^{o}$C for eight days. After eight days of incubation, the mycelial growth was harvested and dried at 70$^{o}$C for 24 hrs in hot air oven. Further, the dry weights were recorded using electronic digital balance in four replicates for each treatment and the average mycelial dry weight was calculated.

**Rearing and maintenance of *S. litura* culture:** Mass rearing of *S. litura* was initiated in the growth chambers of the laboratory with freshly laid egg masses collected from the fields of Agricultural College, Raichur. Growth chamber was sterilized with 10 percent formaldehyde prior to establishing the culture. Ambient environmental conditions *viz.,* 27±1.0$^{o}$C temperature, 75.0±5.0 percent relative humidity and 12h light: 12h dark photoperiod was maintained throughout the rearing. In order to have continuous supply of large number of quality insects, a stock culture of *S. litura* was maintained on leaves of the castor plant, *Ricinus communis* L. The freshly laid egg masses were incubated and maintained in small plastic containers with castor leaves to provide immediate food for the neonates after hatching. First instars neonates hatched from the egg mass were reared in bread boxes (20x10x10 cm). From the fourth instar onwards, larvae were reared in groups of 50-100 in a plastic tray (40x30x6 cm). Later larvae were allowed for pupation in moist and loose soil. The pupae were sexed on the fourth day after the sclerotization and hardening of pupal integument with respect to the position of gonopores. Male pupae had gonopore in the form of two kidney shaped bumps with a black slot in the middle of the ninth abdominal sternum and female pupae had gonopore as a black slot located on the eighth abdominal sternum. Adult moths were emerged in seven to eight days. Generally 10-12 pairs of moth were released in insect cages (45x40x40cm) for mating and oviposition provided with 10 percent honey solution as food for them. Castor leaves were provided as an ovipositional substrate to the female moth within the cage by dipping their petiole in water containing glass beaker (Seth and Sharma, 2002).

Experiments on thermo-tolerance and bioassay

were conducted twice. Though there were very little variations found in the generated data during two seasons, they were pooled and statistically analysed and results were presented as hereunder.

**Bioassay:** Pathogenicity of fungi was determined as the estimated spore concentration required to kill 50 percent of the test insects ($LC_{50}$, expressed as conidia $ml^{-1}$), and its virulence was estimated by the median time to death of infected insects ($LT_{50}$) (Thomas and Elkinton, 2004). The fungal isolates which found temperature tolerant in the previous study were subjected for bioassay studies.

Conidial suspensions were prepared by harvesting conidia from two week old sporulating cultures and suspending them in sterile distilled water containing 0.2 percent Tween 80 in 10 ml glass bottles. The conidial suspensions were filtered through several layers of sterilized muslin cloth and discarded the mycelial mats. Then, spore concentrations were quantified using a haemocytometer (Goettel and Inglis, 1997) and were used for pathogenicity studies. The conidial suspension used for the virulence studies was adjusted to a final concentration of $1\times10^8$ conidia $ml^{-1}$. For the pathogenicity study, five concentrations of conidia including $1\times10^4$, $1\times10^5$, $1\times10^6$, $1\times10^7$ and $1\times10^8$ spore $ml^{-1}$ were used.

**Virulence studies:** Pre-starved third instar of *S. litura* were topically sprayed with one ml of the conidial suspension at $1\times10^8$ conidia $ml^{-1}$ of EPF isolate and placed in separate pertiplate containing moistened filter paper. For each isolate four replications containing 20 larvae per replication were maintained. These treated insects were incubated in a growth chamber with temperature of $27\pm1^0$ C with 75±5 percent relative humidity. The experimental larvae were observed for every 24 hr until death, followed by white mycelial growth on the surface of the cadavers. The total mortality data collected on each day were used for the determination of $LT_{50}$ using probit analysis(Gurvinder and Padmaja, 2008).

**Pathogenicity studies:** Different serial dilutions of conidial suspensions *viz.*, $1\times10^4$, $1\times10^5$, $1\times10^6$, $1\times10^7$ and $1\times10^8$ spore $ml^{-1}$ were prepared in sterile distilled water for promising isolates. Then, twenty third instar of *S. litura* in a petriplate (90 mm diameter) lined with cabbage leaf disc was sprayed with one ml of conidial suspension from each concentration per each isolate by potter's tower at pressure of 10 psi. Treated larvae were maintained in insect growth chamber at the temperature of $27.0\pm1.0^oC$ and relative humidity of 75.0±5.0 percent. For each isolate four replications containing twenty larvae per replication was used and similarly, another four replications containing twenty larvae per replication sprayed with sterile distilled water and 0.01 percent tween 80 was used as a control. Mortality was counted daily and the mortality data were corrected using Abbott's formula. The lethal concentration ($LC_{50}$ and $LC_{90}$) was determined by probit analysis. Only those larvae covered with white mycelia and spores were considered to have died as a result of fungal infection (Goettel and Johnson, 1992).

Percent mortality was calculated according to Abbot's (1925) formula:

$$\% \text{ Mortality} = \frac{\text{Mortality (\%) in treatment — Mortality (\%) in control}}{\text{100 — Mortality (\%) in control}} \times 100$$

….Eq.1

**Statistical analysis:** Data from repeated experiments conducted during 2014-15 and 2015-16 were pooled as ANOVA indicated no significant difference between the experiments. Percentage corrected mortality of the larva was calculated as described by Abbot's (1925). The arcsine transformation was used for normalisation of mortality percentage data before an ANOVA was conducted. Analysis was undertaken on the transformed data and untransformed means ± SE are presented. Entomopathogenic fungal inoculation rates and time effects, and their interactive effects on mortality percentage data were analyzed using WASP 2.0 (ICAR-Goa institute). When ANOVA was significant, comparisons of relevant means were made using Duncan Multiple Range Test (DMRT) significance test at a significance level of 5%. Lethal time and Lethal concentration were analyzed using SPSS 16.0V. Larvae used in the whole study were of age five days which were hatched from single mass of egg.

## RESULTS AND DISCUSSION

### Thermo-tolerance characterization

**Spore germination test:** There were no significant differences among the EPF isolates with re-

**Table 1.** Source of entomopathogenic fungal isolates.

| Entomopathogenic Fungi | Designation of the isolates | Isolated From | Area of collection |
|---|---|---|---|
| *Beauveria bassiana* | Bb 01 | Chrysomelid beetle | GKVK, UAS, Bengaluru |
| | Bb 02 | Sugarcane soil | Channarayapattana, Hassan |
| | Bb 03 | *S. litura* larvae | GKVK, UAS, Bengaluru |
| | Bb 04 | Lepidopteran larvae | Agriculture College, Bheemarayanagudi |
| | Bb 05 | Groundnut soil | UAHS, Shimoga |
| *Metarhizium anisopliae* | Ma 01 | French bean soil | Agriculture College, Chinthamani |
| | Ma 02 | Field bean soil | Hunsuru (Mysore) |

**Table 2.** Mean conidial germination of the selected *B. bassiana* and *M. anisopliae* isolates incubated at different temperatures.

| EPF isolates | Mean number of conidia germinated per 100 conidia at different temperature regimes | | | | |
|---|---|---|---|---|---|
| | At 25° C/24hr | At 28° C/24 hr | At 30° C/24 hr | At 32° C/24 hr | At 34° C/24 hr |
| Bb 01 | 97.12±1.18[a] | 94.13±3.57[a] | 73.25±2.06[a] | 50.13±1.03[a] | 30.50±0.40[c] |
| Bb 02 | 95.87±1.25[a] | 92.88±2.53[a] | 71.63±1.38[a] | 50.75±1.85[a] | 31.75±0.87[b] |
| Bb 03 | 89.75±3.12[a] | 89.75±2.96[a] | 64.00±4.38[b] | 22.75±0.87[b] | 2.38±0.25[e] |
| Bb 04 | 92.25±5.95[a] | 91.88±1.75[a] | 74.00±2.04[a] | 51.13±1.03[a] | 35.00±0.71[a] |
| Bb 05 | 92.25±5.24[a] | 91.25±3.07[a] | 64.00±2.48[b] | 22.88±1.49[b] | 2.63±0.85[e] |
| Ma 01 | 92.12±3.42[a] | 91.50±1.73[a] | 61.25±1.55[b] | 21.00±1.91[b] | 2.75±0.29[d] |
| Ma 02 | 92.87±2.29[a] | 90.75±2.63[a] | 56.88±2.17[c] | 23.00±1.22[b] | 3.75±0.29[e] |
| C.D (p<0.01) | NS | NS | 4.94 | 2.79 | 1.16 |
| C.V (%) | | | 3.73 | 4.05 | 3.74 |

* Means followed by the same letter in a column are not significantly different at P<0.01

**Table 3.** Mean mycelial dry weight of the selected *B. bassiana* and *M. anisopliae* isolates incubated at different temperatures**.**

| EPF isolates | Mean mycelial dry weight (mg) at different temperature regimes at 8 days of incubation period | | | | |
|---|---|---|---|---|---|
| | At 25° C | At 28° C | At 30° C | At 32° C | At 34° C |
| **Bb 01** | 980.13±23.28[a] | 943.25±25.69[a] | 723.13±08.02[b] | 485.00±25.34[b] | 429.63±3.38[b] |
| **Bb 02** | 981.38±15.00[a] | 949.25±10.90[a] | 738.50±17.82[ab] | 527.38±09.01[a] | 429.62±4.46[b] |
| **Bb 03** | 954.88±36.37[a] | 948.75±11.00[a] | 466.25±20.61[c] | 226.00±04.97[c] | 96.00±2.38[cd] |
| **Bb 04** | 985.50±38.61[a] | 966.00±08.64[a] | 759.75±20.22[a] | 543.00±12.09[a] | 443.38±1.25[a] |
| **Bb 05** | 939.25±41.82[a] | 925.25±33.07[a] | 423.88±08.93[d] | 223.88±02.39[c] | 103.13±3.94[c] |
| **Ma 01** | 983.88±10.63[a] | 950.25±09.07[a] | 483.75±38.19[c] | 186.25±14.00[d] | 92.50±10.80[d] |
| **Ma 02** | 979.50±23.53[a] | 950.50±13.00[a] | 491.25±00.32[c] | 198.00±08.12[d] | 98.38±3.06[cd] |
| **C.D (p<0.01)** | **NS** | | 40.55 | 25.80 | 10.16 |
| **C.V (%)** | | **NS** | 3.47 | 3.78 | 2.09 |

* Means followed by the same letter in a column are not significantly different at P<0.01

**Table 4.** Median lethal time ($LT_{50}$) of thermo-tolerant *B. bassiana* isolates against *S. litura* larva ($1x10^8$ conidia/ ml).

| EPF isolates | $LT_{50}$ values of EPFs | | | | |
|---|---|---|---|---|---|
| | $LT_{50}$ (days) | Fiducial limit (95%) | | Slope | $\chi^2$ |
| | | Lower limit | Upper limit | | |
| Bb 01 | 4.76 | 4.31 | 5.21 | 0.16 | 9.91 |
| Bb 02 | 4.44 | 4.20 | 4.67 | 0.15 | 7.62 |
| Bb 04 | 4.08 | 3.85 | 4.30 | 0.16 | 5.12 |

**Table 5.** Mortality of *S. litura* larva exposed to thermo-tolerant *B. bassiana* isolates at various concentration.

| Concentration (conidia per ml) | Per cent mortality | | |
|---|---|---|---|
| | Bb 01 | Bb 02 | Bb 04 |
| $1\times10^4$ | 40.63 (39.58)[d] | 40.00 (39.24)[e] | 46.00 (42.70)[e] |
| $1\times10^5$ | 45.00 (42.12)[d] | 45.00 (42.12)[d] | 51.88 (46.08)[d] |
| $1\times10^6$ | 55.63 (48.23)[c] | 55.00 (47.88)[c] | 61.00 (51.36)[c] |
| $1\times10^7$ | 61.88 (51.87)[b] | 65.00 (53.73)[b] | 69.00 (56.17)[b] |
| $1\times10^8$ | 70.00 (56.79)[a] | 75.00 (60.02)[a] | 80.75 (63.97)[a] |
| Control (0.02% Tween 80) | 0.00 (0.59)[e] | 0.00 (0.59)[f] | 0.00 (0.59)[f] |
| C. D (p<0.01 | 3.59 | 3.15 | 0.70 |
| **C.V (%)** | 2.63 | 2.31 | 0.78 |
| **SEm±** | 0.87 | 0.76 | 0.79 |

*Treatments with the same letter are not significantly different, p <0.01, Figures in the parentheses are arc sine transformed values, Twenty larvae per each replicates were used

spect to mean number of spore germination at the temperatures 25 and 28°C where, spore germinations were ranged from 89.75 to 97.12 (Table-2). However, as the temperature was increased, the rate of spore germination declined. At 30°C, the higher percent spore germination was recorded by the isolate Bb 04 (74.00) as compared to remaining isolates. Similarly at 34°C, Bb 04 recorded the higher spore germination of 35.00 percent and lowest spore germination was recorded in Bb 03 of 2.38 percent.

**Mean dry mycelial weight test:** There were no significant differences in the mean dry mycelial weight among EPF isolates (Table-3) grown at the temperatures 25 and 28°C. But the dry mycelial weight decreased with the increase in temperature. At 30°C, the higher mean dry mycelial weight was recorded in the isolate Bb 04 of 759.75 mg. Similarly, at 34°C, higher mean dry mycelial weight was recorded in Bb 04 of 443.38 mg and mean mycelial dry weight recorded to be lowest in

**Table 6.** Median lethal concentrations ($LC_{50}$ and $LC_{90}$) of thermo-tolerant *B. bassiana* isolates against larva of *S. litura* at various concentrations.

| EPF isolates | $LC_{50}$ (conidia/ml) | Fiducial limit (95%) | | $LC_{90}$ (conidia/ml) | Fiducial limit (95%) | | Slope | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|
| | | Lower limit | Upper limit | | Lower limit | Upper limit | | |
| Bb 01 | $2.45\times10^5$ | $3.81\times10^4$ | $1.39\times10^6$ | $1.38\times10^{11}$ | $1.31\times10^9$ | $3.17\times10^{13}$ | 0.28 | 0.30 |
| Bb 02 | $3.11\times10^5$ | $4.27\times10^4$ | $8.71\times10^5$ | $1.02\times10^{12}$ | $3.17\times10^{10}$ | $2.41\times10^{14}$ | 0.28 | 0.24 |
| Bb 04 | $2.07\times10^4$ | $5.98\times10^3$ | $2.06\times10^5$ | $7.95\times10^9$ | $2.94\times10^8$ | $6.24\times10^{13}$ | 0.29 | 1.07 |

Ma 01 of 92.50 mg.

Unfavorable temperatures are one of the limiting factors of EPF in controlling arthropods. Fungi tolerant to high temperatures are especially important, when fungal isolates are selected for insect pest management in the tropical and subtropical climate. Pathogenicity of most EPF is usually performed at 25-28°C. However, isolates with optimal growth temperatures below these levels may not be successful for application at the higher temperature (30-35°C) as shown by Polar *et al.* (2005). In the current study, Bb 04 recorded the temperature tolerance up to 34°C with higher mean spore germination and higher mean dry mycelial weight. It is also evident in the current study that the maximum temperature required for spore germination and mycelial growth was 32°C for all the isolates, which was in accordance with that of Walstad *et al.* (1970) on the *B. bassiana* and *M. anisopliae* isolates and Fargues *et al.* (1992) on *M. anisopliae* isolates.

**Bioassay studies**

**Estimation of $LT_{50}$ value:** Virulence studies were conducted with isolates *viz.,* Bb 01, Bb 02 and Bb 04 at the higher spore concentration of $1x10^8$. The estimated $LT_{50}$ values for these three isolates were ranged from 4.08 to 4.76 days (Table - 4). The smallest $LT_{50}$ value was recorded for Bb 04 of 4.08 days with fiducial limit of 3.85 to 4.30 days and the larger $LT_{50}$ value was recorded for Bb 01 of 4.76 days with fiducial limit of 4.31 to 5.21 days. The $\chi^2$ value was 9.91 and 5.12 for Bb 01 and Bb 04, respectively.

The estimated $LT_{50}$ value for Bb 04 was 4.08 days @ $1x10^8$ conidia/ml and which was closer to $LT_{50}$ value of 4.80 and 4.00 days recorded by Bb 02 and Bb 09 isolates of (*B. bassiana* from Madurai and Dindigul)on *S. litura* larva at $1.5x10^8$ conidia/ml (Moorthi *et al.,* 2011). Similarly, $LT_{50}$ value of the current studies were much larger than the $LT_{50}$ value estimated by Silva *et al*. (2003) of 1.10 days for *B. bassiana* isolate ESALQ 634 on second instar of *Plutella xylostella* at $1x10^8$ conidia/ml. Virulence of an isolate also depends on the interaction of the host, the pathogen and the environment, where study was done by Santiago *et al.* (2006) on *B. bassiana* to *Bemisia tabaci* reared on cucumber, tomato, melon, green pepper, potato, eggplant, marrow, cabbage, bean or cotton Thomas and Elkinton, 2004 on *Entomophaga maimaiga* for virulence and Faria and Wraight, (2007) based on compiled report of various entomopathogenic fungal isolates over insects of 48 families.

**Percent mortality on fifth day:** *B. bassiana* isolates *viz.,* Bb 01, Bb 02 and Bb 04 caused significantly higher percent mortality of *S. litura* larva at the concentration of $1\times10^8$ condia $ml^{-1}$ on fifth day under controlled environmental conditions compared to control (Table - 5). The highest cumulative mortality of 80.75 percent was recorded with Bb 04 isolate where as, Bb 01 resulted in the lowest cumulative mortality of 70.00 percent.

When EPF spores come in contact with cuticle, they germinate and grow directly through it to inner body of larva. Here fungus proliferates inside, produces toxins and nutrients, eventually kills the larva. In the current study, Bb 04 caused significantly higher percent mortality of *S. litura* larva at the concentration of $1\times10^8$ condia/ml on fifth day. Gupta and Kumar (2014) also reported a mortality of 80.00 percent on third instar of *S. litura*, when *B. bassaiana* applied at concentration of $0.2x10^8$ spore/ml.

**Estimation of $LC_{50}$ and $LC_{90}$ value:** The increasing conidial concentration significantly increased larval mortality as shown in the table 6. There were significant differences in percent mortality of *S. litura* larva at different spore concentration of three tested isolates on five days after treatment. The smallest $LC_{50}$ and $LC_{90}$ values were recorded to be $2.07\times10^4$ and $7.95\times10^9$ conidia $ml^{-1}$ with fiducial limit of $5.98\times10^3$ to $2.06\times10^5$ and $2.94\times10^8$ to $6.24\times10^{13}$ conidia $ml^{-1}$, respectively in the isolate Bb 04. While, the larger $LC_{50}$ and $LC_{90}$ values were $3.11\times10^5$ and $1.02\times10^{12}$ conidia $ml^{-1}$ with fiducial limit of $4.27\times10^4$ to $8.71\times10^5$ and $3.17\times10^{10}$ to $2.41\times10^{14}$ conidia $ml^{-1}$, respectively in the isolate Bb 02. The $\chi^2$ values were 0.24 and 1.07 for Bb 02 and Bb 04, respectively.

The estimated $LC_{50}$ value of Bb 04 was $2.07\times10^4$ conidia/ml at five days after treatment. The estimated $LC_{50}$ value of current bioassay studies were closer to $LC_{50}$ value of $2.1x10^6$, $3.6x10^7$ and $1.2x10^7$ conidia/ml on third instar of *S. litura*, registered by *B. bassiana* isolates (from Madurai and Dindigul) *viz.,* Bb 02, Bb 09 and Bb 10, respectively (Moorthi *et al*., 2011). The difference in pathogenicity between the isolates of current study and experimental isolates of Moorthi *et al.,* 2011 may be due to the susceptibility of larval stages, virulence of EPF isolates used for bioassay and laboratory conditions.

## Conclusion

Isolates of the same species of *B. bassiana* and *M. anisoplae* varied in their response to elevated temperatures with regard to spore germination and dry mycelial weight. All EPF isolates (Five of *B. bassiana* and two of *M. anisoplae*) were capable to grow at temperatures of 25-32$^{o}$C with apparent differences among isolates in their tolerance to the maximum temperature of 32$^{o}$C. At this temperature, Bb 04 showed higher germination rate of 51.13 percent spores and the higher dry mycelial weight of 543.00 mg. Subsequently, temperature tolerant EPF isolate, Bb 04 was subjected to bioassay against third instar of *S. litura,* where, Bb 04 showed the higher virulence ($LT_{50}$) and pathogenicity ($LC_{50}$). These results suggested that Bb 04 was a suitable EPF isolate for management of *S. litura,* where hot temperature prevails.

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