I J T A
© Serials Publications
National Academy of Agricultural Science (NAAS) Rating : 3. 03

# Bioassay of Entomopathogenic fungi (*Beauveria bassiana*) against tomato fruit borer under *in vitro* conditions

**P. Lakshmidevi[1]*, P. Gopalakrishnan[1] and A. Sridharan[1]**

***ABSTRACT:*** *Fruit borer (Helicoverpa armigera) is one of the most important pest of tomato which causes yield loss up to 60 percent. Management of this pest in tomato using pesticides leads to several disadvantages. Under these circumstances, inclusion of management strategies using biocontrol agent namely Beauveria bassiana play major role in managing the fruit borer in tomato. In the current study, twenty isolates of B. bassiana were collected and tested for their pathogenicity against H. armigera. The isolates with high mortality were selected and subjected invitro studies for fixing effective dosage. The results of the experiments revealed that, all twenty isolates of B. bassiana showed pathogenicity towards H. armigera with varied mortality ranged from 93.33% to 26.6%. Further, Bb 8 isolate with conidial concentration of $1\times10^8$ showed the highest per cent mortality (75.3%) when compared to Bb 2 and control treatments. Hence this study concludes that, Bb8 isolate can be used as good biocontrol agent for the development of different biofomulation of entomopathogenic fungi to manage fruit borer in tomato.*

***Keywords:*** *B. bassiana, Bioassay of insects, Entomopathogenic fungi, Helicoverpa armigera.*

## INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a major contributor to the fruit vegetable diet of humans. It is cultivated in almost all countries either in field or in green house system. It is a rich source of minerals, vitamins, essential amino acids, organic acids, iron and phosphorus, sugars and dietary fibres (Akrami and Yousefi, 2015). This tomato crop is affected by various pests which causes considerable yield loss. Among the pests, fruit borer (*Helicoverpa armigera*) is one of the most important pest which recorded a yield loss up to 60 percent. Tomato is very much susceptible to *H. armigera* and it has been reported to cause damage to an extent of about 50-60 per cent fruits (Singh, 1985). The key pest status of *H. armigera* is due to the larval preference for feeding on plant parts rich in nitrogen such as reproductive structures and growing tips. These structures are highly suitable for larval development (Fitt, 1989).

Management of this pest in tomato by using pesticides has led to the development of resistance in the insect besides environmental pollution as well as human health's hazards (Singh *et al.*, 2001). Under these circumstances, inclusion of management strategies using biocontrol agent namely *Beauveria bassiana* played major role in managing the pests in crop plants (Senthilraja *et al.*, 2010; Wraight *et al.*, 2010) has been used extensively for the control of wide range of insect pests. This fungus is natural inhabitants of soil and has been used as biocontrol agents for many insect pests (Milner *et al.*, 1993; Sharma *et al.*, 1999).

Entomopathogenic fungi infects insects by breaching the host cuticle and this is more advantageous from the point of view of pest control because propagules don't have to be ingested and thus are active against the non-feeding stages of insects. The fungal hyphae produce enzymes and toxins. Host insects are infected when getting into contact with conidiospores (conidia) which are usually passively distributed by wind. After attaching to the insect (Bateman *et al.*, 1996), conidia penetrate the cuticle with the help of enzymatic degradation and pressure of the germ tube (Starnes

[1] Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore-641 003.
* Corresponding Authors. *E-mail : lakshmidevisp@gmail.com* *Telephone : 91-422-6611446, Fax : 91-422-6611437*

**Table 1**
**Isolates of *Beauveria bassiana* (Bb) collected from different places of Tamil Nadu**

| S. No. | Isolate code | Place | Source | District |
|---|---|---|---|---|
| 1. | Bb 1 | Parangipettai | Soil | Cuddalore |
| 2. | Bb 2 | Arachalore | Infected larva | Erode |
| 3. | Bb 3 | kadhuaruthanmedu | Infected larva | Villupuram |
| 4. | Bb 4 | Chinnasalem | Soil | Villupuram |
| 5. | Bb 5 | Veerachozhapuram | Soil | Villupuram |
| 6. | Bb 6 | Kallakuruchi | Infected larva | Villupuram |
| 7. | Bb 7 | Kattukotai | Soil | villupuram |
| 8. | Bb 8 | Udumalai | Infected larva | Tirupur |
| 9. | Bb 9 | Kinathukadavu | Infected larva | Coimbatore |
| 10. | Bb 10 | Malumichanpatti | Soil | Coimbatore |
| 11. | Bb 11 | Thazhiyur | Infected larva | Coimbatore |
| 12. | Bb 12 | Pollachi | Infected larva | Coimbatore |
| 13. | Bb 13 | Coimbatore | Soil | Coimbatore |
| 14. | Bb 14 | Karamadi | Infected larva | Coimbatore |
| 15. | Bb 15 | Pudukkottai | Infected larva | Pudukottai |
| 16. | Bb16 | C.K.Valasu | Soil | Dindigul |
| 17. | Bb 17 | Dindigul | Infected larva | Dindigul |
| 18. | Bb 18 | Vangudi | Infected larva | Kattumanar-koil |
| 19. | Bb 19 | Valliyur | Infected larva | Tirunelveli |
| 20. | Bb 20 | Keezhpinnathur | Soil | Tiruvanna-malai |

**Table 2**
**Pathogenicity of *Beauveria* isolates against *H. armigera* under *in vitro* conditions**

| S. No | Isolates | Percentage of larval mortality |
|---|---|---|
| 1. | Bb 1 | $43.33^{gh}$ (41.16) |
| 2. | Bb 2 | $86.66^{b}$ (68.75) |
| 3. | Bb 3 | $55.33^{e}$ (48.06) |
| 4. | Bb 4 | $50.00^{ef}$ (44.99) |
| 5. | Bb 5 | $73.33^{c}$ (58.94) |
| 6. | Bb 6 | $46.66^{jk}$ (41.16) |
| 7. | Bb 7 | $30.00^{k}$ (33.20) |
| 8. | Bb 8 | $93.33^{a}$ (75.74) |
| 9. | Bb 9 | $40.00^{hi}$ (39.22) |
| 10. | Bb 10 | $53.33^{fg}$ (46.91) |
| 11. | Bb 11 | $23.33^{c}$ (28.87) |
| 12. | Bb 12 | $31.66^{l}$ (34.24) |
| 13. | Bb 13 | $40.00^{hi}$ (39.22) |
| 14. | Bb 14 | $36.66^{ij}$ (37.26) |
| 15. | Bb 15 | $66.66^{e}$ (54.75) |
| 16. | Bb 16 | $76.66^{c}$ (61.16) |
| 17. | Bb 17 | $26.66^{kl}$ (31.06) |
| 18. | Bb 18 | $43.33^{gh}$ (41.16) |
| 19. | Bb 19 | $50.00^{ef}$ (44.99) |
| 20. | Bb 20 | $53.33^{e}$ (46.91) |

Values are mean of three replications.
Figures in parentheses represent arcsine transformation. Means in a column followed by same superscript letters are not significantly different according to DMRT at $P \leq 0.05$

*et al.*, 1993). Extracellular chitinases have been reported to be a major virulence factor in fungal entomopathogenicity. The production of cuticle degrading enzymes by entomopathogenic fungal pathogens enhances the pathogenicity on insect hosts.

Various formulated conidia of *B. bassiana* B2 has been extensively studied against various insect pests like, rice leaffolder (Sivasundaram *et al.*, 2008; Karthiba *et al.*, 2010), chickpea pod borer (Saravanan, 2007), groundnut leafminer (Senthilraja *et al.*, 2010) and found that all these insects were highly susceptible to the *B. bassiana* B2 under *in vitro*. Combined application of endophytic *Bacillus* and *Beauveria* significantly reduced the *Fusarium* wilt disease and fruit borer in tomato under field conditions (Prabhukarthikeyan *et al.*, 2013).

With this supporting information, the present investigation was undertaken to evaluate the different isolates of *B. bassiana* against fruit borer of tomato under *in vitro* conditions.

## MATERIALS AND METHODS

Isolation of entomopathogenic fungal isolates from soil and cadavers

For the isolation of entomopathogenic fungi existing in soil, the insect-bait method was used. Bait for this method was larvae of *H. armigera* and soil samples were collected from different tomato growing regions of Tamil Nadu. Each soil sample was placed in four separate Petri dishes of 35 mm in diameter and a small quantity of sterilized water was added to the dish. Three larvae were placed in each dish and the dishes were kept at room temperature. Each larva was taken out of the dish after 24 h of burying, transferred to a test tube of 18 mm × 180 mm covered with cheese cloth and fed with leaf of tomato. These larvae were checked daily for mortality and dead ones were placed in 35 mm Petri dishes with moistened filter paper after 2-3 days of drying in the tube. To isolate the fungus, SDY medium (Sabouraud's dextrose with 1 % yeast extract medium) was used which contains ingredients viz., (Barley flour 50 g; Dextrose 10g; Neo peptone 4g; Yeast extract 2g; Agar Agar 18g; Distilled water 1000 ml.

Conidia of the pathogenic fungi formed on the cadavers were taken by a mycological loop and streaked on SDY medium. After incubation at room temperature 28 ± 2°C for a week, the colonies obtained were transferred to SDY slant for preservation. The isolate was identified by microscopically inspecting the conidia forming mycelia for conidiogenous structure and conidial morphology.

**Table 3a**
**Concentration-mortality responses of third instar larvae of *H. armigera* to *B. bassiana* (Bb8) LC50**

| | | | Fiducial limit (95%) | | |
|---|---|---|---|---|---|
| *Conidial concentration* | *% mortality* | *LC50 (spore/ml)* | *lower* | *upper* | *Slope (± S.E)* |
| $1 \times 10^2$ | 31.6 (32.18)[d] | $5.3 \times 10^4$ | 0.2 | 32.9 | 0.20 |
| $1 \times 10^4$ | 44.9 (46.54)[c] | – | – | – | – |
| $1 \times 10^6$ | 63.6 (68.96)[b] | – | – | – | – |
| $1 \times 10^8$ | 75.3 (85.10)[a] | – | – | – | – |
| Control | 0.00 (0.00)[e] | – | – | – | – |

**Table 3b**
**Concentration-mortality responses of third instar larvae of *H. armigera* to *B. bassiana* (Bb 2) LC50**

| | | | Fiducial limit (95%) | | |
|---|---|---|---|---|---|
| *Conidial concentration* | *% mortality* | *LC50 (spore/ml)* | *lower* | *upper* | *Slope (± S.E)* |
| $1 \times 10^2$ | 30.60 (31.13)[d] | $5.78 \times 10^4$ | 0.5 | 68.0 | 0.19 |
| $1 \times 10^4$ | 42.90 (44.33)[c] | – | – | – | – |
| $1 \times 10^6$ | 61.10 (65.73)[b] | – | – | – | – |
| $1 \times 10^8$ | 71.10 (79.09)[a] | – | – | – | – |
| Control | 0.00 (0.00)[e] | – | – | – | – |

### Bioefficacy of *B. Bassiana*

Twenty isolates of *B. bassiana* were tested against the larvae of tomato fruit borer with different conidial concentration in order to determine the median lethal time ($LT_{50}$) and median lethal concentration ($LC_{50}$) for the virulent isolates.

### Preparation of Conidial Suspension

Mycelial discs of different isolates of *B. bassiana* were inoculated in SDY broth (Sabouraud's dextrose with 1% (w/v) yeast extract without agar) and incubated at 25 ± 1°C for 48 h with shaking at 180 rpm.

### Preparation of Conidial Concentration

The fungal pathogenic isolates were cultured on SDY medium and the Petri dishes were incubated for 7-10 days at 25 ± 1°C. The fungal spores were harvested in 25-30 ml of sterilized distilled water containing 0.05% Tween. The spore count of this stock suspension was estimated with Neubaur haemocytometer. The spore concentration of the suspension was adjusted to $10^8$ spores/ml with sterile distilled water and they were used for bioassay against tomato fruit borer. The experiments were repeated three times over time. The best performing fungal strain was then selected for subsequent experiments.

### Fixing Effective Dose of *B. Bassiana* Against Fruit Borer Under *in Vitro*

Third instar larvae of *H. armigera* were bioassayed for their susceptibility to best isoalate of *B. bassiana*. Ten larvae were taken in a Petri dish which was lined by a filter paper at the bottom for absorbing excess moisture. Ten ml of four different concentrations *viz.*, $1 \times 10^2$, $1 \times 10^4$, $1 \times 10^6$ and $1 \times 10^8$ conidia/ml was directly sprayed on the larvae using a hand atomizer. Four replicates of ten larvae were used in each case. Four lots of ten larvae sprayed with 10 ml of sterilized distilled water with 0.05% Tween 20 served as control. The larvae were air dried by keeping them in laminar air flow for five min and carefully transferred to individual clean sterile plastic Petri dish containing fresh leaves of tomato. These Petri dishes were then kept inside the BOD incubator at 25 ± 1°C. The larval mortality was recorded at 24 h interval until 14 days of treatment. The per cent larval mortality due to mycosis was calculated and the results of the assay were subjected to probit analysis and the median lethal time ($LT_{50}$) and median lethal concentration ($LC_{50}$) for the virulent isolate were determined.

## RESULTS

### Isolation of Entomopathogenic Fungus

Totally twenty isolates of *B. bassiana* were isolated from the soil samples and insect cadavers collected from different regions of Tamil Nadu (Table 1). The fungus was identified based on the conidial structure observed under microscope. The colony growth ranged from dull white to pure white in colour. The fungi had produced millions of conidia, which were hyaline, globose and single celled. They were borne in a cluster on a very short conidiogenous rachis. The idenfied isolates were designated as Bb 1 to Bb 20.

### Pathogenicity of *B. Bassiana* Against *H. Armigera* in Tomato

The tested isolates of *B. bassiana* showed pathogenicity towards *H. armigera* with varied mortality rates (Table 1). All the *B. bassiana* isolates were found pathogenic to larvae of

*H. armigera*. Among which, the Bb8 isolate showed higher per cent mortality (93.3%) against *H. armigera* followed by Bb 2 (86.6%). The isolate Bb 17 was least effective against

*H. armigera* which recorded only 26.6% mortality (Table 2).

**Bioassay of *B. Bassiana* Against Tomato Fruit Borer Under *in Vitro* Conditions**

The studies revealed that the larval mortality was proportional to the concentration of conidial suspension. Bioassay of *B. bassiana* (Bb8) against *H. armigera* recorded the least larval mortality of 30.60% with $1 \times 10^2$ conidial concentration. The conidial concentration of $1 \times 10^8$ showed the highest per cent mortality (75.3%). Whereas, in case of *B. bassiana* (Bb2) showed the lesser per cent mortality (71.1%) against the third instar larvae of *H. armigera* with the conidial concentration of $1 \times 10^8$ (Table 3a and 3b)

## DISCUSSION

The primary means of controlling the insect pests is through the application of broad-spectrum insecticides. At this juncture, several entomopathogens offer effective means of microbial control (Lacey and Shapiro-Ilan, 2008). In addition, microbial control agents are safe for the environment, beneficial insects, applicators, and the food supply and they can be applied just prior to harvest (Kaya and Lacey, 2007). Entomopathogenic fungi contribute to the natural regulation of insect, tick and mite populations (Kleespies *et al*., 2008).

In the current study, twenty *B. bassiana* strains were isolated from the soil and dead insects collected from various regions of Tamil Nadu. Isolation of these entomopathogenic fungal pathogens from the soil and insect was previously studied by several authors. Strains of entomopathogenic fungi *B. bassiana* have been isolated from the soil and insects of various crops in different countries by several workers (Kulkarni *et al*., 2008; Thakur and Sandhu, 2009) and tested against several insect pests (Wraight *et al*., 2010; Campos *et al*., 2010).

*B. bassiana* (Bb8) was highly effective on third instar larvae of *H. armigera* with a larval mortality of 93.3% under *in vitro* conditions. Based on the mortality rate, the two isolates Bb8 and Bb2 were taken for further studies. Many workers are of the opinion that the isolates originating from the same host were more virulent against that target pest than others. Gerritsen *et al*. (2000) also reported the pathogenicity of *B. bassiana* to Western flower thrips, *Frankliniella occidentalis* and showed that isolates of *B. bassiana* isolated from thrips caused higher mortality than *M. anisopliae*. Senthilraja *et al*. (2010) also reported that *B. bassiana* (B2) recorded the highest per cent mortality (68.90%) against larvae of groundnut leafminer (*Aproaerema modicella*).

In our study, all twenty tested strains showed pathogenicity against third instar larvae of

*H. armigera* in the screening tests, corroborating previous findings of Nguyen *et al.* (2007). However, the two *B. bassiana* strains *viz*., Bb8 and Bb2 were more virulent than the other tested *B. bassiana* strains. These results were similar to previous laboratory findings demonstrating high virulence of *B. bassiana* against *H. armigera* larvae (Sandhu *et al*., 2001). In this present study, we observed that $1 \times 10^8$ spores/ml is desirable for the control of *H. armigera*. Similarly, the feeding ability of fruit borer was significantly affected by entomopathogenic fungal pathogens treatments. Ekesi *et al*. (2002a) reported the ovicidal activity of eight isolates of entomopathogenic hyphomycetes against *M. vitrata* and *Clavigralla tomentosicollis* at a concentration of $1 \times 10^8$ conidia/ml under *in vitro* conditions.

In conclusion, among various isolates of *B. bassiana* Bb 8 has the potential to enhance the mortality rate of *H. armigera* and could be used as a potential biocontrol agent for the management of tomato fruit borer.

## REFERENCE

Akrami, M. and Yousefi, Z. (2015), Biological Control of *Fusarium* wilt of Tomato (*Solanum lycopersicum*) by *Trichoderma* spp. as Antagonist Fungi. *Biol. Forum-An Intrrn. J.* **7**(1): 887-892.

Bateman, R.P., Carey, M., Batt, D., Prior, C., Abraham, Y., Moore, D., Jenkins, N. and Fenlon, J. (1996), Screening for virulent isolates of entomopathogenic fungi against the desert locust, *Schistocerca gregaria* (Forskal). *Biocont. Sci. Technol.*, **6**: 549-560.

Campos, R.A., J.T. Boldo, J.T., I.C. Pimentel, I.C., Dalfovo, V., W.L. Araújo, W.L., J.L. Azevedo, J.L., Vainstein, M.H. and Barros, N.M. (2010), Endophytic and entomopathogenic strains of *Beauveria* sp to control the bovine tick *Rhipicephalus* (*Boophilus*) *microplus*. *Genet. Mol. Res*. **9**(3): 1421-1430.

Ekesi, S., Adamua, R.S. and Manianiab, N.K. (2002a), Ovicidal activity of entomopathogenic hyphomycetes to the legume pod borer, *Maruca vitrata* and the pod sucking bug, *Clavigralla tomentosicollis. Crop prot*., **21**: 589–595.

Fitt, G.P. (1989), The ecology of *Heliothis* in relation to agroecosystems. *Annu. Rev. Entomol*., **34**: 17–52.

Gerritsen, L.J.M., Wiegers, G.L., Sommeijer, M.J. and Meeuwsen, F.J.A.J. (2000), Pathogenicity of the fungi

*Metarhizium anisopliae* and *Beauveria bassiana* to the western flower thrips, *Frankliniella occidentalis*. Proceedings of the 11th meeting of experimental and applied entomologists in the Netherlands, Wageningen, Netherlands, 17 December 1999. Proceedings of the section experimental and applied entomology of the Netherlands Entomological Society., **11**: 37–40.

Karthiba, L., Saveetha, K., Suresh, S., Raguchander, T., Saravanakumar, D. and Samiyappan, R. (2010), PGPR and entomopathogenic fungus bioformulation for the synchronous management of leaffolder pest and sheath blight disease of rice. *Pest Manag. Sci.,* **66**: 555-564.

Kaya, H.K. and Lacey, L.A. (2007), Introduction to microbial control. In: *Field Manual of Techniques in Invertebrate Pathology: Application and Evaluation of Pathogens for Control of Insects and Other Invertebrate Pests*. (Eds. Lacey, L.A. and Kaya, H.K.) Springer. 2nd ed., Dordrecht. pp 3-7.

Kleespies, R.G., Huger, A.M. and Zimmermann, G. (2008), Diseases of Insects and other Arthropods: Results of Diagnostic Research over 55 Years. *Biocont. Sci. Tech.*, **18**: 439-484.

Kulkarni, S.A., Ghormade, V., Kulkarni, G., Kapoor, M., Chavan, S.B., Rajendran, A., Patil, S.K., Shouche, Y. and Deshpande, M.V. (2008), Comparison of *Metarhizium* isolates for biocontrol of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in chickpea. *Biocont. Sci. Tech..,* **18**(8): 809-828.

Lacey, L.A. and Shapiro-Ilan, D.I. (2008), Microbial Control of Insect Pests in Temperate Orchard Systems: Potential for Incorporation into IPM. *Annu. Rev. Entomol.*, **53**: 121-44.

Milner, R.J., Mc Rac, C., M.N., Huppatz, R.J. and Brier, H. (1993), Preliminary evaluation of the use of *Metarhizium anisopliae as* a mycopesticide for control of peanut scarabs. In: *Pest control in sustainable agriculture*, Melbourne, Australia CSIRO, pp. 235-255.

Nguyen, N.T.H., Borgemeister, C., Poehling, H.M. and Zimmermann, G. (2007), Laboratory investigations on the potential of entomopathogenic fungi for biocontrol of *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae and pupae. *Biocont. Sci. Tech.,* **17**(8): 853-864.

Prabhukarthikeyan, S. R., D. Saravanakumar and T. Raguchander. (2014), Combination of endophytic *Bacillus* and *Beauveria* for the management of *Fusarium* wilt and fruit borer in tomato. *Pest Management Science*. **70**: 1742-1750.

Sandhu, S.S., Unkles, S.E., Rajak, R.C. and Kinghorn, J.R. (2001), Generation of benomyl resistant *Beauveria bassiana* strains and their infectivity against *Helicoverpa armi-gera*. *Biocont. sci. Technol.*, **11**: 245-250.

Saravanan, A. (2007), Endophytic *Beauveria bassiana* (Bals.) Vuill. mediated Induced systemic resistance (ISR) for the management of major pest and diseases of pigeonpea and chickpea. *M.Sc., (Ag.). Thesis,* Tamil Nadu Agricultural University, Coimbatore, India. pp. 168.

Senthilraja, G., Anand, T., Durairaj, C., Kennedy, J. S., Suresh, S., Raguchander, T. and Samiyappan, R. (2010), A new microbial consortia containing entomopathogenic fungus, *Beauveria bassiana* and plant growth promoting rhizobacteria, *Pseudomonas fluorescens* for simultaneous management of leafminers and collar rot disease in groundnut. *Biocont. Sci. Tech.*, **20**(5): 449 -464.

Sharma, Shashi, Gupta, R.B.L. and Yadava, C.P.S. (1999), Mass multiplication and formulation of entomopathogenic fungi and their efficacy against white grubs. *J. Mycol. Pl. Pathol.*, **29**: 299-305.

Singh, R.S. (1985), Diseases of Vegetable Crops. Oxford and IBH Publishing Co., New Delhi, Bombay, Calcutta. pp. 346.

Singh, V.K. and Kumar, A, (2001), PCR Primer design. *Mol. Biol. Today.* **2**: 2732.

Sivasundaram, V., Rajendran, L., Muthumeena, K., Suresh, S., Raguchander, T. and Samiyappan, R. (2008), Effect of talc-formulated entomopathogenic fungus *Beauveria* against leaffolder (*Cnaphalocrosis medinalis*) in rice. *World J. Microbiol. Biotechnol.* **24**: 1123-1132.

Starnes, R.L., Liu, C.L. and Marrone, P.G. (1993), History, Use and Future of Microbial Insecticides. *American Entomologist,* 83-91.

Thakur, R. and Sandhu, S.S. (2009), Distribution, occurrence and natural invertebrate hosts of indigenous entomopathogenic fungi of Central India. *Indian. J. Microbiol.,* 1-8.

Wraight, S.P., Ramos, M.E., Avery, P.B., Jaronski, S.T. and Vandenberg, J.D. (2010), Comparative virulence of Beauveria bassiana isolates against lepidopteran pests of vegetable crops. *J. Invertebr. Pathol.*, **103**: 186-199.

❍❍❍