Hexapoda **16(2):** 107-113, 2009 ISSN 0973-8592

EFFICACY OF *Beauveria basssiana* (BALSAMO) AGAINST RED FLOUR BEETLE, *Tribolium castaneum* (HERBST)

S. Malarvannan*, K. Priyadarshan, M. Arumugam Prasietha, L. Saran Prakash, V. R. Prabavathy and Sudha Nair

M. S. Swaminathan Research Foundation, III Cross Street,
Taramani Institutional Area, Chennai 600 113. *Corresponding author e-mail: malar@mssrf.res.in

ABSTRACT: White muscardine fungus, *Beauveria bassiana,* was evaluated for its insecticidal activity against grubs, pupae and adults of the red flour beetle, *Tribolium castaneum.* In general, prolongation in grub and pupal duration was observed. As a subsequent effect, malformed adults were evident. The adult mortality (%) increased with the increase in exposure period and conidia concentration. This proved that the time of dipping and spore concentration greatly influenced the insecticidal effect of the fungus. The bioassay revealed that adults seem to be less susceptible to *B. bassiana* when compared to grubs. Malformed adults were observed in pupae treated with *B. bassiana*.

Key words: *Beauveria bassiana*, *Tribolium castaneum*, red flour beetle

INTRODUCTION

The red flour beetle, *Tribolium castaneum* (Herbst), is a cosmopolitan pest and considered as one of the major pest of stored and processed grains with high rate of movement among food patches (Dal Bello, 2001) and reported to cause heavy loss throughout the world (Delobel and Tran, 1993)**.** In addition, deterioration in the quality is observed due to the tendency to taint food substrates with secretions by both grubs and adults especially to broken or damaged grains. The widespread use of chemical insecticides for decades as preventive measure has led to resistance development and also human health hazards. Such ill effects have forced the biologists to search for viable and sustainable alternatives. Biological control, using microbial pathogens and in particular entomopathogenic fungi is a potential alternative to chemical insecticides. Though several species of entomopathogenic fungi have been already tested against adult stages of different stored product pests (Batta, 2004; Lord, 2005), the efficacy of fungal pathogens to different developmental stages of *T. castaneum* have been less explored. Hence in the present study, the efficacy of *Beauveria bassiana* against grub, pupal and adult stages of *T. castaneum* was tested.

MATERIALS AND METHODS

Insect rearing (Batta and Abu Safieh, 2005)

Tribolium castaneum Herbst. was reared on dried healthy mature pearl millet grains in plastic basins (35 cm dia and 10 cm depth) under the insectary conditions (25 ± 2°C and 75 ± 5 % R.H. and 16 h of illumination per day). The basins were then covered with cheese cloth fastened by a rubber band to prevent the escape of insects and to ensure proper ventilation.

Fungal source

B. bassiana culture was obtained from the Regional Coffee Research Station, Thandigudi, India and the original source of the isolate is from coffee berry borer, *Hypothenemus hampei*. The culture was maintained on Potato Dextrose Agar + 1% (w/v) Yeast (PDAY).

Bioassay

Preparation of spore suspension: Spore suspension was prepared from 15 days old culture of *B. bassiana* grown on PDAY. The fungal surface was scraped using 10 ml of sterile distilled water supplemented with 0.02% (v/v) Tween 80 as a wetting agent (Rombach *et al*., 1986). The suspension was then filtered through two layers of sterile muslin cloth to eliminate the medium (Sasidharan and Varma, 2005). Spore concentration of the filtrate was determined using a Neubauer haemocytometer. This served as the stock suspension. Different spore concentration was prepared by adding sterile 0.02% (v/v) Tween 80 in distilled water. Spore suspension at five different concentrations 2.4×10^7, 2.4×10^6, 2.4×10^5, 2.4×10^4 and 2.4×10^3 conidia/ml of *B. bassiana* was prepared and tested for efficacy on different stages of *T. castaneum*.

a) Growth inhibition of grubs (Pandey and Kanauja, 2004): Fourth instar grubs of *T. castaneum* were collected using a camel brush. The grubs were treated by spraying 2 ml of different spore concentrations of *B. bassiana.* Ten grubs were used per replication. Each treatment was replicated thrice. The grubs treated with sterile distilled water, 0.02 % (v/v) Tween 80 and 0.006 % (v/v) cypermethrin served as controls. After treatment, the grubs were allowed to feed on broken rice. The grub mortality was recorded until adult emergence. Growth parameters namely grub duration (days), pupation (%), pupal duration and adult emergence (%) were recorded.

b) Growth inhibition of pupae: The final instar grubs were separated and reared in plastic bowl containing pearl millet and the pupae formed on the same day were used for bioassay. Five different spore concentrations of *B. bassiana* with three replications each was used for treating the pupa. In each replication ten pupae were taken and were sprayed with 2 ml of respective fungal spore suspensions. The pupae treated with sterile distilled water, 0.02% (v/v) Tween 80 and 0.006 % (v/v) cypermethrin served as the controls. After every 24 h the pupal mortality was assessed. The growth of surviving pupa was recorded up to adult emergence for the parameters such as pupal duration (days) and adult emergence (%).

c) Efficacy of test fungi against adults (Gopalakrishnan *et al*., 2002): Healthy adults of the same age were collected and starved for 24 hr. The adults (20 nos.) were dipped in the different concentration of *B. bassiana* spores with the help of forceps for 5 and 20 seconds. Five ml of spore suspension was used for treating a set of 20 adults. Adults dipped similarly in sterile distilled water, 0.02% (v/v) Tween 80 and 0.006 % (v/v) cypermethrin served as controls. After treatment, the adults were allowed to feed on broken rice. Mortality of the adults was assessed at 48 hr interval. Triplicates were maintained for each treatment.

Statistical analysis: The data were statistically analysed using AGRES package version 4.

RESULTS

Efficacy of *B. bassiana* against different stages of *T. castaneum*

A) Grub: The grubs treated with different spore concentrations of *B. bassiana* was assessed for various developmental parameters such as pupation (%), grub duration (days), pupal duration (days) and adult emergence (%).

The least pupation (16.66 %) was observed at conidial concentration of 2.4×10^7 and 2.4×10^6 conidia/ml (Table 1). This was followed by 20 % in 2.4×10^5 conidia/ml. The reduction in pupation as against controls, ranged from 70 to 83.33% at different conidia concentration of *B. bassiana* tested. From the results, it was clear that the pupation (%) depended on concentration of *B. bassiana*. The maximum pupation (%) was observed in the controls (distilled water and Tween 80). In addition, white fluffy growth of mycelium was observed in the infected larvae (Plate 1 a). Reduced feeding, sluggishness, prolonged grub duration, grub-pupal intermediates and grub shrinkage (Plate 1 b and c) were few indicators observed in the *B. bassiana* treated grubs in addition to the grub mortality.

The pupal duration was increased by 1.9 days in those treated with 2.4×10^7 conidia/ml as against controls (4.3 days). Pupal duration was prolonged irrespective of the different concentration of *B. bassiana* which disrupted the insect's metamorphosis. This resulted in higher degree of malformed / dead adults (Plate 1 d).

The adult emergence was nil in all the spore concentrations tested except for 4.76 % in 2.4×10^6 conidia/ml of *B. bassiana* and 66.66 % for 0.006 % (v/v) cypermethrin treatment. The adult emergence was normal (100%) in controls.

B) Pupa : The pupae treated with *B. bassiana* was assessed for various parameters such as pupal mortality (%), pupal duration (days) and adult emergence (%) (Table 2).

The pupae treated with *B. bassiana* showed an increase in pupal mortality with increase in spore concentration. The maximum pupal mortality of 36.6% was observed with 2.4 x 10^7spores/ml followed by 26.6 % in 2.4 x 10^6 and 2.4 x 10^4 spores/ml. In general, the pupal duration was found to prolong (6.5 days) in highest conidial concentration (2.4 x 10^7 spores/ml) as against control (distilled water) (4.05 days). An obvious malformation was observed among the adults which varied from 15.8 to 31.7 % with different concentration of 2.4 x 10^3 to 2.4 x 10^7 spores/ml.

C) Adult: The mortality of adults dipped in different spore concentrations of *B. bassiana* for different exposure time (Fig. 1) was recorded. The results obtained indicated that, in general, the adult mortality (%) increased with the increase in the exposure time and spore concentrations.

Maximum and minimum mortality of 21.6 % and 6.66 % respectively was obtained with those dipped in 2.4 x 10^7 and 2.4 x 10^3 spores/ml of *B. bassiana* for 20 seconds. The mortality of adults increased in all the concentrations except in 2.4 x 10^5 spores/ml with increasing exposure period from 5 sec to 20 sec (fig. 1). The mycelial growth was observed over dead infected adults (Plate 2a).

DISCUSSION

The results obtained for pathogenicity of *B. bassiana* against grubs (Table 1), showed that *B. bassiana* is efficient against *T. castaneum* grubs. *B. bassiana* has already been reported to be pathogenic to various grub species by Jayaramaiah and Veeresh, 1983 and Sharma *et al.* (1998). From the results, it was clear that the pupation (%) decreases with increase in spore concentration of *B. bassiana*. Akbar *et al.* (2004) also reported that the larval stages of *T. castaneum* showed a dose dependent mortality with *B. bassiana*. In the present study, reduced feeding was observed in grubs treated with spore suspension of *B. bassiana*. Sivasundaram *et al.* (2007) also reported that *Beauveria* strains altered the feeding behavior of the rice leaf folder, *Cnaphalocrosis medinalis.*

In general, prolongation in grub duration was observed in those treated with *B. bassiana*. This is in confirmation with prolonged larval duration in *Serangium parcesetosum* treated with *B. bassiana* reported by Poprawski *et al.* (1998). Sivasundaram *et al.* (2007) also reported prolonged pupation period, malformed pupa and adult emergence from *C. medinalis* treated with *B. bassiana.* Throne and Jeffrey (2004), hypothesized that *B. bassiana* would be more efficacious when attacking an insect that had a longer immature developmental period which would allow more time for completion of the hyphal penetration process and result in higher rates of mortality.

The nil adult emergence in grubs treated with spore suspension of *B. bassiana* proved that the entomopathogenic fungi may be equally efficient as chemical pesticides in biocontrol. Akey *et al*. (1998) in a field experiment showed that treatments with *B. bassiana* was as effective in controlling the silver leaf white fly, *Bemisia argentifolii* as best chemical control regimes.

In the case of pupae treated with *B. bassiana*, the percentage of adult emergence showed a highly progressive decrease with the increase of spore concentration. An obvious malformation was observed among the emerged adults after treatment of the pupae in all the spore concentrations of *B. bassiana.* The duration of the treated pupae was significantly prolonged at concentrations of 2.4 x 10^7 to 2.4 x 10^3 spores/ml as compared with the control. These results are in agreement with Hafez *et al*. (1994) who also reported that *B. bassiana* treated pupae of potato tuber moth, *Phthorimaea operculella* resulted in an obvious increase in the pupal duration and malformed adults.

Several species of entomopathogenic fungi have been already tested against adult stages of different stored product pests (Batta, 2004; Lord, 2005). From the Figure 1 it is clear that a positive correlation exist between the adult mortality and the fungal concentration. Further it was also observed that the time of dipping also greatly influenced the effect of both the fungi against the adult beetles The results obtained are in harmony with results gained by Klingen *et al*. (2001) and El-Sinary (2005) who stated that the greatest the fungal concentration the highest the reduction in viability of tested insect. When compared to the efficacy of the pathogen against grubs (Table 1), the adults seem to be less susceptible. Akbar *et al*. (2004) also reported that the adults of *T. castaneum* proved to be tolerant to *B. bassiana* and their use does not seem to be promising option against adults because of the high application rates required.

CONCLUSION

In the present study, the effect of *B. bassiana* was observed in almost all stages of *T. castaneum* either by direct lethality or indirect developmental changes. The lack of toxicity of *B. bassiana* to mammals and humans (Langewald *et al*., 1997; USEPA, 1999) renders it as a potential candidate for biological control.

ACKNOWLEDGEMENTS

The authors acknowledge Dorabji Tata Trust, Mumbai for the financial support. The support from colleagues Mr. S. Sekar and Ms. Valarmathi is also acknowledged.

REFERENCES

Akbar, W., Lord, J. C., Nechols, J. R. and Howard, R. W. 2004. Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. *Journal of Economic Entomology*, **97:** 273-280.

Akey, D. H., Henneberry, T. J., Dugger, P. and Richter, D., 1998. In**:** *Proceedings Beltwide Cotton Conference*, SanDiego, pp.1073-1077.

Batta, Y. A. 2004. Control of rice weevil, *Sitophilus oryzae* L., (Coleoptera: Curculionidae) with various formulations of *Metarhizium anisopliae*. *Crop Protection*, **23:**103-108.

Batta, Y. A. and Abu Safieh, D. I. 2005. A study of treatment effect with *Metarhizium anisopliae* and four types of dusts on wheat grain infestation with red flour beetles (*Tribolium castaneum* Herbst. Coleoptera: Tenebrionidae). *Journal of Islamic University, Gaza,* **13:**11-22.

Dal Bello, G., Pandin, S., Lopez, L. and Fabrizio, M**.** 2001**.** Laboratory evaluation of chemical biological control of rice weevil (*Sitophilus oryzae* L.) in stored grains. *Journal of Stored Product Research,* **37**: 77-84.

Delobel, A. and Tran, M. 1993. The Coleopterans of food stuffs stored in the hot regions. ORSTOM/CTA. Paris 242 p.

El-Sinary, N. H. 2005. Influence of wet and dry inocula of the entomopathogenic fungus cavity clustered beside blastospores with the treatment by *Metarhizium anisopliae* (Matschnikoff) against the mature larvae of the potato tuber moth, *Phthorimaeae operculella* (Zeller) combined with neem and gamma irradiation, Egypt. *Journal of Biotechnology*, **21**: 56-64.

Gopalakrishnan, C., Narayanan, K. and Anusuya, D. 2002. Determination of LD_{50} and LT_{50} of *Paecilomyces farinosus* (Homskiold) Brown and Smith on larval instars of *Plutella xylostella* L. *Entomon*, **27:**249-254.

Hafez, M., Zaki, F N., Moursy, A. and Sabbour, M. 1994. Biological effects of the entomopathogenic fungus, *Beauveria bassiana* on the potato tuber moth, *Phthorimaea operculella* (Seller). *Journal of Islamic Academy of Sciences*, **7:**211-214.

Jayaramaiah, M. and Veeresh, G. K. (1983). Fungal pathogens of white grubs in Karnataka. *Journal of Soil Biology and Ecology,* **3:** 83-87.

Klingen, L., Meadow, R. and Aandal, T. 2001.Mortality of *Delia floralis*, *Galleria melonella* and *Mamestra brassicae* treated with insect *pathogenic* hyphomycetous fungi. *Journal of Applied Entomology,* **126**: 231-237.

Langewald, J., C. Kooyman, O. Duoro-Kpindou, C. J. Lomer, A. O. Dahmoud, and H. O. Mohammed. 1997. Field treatment of desert locust (*Schistocerca gregaria* Forskål) hoppers in the field in Mauritania with an oil formulation of the entomopathogenic fungus *Metarhizium flavoviride*. *Biocontrol Science and Technology,* **7**: 603 – 611. Cross Ref, CSA.

Lord, J. C. 2005. Low humidity, moderate temperature, and desiccant dust favor efficacy of *Beauveria bassian*a (Hyphomycetes: Moniliales) for the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bruchidae). *Biological Control,* **34**:180-186.

Pandey, A. K. and Kanaujia, K. R. 2004. Effect of supplementation of medium with larval extract on the virulence of *Beauveria bassiana* (Balls.) Vuill. against *Spilosoma obliqua* Walker. *Journal of Biological Control,* **18:** 173-178.

Poprawski T. J., Legaspi, J. C. and Parker, P. E. 1998. Influence of entomopathogenic fungi on *Serangium parcesetosum* (Coleoptera: Coccinellidae), an important predator of white flies (Homoptera: Aleyrodidae. *Environmental Entomology*, **27**: 785-795.

Rombach, M. C., Aguda, R. M., Shepard, B. M. and Roberts, D. W. 1986. Infection of rice brown plant hopper, *Nilaparvata lugens* (Homoptera: Delphacidae), by field application of entomopathogenic Hyphomycetes (Deuteromycotina). *Environmental Entomology*, **15**: 1070-1073.

Sasidharan, K. R. and Varma, R. V.2005. Laboratory evaluation of *Beauveria bassiana* (Balsamo) Vuillemin against *Indarbela quadrinotata* Walker (Lepidoptera: Metarbelidae)-a key pest of *Casuarina equisetifolia* L.in Tamil Nadu. *Journal of Biological Control*, **19**: 197-200.

Sharma, S., Gupta, R. B. L. and Yadav, C. P. S. 1998. Effect of temperature on growth, sporulation and bioactivity of entomopathogenic fungi against white grub (*Holotrichia consanguinea*). *Indian Journal of Entomology*, **60:** 1-7.

Sivasundaram, V. L.Rajendran, K.Muthumeena, S.Suresh, T.Raguchander and R.Samiyappan. 2007. Effect of talc-formulated entomopathogenic fungus *Beauveria* against leaf folder (*Cnaphalocrosis medinalis*) in rice. *World Journal of Microbiology and Biotechnology*, **24**: 7, 1123-1132.

Throne, J. E., and Jeffrey, J. C. 2004. Control of saw-toothed grain beetles (Coleoptera: Silvanidae) in stored oats by using an entomopathogenic fungus in conjunction with seed resistance. *Journal of Economic Entomology*, **97:** 1765-1771.

USEPA. 1999. Economics Background Document: Economic Assessment of the USEPA's 1999. Proposed Hazardous Waste Identification Rule (HWIR), Office of Solid Waste, 29 Oct 1999, 88 pp.

Original not seen

Table 1. Efficacy of *B. bassiana* against grubs of *T. castaneum*

Treatments		Larval development		Pupal duration (days)	Malformed / dead adults (%)
		Pupation (%)	Grub duration (days)		
Control	Distilled water	100.0^{f}	12.8^{a}	4.3^{c}	0.0^{c}
	0.2%(v/v) Tween 80	96.6^{f}	12.4^{a}	4.3^{c}	0.0^{bc}
	0.006%(v/v) Cypermethrin	6.6^{a}	6.6^{b}	2.0^{d}	33.3^{c}
B. bassiana (spore/ml)	2.4×10^{7}	16.6^{ab}	13.2^{a}	6.5^{a}	100.0^{ca}
	2.4×10^{6}	16.6^{ab}	13.0^{a}	6.2^{ab}	95.2^{a}
	2.4×10^{5}	20.0^{ab}	13.6^{a}	5.3^{abc}	100.0^{a}
	2.4×10^{4}	23.3^{abc}	13.7^{a}	5.2^{bc}	100.0^{a}
	2.4×10^{3}	30.0^{bc}	12.2^{a}	4.4^{c}	100.0^{a}
(CD = 0.01)		22.68	3.96	1.62	38.19

Each value mean of triplicate; Different letters in each column differ significantly (5%) by DMRT

Table 2. Efficacy of *B. bassiana* against pupae of *T. castaneum*

Treatments		Pupal duration (days)	Pupal mortality (%)	Malformed /dead adult (%)
Control	Distilled water	4.0^{cde}	0.0^{c}	0.0b
	0.2%(v/v) Tween 80	3.9^{de}	0.0^{c}	0.0^{b}
	0.006%(v/v) Cypermethrin	2.6^{f}	43.3^{ab}	28.3^{a}
B. bassiana (spore/ml)	2.4×10^{7}	6.1^{a}	36.6^{ab}	31.7^{a}
	2.4×10^{6}	5.1^{abc}	26.6^{b}	28.5^{a}
	2.4×10^{5}	5.4^{ab}	23.3^{b}	29.1^{a}
	2.4×10^{4}	5.0^{abc}	26.6^{b}	16.2^{ab}
	2.4×10^{3}	5.0^{abcd}	3.3^{b}	15.8^{ab}
(CD = 0.01)		1.5	28.6	29.3

Each value mean of triplicate. Different letters in each column differ significantly (5%) by LSD

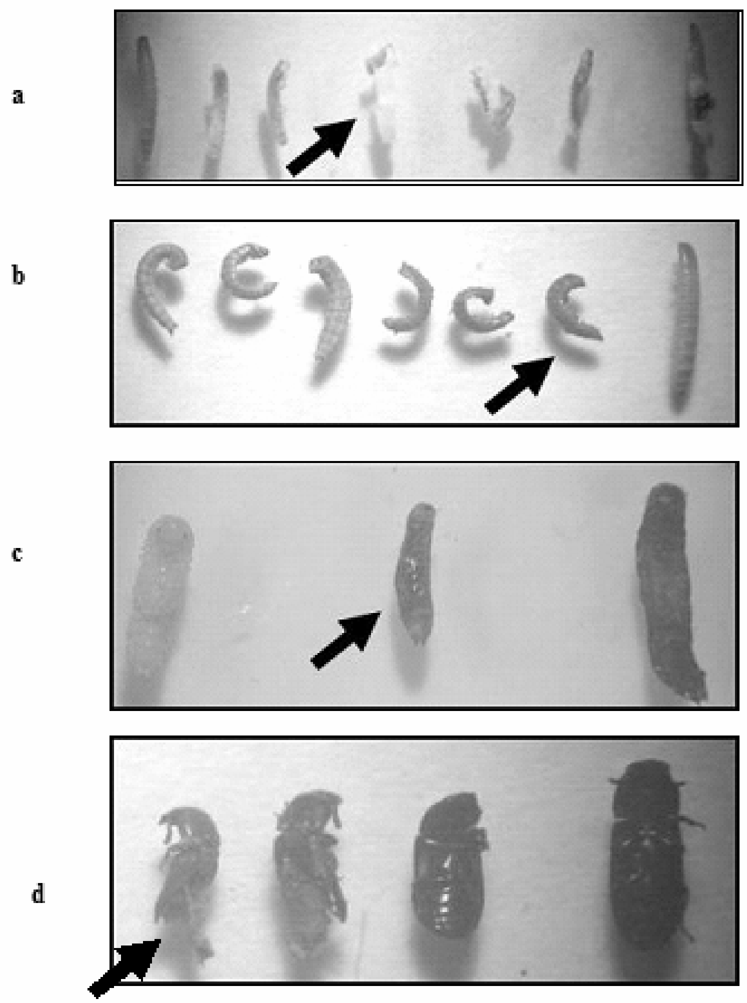

Figure 1

Plate 1

Plate 1. Impact of *B. bassiana* infection in *T. castaneum* grubs
a- infections on grubs; b – shrinkage; c – grub-pupal intermediate and
d – malformed adult (arrows)

Plate 2

Plate 2. Mycelial growth of *B. bassiana* over the adult of *T. castaneum* died following infection

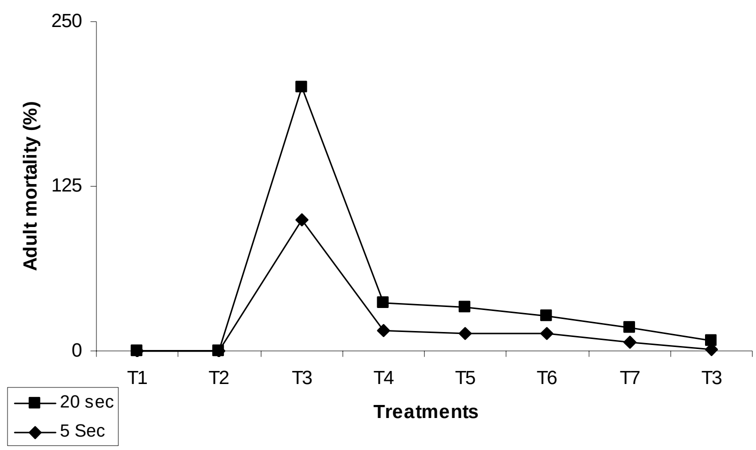

Fig. 1. Bioefficacy of *B. bassiana* conidia suspension against *T. castaneum* adult
T1 – Distilled water; T2 – Tween 80; T3 – Cypermethrin; T4 – 2.4×10^7 ;
T5 – 2.4×10^6 ; T6 – 2.4×10^5 ; T7 – 2.4×10^4 ; T8 -2.4×10^3conidias/ml