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Impact of entomopathogenic fungus, *Beauveria bassiana* on stored grains **pest, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)**

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ABSTRACT

Laboratory bioassay was carried with five different concentrations of *Beauveria bassiana* ($1x10^4$ to $1x\ 10^8$ ml-1) against the pulse beetle, *Callosobruchus maculatus.* Stored seeds were severely attacked by the pulse beetle, *Callosobruchus maculatus,* a coleopteran pest.At the larval stage, it penetrated into stored seeds and fed on cotyledons. Entomopathogenic fungus, *Beauveria bassiana* caused maximum oviposition reduction and 100% adult mortality was obtained at higher concentrations. Mortality declined with the decrease in concentrations. At higher concentrations, oviposition reduction was 60.58%, and adult mortality was 99.44% at 92h respectively. It showed higher virulence compared to other isolates.At lower concentrations, oviposition reduction was 23.82% and adult mortality was 81.93% at 92 h respectively.

Key words: *Beauveria bassiana ; Callosobruchus maculatus;* oviposition deterrent, adult mortality.

INTRODUCTION

Callosobruchus maculatus is one of the important storage pests that affects the shelf life of stored pulses like blackgram.The post harvest loss of blackgram has to be minimized from the attack of storage pests. So instead of chemical treatments ecofreindly and safe biocontrol strategies are adopted to protect the storage of blackgram. In the present study the entomopathogenic fungus *Beauveria bassiana* was tested for its efficacy to control the oviposition and the adult mortality of *C.maculatus.*

In this era of integrated pest management, microbial pest control agents are used in various crops. Microbial pesticides ensure economic viability and ecofriendly nature. The use of antagonistic bacteria to combat pests had been well explored (Ramesh *et al.*, 2009; Rong-lintte *et al.*, 2009; Uma *et al.*, 2010; Dhahira beevi and Quadri, 2010). Like the control of plant pests, it has become imperative to control pests in post harvested products. Although several physical and chemical methods are available, microbial pest control has been preferred to the others. Regarding microbes, entomopathogenic fungus became a reliable alternative for coleopteran pest control as an alternative to chemical controls (Murad *et al*., 2007). *Beauveria bassiana* and *Metarhizium anisopliae* were world wide evaluated according to their virulence towards storage, maize bruchid pests (Cherry *et al*., 2005). Murad *et al*. (2006) screened ten *Metarhizium anisopliae* isolates with virulence against *C.maculatus* and also performed enzymatic and proteomic analysis by two dimensional sets of secretions produced in the presence of *C.maculatus* shells. Like the entomopathogenic fungi, bacterial isolates like *Bacillus thuringiensis* of subspecies *Tenebrionis* and *Sandiego* had been reported to be effective in controlling *C.maculatus* (Dulmage, 1981). Hence in the present study attempts were made to utilize the microbe *Beauveria bassiana* as pathogenic agent against *C.maculatus.*

MATERIALS AND METHODS

Collection and rearing of *C.maculatus*

A laboratory colony of *C.maculatus* was established from adult beetles sieved from samples of black gram seeds obtained from a local market in Tirunelveli. The black gram seeds were stored at -20ºC for 2 weeks to eliminate natural infestations and then exposed to ambient conditions for 7 days until grain moisture content (g.m.c) stabilized at approximately 13%. Black gram seeds were contained in glass jars with metal gauze lids at a rate of 25g per jar. Ten adult of *C.maculatus* females were introduced to each jar to allow oviposition and then removed by sieving 48 h later. Infested seeds were incubated at 28± 2ºC and 65±5% relative humidity (R.H) with a natural photoperiod.

Bioassay

In order to control the stored product pest *C.maculatus*, experiments were conducted to assess the efficacy of the entomopathogenic fungus *Beauveria bassiana*. *B. bassiana* utilized in bioassays were grown in PDA (Potato Dextrose Agar) medium for 15 days at 20ºc. For initial screening conidia were suspended in 0.1% Tween 80 to obtain a dilution of $1x10^4$ to 10^8 conidia / ml^{-1} conidial solutions (2.0 ml) were sprayed upon insects using a sprayer. Negative control treatment was done spraying 2 ml of 0.1% Tween 80 solution (Malarvannan *et al*., 2010). Treated insects were incubated in 5 groups of 10 pairs of female in transparent plastic petridishes, each containing 25 g blackgram seeds for 10 days at 28±2ºC , 65±5% RH and with a natural photoperiod. Oviposition inhibition and adult mortality in this experiment was assessed at 24 h interval up to 120 h. The number of eggs laid and adult mortality were recorded. Triplicates were maintained for each treatment.

Oviposition deterrence activity

Laboratory tests for oviposition inhibition effects were conducted by the following method of with some modifications.

Five pairs of *C. maculatus* were released in each plastic container and covered with a lid. They were allowed to remain in the container for 7 days till they lay eggs. One week after oviposition the number of eggs laid were counted using hand lens. The number of eggs laid on treated seeds (Et) and control seeds (Ec) were recorded and the percentage of oviposition deterrence (POD) was calculated using the following formula.

$$\text{Egg laying (\%) - POD} = \frac{Ec - Et}{Ec} x100$$

where, POD = percentage of oviposition deterrence, Ec = control seeds and Et = treated seeds

Adult mortality

The adult mortality was recorded at alternative days and the per cent mortality was calculated using Abott's formula .The adult mortality of *C.maculatus* was observed after 1, 3, 5, 7 and 9 days treatment. For mortality test the original data were corrected making use of Abbott's formula.

RESULTS AND DISCUSSION

Oviposition of *C. maculatus*

The entomopathogenic fungus *Beauveria bassiana* was tested for its efficacy to inhibit the oviposition of the stored grain pest *C. maculatus*. In the present study 5 different conidial concentrations of *B.bassiana* were experimented. In a dilution of 1 x 10^4, the mean oviposition of *C. maculatus* was low and slightly increased in spore concentrations dependent manner. However, in the control, the mean

Table 1. Inhibition of oviposition (number of egg/25 seeds) (X ± SD) *C. maculatus* due to the treatment of different spore concentrations of *Beauveria bassiana*

Conidia Concentration of *Beauveria bassiana* (Conidia ml^{-1})	No of eggs *C.maculatus*/25 seeds (Mean ± SD)	Percentage reduction in oviposition over control
$1x10^4$	35.56±2.93*	60.58
$1x\ 10^5$	44.44±1.84*	50.74
$1x\ 10^6$	56.66±5.04*	37.19
$1x10^7$	60.11±2.62	33.38
$1x10^8$	68.72±1.91	23.82
-	90.21±1.61	-

* Shows significance at 5% level.

Table 2. Mean cumulative mortality (%) of pulse beetle *C. maculatus* treated with *B. bassiana*

Treatment	Cumulative mean mortality									
	24 h		48 h		72 h		92 h		120 h	
	Mean±SD	% mortality over control	Mean±SD	% mortality over control	Mean±SD	% mortality over control	Mean ±SD	% mortality over control	Mean ±SD	% mortality over control
T1	1.33±0.66	13.33	4.00±1.15	39.99	9.33±0.66	92.84*	10.00±0.00	99.44*	-	-
T2	4.00±0.00	40.00	8.00±0.00	80.00*	8.66±0.66	85.70*	9.33±0.66	91.52*	10.00±0.00	97.90*
T3	2.66±1.33	26.66	6.00±3.05	59.99	8.66±0.66	85.69*	9.33±0.66	91.50*	10.00±0.00	97.87*
T4	1.33±0.66	13.33	3.33±0.66	33.33	7.33±1.76	71.42*	7.33±1.76	71.42*	10.00±0.00	97.78*
T5	0.00±0.00	-	0.00±0.00	-	5.33±0.66	53.33	8.66±0.66	81.39*	10.00±0.00	93.90*
Control	0.00±0.00	-	0.00±0.00	-	0.66±0.33	-	1.00±0.57	-	1.66±0.33	-

*Shows significance at 5% level

oviposition was light *B. bassiana* has been reported to control many pests particularly *C. maculatus* (Cherry *et al.,* 2005; Murad *et al.,* 2007).

B. bassiana is also capable of penetrating through the insect cuticle, secreting hydrolytic enzymes such as chitinases, proteinases and lipases, commonly referred to as cuticle degrading enzymes (Leger *et al* ., 1986; Moraes *et al*., 2003; Fang *et al*., 2005) being effective towards several bruchids (Shah and Pell, 2003; Cherry *et al*., 2005). The experimental results proved that biopesticides, particularly microbial pesticides can be used as an alternative to control pest. Its wide application as biological pesticides could be taken up after exploring its toxicity and field trials.

Mortality of *C. maculatus*

In treatment I – IV, the mortality was Conidia and exposure from 24 h, the mortality started (40%). At 48 h time dependent manner (Table 2). However, in treatment T5, there was no mortality till 48 h. In the 72 h exposure mortality was 53.3%. From the results it is quite evident that the *B.bassiana* was able to inflict mortality on *C.maculatus* and this can be used as a biocontrol agent for stored pests Vilas Boas *et al.* (1996) tested *Beauveria bassiana* isolates against *C.maculatus* adults by immersion. *Beauveria bassiana* was used as a grain treatment for stored cowpea and it significantly reduced the population of *C.maculatus* adults (Cherry *et al*., 2005). At this concentration, *B.bassiana* also gave appreciable reduction in population showing 96.66%. From the present study it is quite evident that the fungus *B.bassiana* is very effective in controlling the survival and oviposition of the stored grain pest *C.maculatus* that causes serious threat to blackgram.

REFERENCES

Cherry, A. J., Abalob, P. and Hella, K. 2005. A laboratory assessment of the potential of different strains of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) *Vuillemin* and *Metarhizium anisopliae* (Metschnikoff) to control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in stored cowpea. *Journal of Stored Product Research*, **41**: 295–309.

Dhahira Beevi, N. and Qadri, S. M. H. 2010. Biological control of mulberry root rot disease (Fusarium spp) with antagonistic microorganisms.*Journal of Biopesticides,* **3**: 90 – 92.

Dulmage, H. T. 1981. Insecticidal activity of isolates of *Bacillus thuringiensis* and their potential for pest control. In (H.D. Burges ed.) *Microbial control of pests and plant diseases*, 1970 - 1980. Academic Press, London. 193 - 222 **PP**.

Fang, W., Leng, B., Xiao, Y., Jin, K., Ma, J., Fan, Y., Feng, J., Yang, X., Zhang, Y. and Pei, Y. 2005. Cloning of *Beauveria bassiana* chitinase gene Bbchit1 and its application to improve fungal strain virulence. *Applied Environmental Microbiology*, **71**:363–370.

Leger, R. J. S., Cooper, R. M., Charnley, A. K. 1986. Cuticle degrading enzymes of entomopathogenic fungi: regulation of production of chitinolytic enzymes. *Journal of Genetic Microbiology*, **132**: 1509–1517.

Malarvannan,S., Murali,P. D., Shantha kumar, S. P., Prabavathy,V. R. and Sudha Nair. 2010. Laboratory evaluation of the entomopathogenic fungi, *Beauveria bassiana* against the tobacco caterpillar, *Spodoptera litura*(F). *Journal of Biopesticides,* **3**: 126-131.

Moraes, C. K., Schrank, A., Vainstein, M. H., 2003. Regulation of extracellular chitinase and proteases in the entomopathogen and acaricide *Metarrhizium anisopliae*. *Current Microbiology,* **46**: 205–210.

Murad, A. M., Laumann, R. A., Lima, Tde A., Sarmento, R. B. C., Noronha, E. F., Rocha, T. L., Valadares-Inglis, M. C. and Franco, O. L. 2006. Screening of entomopathogenic *Metarhizium anisopliae* isolates and proteomic analysis of secretion synthesized in response to cowpea weevil (*Callosobruchus maculatus*) exoskeleton. *Comp Biochem Physiol C Toxicol Pharmacol,* **142**: 365–370.

Murad, A. M., Laumann, R. A., Mehta, A., Noronha, E. F. and Franco, O. L. 2007. Screening and secretomic analysis of enthomopatogenic *Beauveria bassiana* isolates in response to cowpea weevil (*Callosobruchus maculatus)* exoskeleton. *Comp Biochem Physiol C Toxicol Pharmacol,* **145**: 333–338

Ramesh, R., Joshi, A. A. and Ghanekar, M. P. 2009. *Pseudomonas*: Major endophytic bacteria to suppress bacterial wilt pathogen *Ralstonia solanacearum* in the egg plant (*Solanum melongena* L.). *World Journal of Microbiology and Biotechnology*, **25**: 47-55.

Rong-lin He, Guo-ping Wang, Xiao-Hong Liu, Chu-long Zhang and Fu-cheng Lin, 2009.Antagonistic bioactivity of an endophytic bacterium isolated from *Epimedium brevicornu* Maxim. *African Journal of Biotechnology*, **8**: 191-195.

Shah, P. A., Pell, J. K. 2003. Entomopathogenic fungi as biological control agents. *Applied Microbiology and Biotechnology*, **61**:413-423.

Uma, G. P., Prabhuraj, A. and Vimala, 2010.Effect of the entomopathogenic bacterium *Photorhabdus luminescens* to *Aphis gossypii* Glover. *Journal of Biopesticides,* **3**:100 – 104.

Vilas Boas, A. M., Oliveira, J. V., Campos, A. L., Andrade, R. M., Silva, R. L. X., 1996. (Pathogenicity of wild strain and mutants of *M. anisopliae* and *Beauveria bassiana* to *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Arquivos de Biologia e Tecnologia*, **39**: 99–104 (Portuguese with English abstract).

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