Efficacy of *Beauveria bassiana* for control of the rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)

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Abstract

An entomopathogenic fungus, *Beauveria bassiana* was tested against the rice weevil *Sitophilus oryzae*. Bioassays were conducted by introducing 25 adult insects on 50 g of *B. bassiana* mixed rice in glass jars maintained at $28 \pm 2^\circ\text{C}$, and 70% RH. Mortality was monitored at five-day intervals until 25 days. At higher doses (7.6 log conidia/ml), *B. bassiana* produced a higher percentage of mortality up to 75.8% and F_1 adult emergence was reduced by 86.2%. At lower concentrations *B. bassiana* was not effective against *S. oryzae*. The study stresses the need for higher numbers of conidial inoculation for maximum mortality.

Key words: Bioassay, rice weevil, *Sitophilus oryzae*, *Beauveria bassiana*, mortality

INTRODUCTION

The rice weevil, *Sitophilus oryzae* (L.), is the most important storage pest of raw cereals throughout the world. The female rice weevil oviposits directly into seeds and the larvae complete development inside the seeds and emerge as adults (Champ and Dyte, 1976). To control these pests, synthetic chemical insecticides are used during storage of grains (Rice and Cogburn, 1999). Because of the adverse effects on humans (Matsumura, 1980) and the environment (Matsumura et al., 1972), health authorities are reluctant to allow chemical insecticides and their residues on grains (Thaung and Collins, 1986). Also, *S. oryzae* has been reported to develop resistance to synthetic chemicals (Moina et al., 1998). The growing need for research on biological protectants has revealed the positive role played by microbial insecticides. Susceptibility of stored insect pests to *Bacillus thuringiensis* (McGaughey, 1985) and *Beauveria bassiana* (Hluchy and Samsinakova, 1989; Rice and Cogburn, 1999) has been demonstrated by various workers. The types of strains involved are also essential to an understanding of the considerable differences in insecticidal activity (Samsinakova and Kalalova, 1983). *B. bassiana* has also been used to control the boll weevil (Wright, 1993), rice

water weevil (Rice et al., 1993) and stored grain pests (Searle and Doberski, 1984; Adane et al., 1996; Moina et al., 1998). Even though many *B. bassiana* strains are maintained by various Institutes and commercial organizations, their availability is restricted. Hence, in the present study we studied whether the strain, *B. bassiana* (MTCC 2028) which is universally accessible, is pathogenic to the rice weevil *S. oryzae*, so that this strain can be used later at the mass level.

MATERIALS AND METHODS

Sitophilus oryzae was collected from infested rice obtained from a local market and reared in glass jars under laboratory conditions of $28 \pm 2^\circ\text{C}$ and RH $70 \pm 5\%$. Twenty four hour old *S. oryzae* were used for subsequent experiments. The *B. bassiana* (MTCC 2028) strain used in this experiment was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The strain was stored under cryogenic conditions. The spore stocks were inoculated in solid medium plates of Sabouraud-dextrose agar containing 1% (w/v) yeast extract. Conidia were collected through surface scrapings of a 15-day-old culture and suspended in 0.1% Tween 80. The stocks were titered by dilution (1, 1 : 10, and 1 : 100) and direct counts

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were made using a haemocytometer. Concentrations were made containing log 5.5 (3.2×10^5), 6.4 (2.5×10^6) and 7.6 (3.9×10^7) conidiospores/ml.

Hull-removed medium-sized grain rice (IR 50) was divided into 50 g lots and placed in glass jars. *B. bassiana* (1 ml) in the dose mentioned above was mixed with the rice. The rice was tossed for 10 min in a mixer to thoroughly mix the conidia with the grain. Twenty-five unsexed adult rice weevils were introduced into the jars. Mortality of the rice weevil was recorded after 5, 10, 15, 20 and 25 days and all dead adults were removed after each count. After 25 days F_1 adults emerged from the treated seeds were counted. Ten replications were done for each treatment. Rice treated with 0.1% Tween 80 was used as a control.

Data collected from the experiments were subjected to one way Analysis of Variance and the individual means were compared using "Tukey's honestly significant different test" (Zar, 1984). Mortality of the adult insects was corrected using the Abbott formula (Abbott, 1925), subjected to probit analysis and the lethal time (LT_{50}) was calculated (Finney, 1971).

RESULTS

B. bassiana was found to be pathogenic to the *S. oryzae* tested. Mortality produced by the fungus was confirmed on the basis of the visual observation of mycelial growth on cadavers.

Although the results were insignificant between concentrations of 1:1 and 1:10 on day 10, a significant difference in mortality ($p < 0.05$) was observed at all three dosage levels when compared to the control. Mortality was high (75.8%) in the treatment of 7.6 log conidia/ml after 25 days (Table

1). In general the mortality was incremental, thus producing a sigmoid curve, with increasing concentrations of treatments as well as exposure period.

Based on the periodicity and the mortality data of the individual treatments, we calculated the equation for the regression line and lethal time (LT_{50}). The equation for the regression line drawn based on the *B. bassiana* concentration and mortality showed a positive correlation, and lethal time (LT_{50}) values were 38.5–13.5 days for the treatments studied (Fig. 1). A significant difference in F_1 adult emergence was observed in the treatments compared to the control. The percentage reduction in adult emergence ranged 26.4–86.2% for the treatments (Table 2).

DISCUSSION

Use of *B. bassiana* to control *S. oryzae* has been studied by various people worldwide (Hluchy and Samsinakova, 1989; Rice and Cogburn, 1999). *B. bassiana* was found to be an effective controlling agent for rice weevil on IR50 rice variety. However, there existed a range in mortality according to the concentration of conidia incorporated in the treatment. Early reports showed an increase in mortality at the higher dosages (Adane et al., 1996; Rice and Cogburn, 1999). The present study agrees with the reports where higher doses produced the highest percentage of mortality. The identification of strains involved and the type of formulation used are also essential to an understanding of the considerable differences in the activity of individual strains. The strain used in this study was found to work well on rice weevil at $28 \pm 2^\circ\text{C}$ and RH 70 $\pm$ 5% where the mortality stood at 75.8% after 25

Table 1. Mean percentage mortality of *Sitophilus oryzae* in *Beauveria bassiana* treated rice^a

Treatments	Exposure period in days ^b				
	5	10	15	20	25
Control	0.0 $\pm$ 0.00 a	0.0 $\pm$ 0.00 a	0.0 $\pm$ 0.00 a	5.6 $\pm$ 2.80 a	7.6 $\pm$ 3.50 a
5.5 log conidia/ml	7.6 $\pm$ 2.95 b	16.8 $\pm$ 3.68 b	20.8 $\pm$ 3.68 b	26.8 $\pm$ 3.29 b	28.0 $\pm$ 3.77 b
6.4 log conidia/ml	12.0 $\pm$ 2.67 c	20.0 $\pm$ 3.27 b	27.2 $\pm$ 2.52 c	40.8 $\pm$ 4.13 c	48.4 $\pm$ 2.95 c
7.6 log conidia/ml	35.2 $\pm$ 3.29 d	41.6 $\pm$ 3.75 c	54.0 $\pm$ 3.40 d	61.2 $\pm$ 3.79 d	75.8 $\pm$ 5.53 d

^a Values are mean $\pm$ standard deviation of ten replicates.

^b Means followed by same letter within each column do not differ significantly (Tukey test) at 5% level; df=36, 3; SE=(0.82) 5th day, (0.98) 10th day, (0.89) 15th day, (1.12) 20th day, (1.28) 25th day; critical q distribution (q 0.05, 30, 3) value=3.486.

Fig. 1. *Beauveria bassiana* concentration based probit regression lines for mortality of *Sitophilus oryzae*.

days. These results corroborate the findings of Searle and Doberski (1984) where 100% mortality was recorded 20 days after inoculation at 100% RH and 25°C. Emergence of adult rice weevil progeny was reduced by 86.2% at the higher dose (7.6 log conidia/ml). Rice and Cogburn (1999) using *B. bassiana* for the control of *S. oryzae* also reported results that complement the present findings.

Though the direct exposure of neonates to conidia is much less when considering the life cycle behaviour of *S. oryzae*, the present results are encour-

Table 2. Mean number of F₁ adults emerged from *Beauveria bassiana* treated rice^a

Treatments	F ₁ adult emergence ^b	Total reduction (%)
Control	150.0±17.27 a	—
5.5 log conidia/ml	111.0±9.49 b	26.39
6.4 log conidia/ml	85.7±5.87 c	43.17
7.6 log conidia/ml	20.0±3.30 d	86.21

^a Values are mean±standard deviation of ten replicates.

^b Means followed by same letter within each column do not differ significantly (Tukey test) at 5% level; df=36, 3; SE=3.3; critical *q* distribution (*q* 0.05, 30, 3) value= 3.486.

aging. In future, synergistic studies on the use of botanicals with *B. bassiana* would throw much light on effective biological control measures. Moreover, analysing the seeds for organoleptic properties would enable one to understand the part played by the fungus on the seed quality. Based on the findings of these studies, it is recommended that this strain could be used in Insect Pest Management. An integrated approach to isolate and test similar strains would be an advantageous proposition for effective control of storage pests.

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