

EFFECTS OF DUST FORMULATIONS OF THREE ENTOMOPHATOGENIC FUNGAL ISOLATES AGAINST *SITOPHILUS ORYZAE* (COLEOPTERA: CURCULIONIDAE) IN RICE GRAIN

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Abstrak: Tiga pencilan kulat entomopatogen telah dinilai sebagai konidia kering terhadap kutu beras, *Sitophilus oryzae*. Berasaskan kecuraman gradien dan disokong oleh nilai EC_{50} dan EC_{95} yang rendah, ujian patogenisiti menunjukkan bahawa pencilan *Beauveria bassiana* (BbGc) adalah paling berkuasa menjangkiti kutu beras. Bancuhan pencilan kulat BbGc dan BbPs bersama kaolin, talkum atau tepung ubi kayu (20% w/w b.a.) yang diaplikasikan pada kadar terendah sebanyak 0.05 g b.a. dan digaul sama rata dengan beras panjang di dalam bekas plastik (8 cm diameter dengan kedalaman 5 cm) telah menghasilkan kematian kutu beras melebihi 80% 7 hari selepas pendedahan. Sebagai bandingan, bancuhan pencilan *Metarhizium anisopliae* (MaPs) yang diaplikasikan pada kadar yang sama telah menghasilkan kematian yang rendah terhadap kutu beras. Formulasi kulat dalam kaolin dan talkum memberi perlindungan yang lebih berkesan terhadap kutu beras dengan mengakibatkan kematian signifikan berbanding formulasi dalam tepung ubi kayu atau kawalan yang tidak diformulasikan. Apabila diaplikasi pada kadar tidak kurang daripada 0.1 g b.a. dalam 50 g beras, kesemua pencilan dalam bancuhan kaolin telah memberi kematian tinggi secara konsisten; contohnya BbGc dalam kaolin mengakibatkan 98.75% kematian yang signifikan 7 hari selepas rawatan. Pada amnya, bancuhan pencilan dalam tepung ubi kayu agak lemah dalam melindungi beras; kehilangan berat beras yang signifikan telah direkodkan berbanding kaolin atau talkum setelah disimpan selama 4 bulan.

Abstract: Three isolates of entomopathogenic fungi were evaluated as dried conidia against the rice weevil, *Sitophilus oryzae*. Based on the steepness of the gradients and supported by low EC_{50} and EC_{95} values, the test for pathogenicity indicated that the isolate of *Beauveria bassiana* (BbGc) was the most infectious against the rice weevil adults. Admixtures of the isolates BbGc and BbPs with either kaolin, talc or tapioca flour (20 % w/w a.i.) applied at the lowest rate of 0.05 g a.i. and thoroughly mixed with long grain rice in plastic cups (8 cm diameter by 5 cm high) resulted in excess of 80% mortality to the adult weevils by the 7th day of exposure. In comparison the admixture of *Metarhizium anisopliae* (MaPs) applied at the same dosage gave lower percentage mortality of the adult weevils. Fungal formulations in kaolin and talc provided better protection against the rice weevil by giving significantly better kill compared to those formulated in tapioca flour or the unformulated control. When applied at the rate of at least 0.1 g a.i. in 50 g rice grain, kaolin admixtures of all the three isolates consistently gave the highest kill; for example BbGc in kaolin gave significantly 98.75% mortality 7 days after treatment. Generally, admixtures of the test isolates formulated in tapioca flour provided poor protection of the rice grain; a significantly higher grain weight loss was recorded compared with that of kaolin or talc after 4 months of storage.

Keywords: Entomopathogenic Fungi, Admixtures of Dust Formulation, Rice Weevil, Grain Weight Loss

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INTRODUCTION

The rice weevil, *Sitophilus oryzae* (L.), is one of the most serious stored grain pests worldwide. This pest of whole grain, originated in India, has spread worldwide by commerce and now has a cosmopolitan distribution. Being an ubiquitous pest of economic importance, *S. oryzae* feeds internally by boring into stored grain. The adults feed mainly on the endosperm thus reducing the carbohydrate content, while the larvae feed preferentially on the germ of the grain thus removing a large percentage of the proteins and vitamins (Belloa *et al.* 2000).

Application of insecticides is one means of preventing some losses during storage. However, the choice of insecticides for storage pest control is very limited because of the strict requirements imposed for the safe use of synthetic insecticides on or near food (Padin *et al.* 2002). The continuous use of chemical insecticides for control of storage grain pests has also resulted in serious problems such as resistance to the insecticides, pest resurgence, elimination of economically beneficial insects, and toxicity to humans and wildlife (Khan & Selman 1989; Adane *et al.* 1996; Padin *et al.* 2002).

There is no record of natural infection of rice weevils by entomopathogenic fungus. Investigations since mid-1980s by Tanya and Doberski (1984) followed by Adane *et al.* (1996), Hidalgo *et al.* (1998), Rice (1999), Smith *et al.* (1999), Belloa *et al.* (2000), Ekese *et al.* (2001) and Padin *et al.* (2002) suggested that isolates of *Beauveria bassiana* and *Metarhizium anisopliae* are potential microbial control agents against some stored product pests.

MATERIALS AND METHODS

Culture of Insect

Plastic containers (17.5 × 10.5 × 9.5 cm) covered with muslin cloths were used to mass rear *S. oryzae* on rice in an ambient environment of 28 ± 2°C and 60–95% RH. A cohort of 1,000 *S. oryzae* adults were introduced in 200 g rice grain of 14% M.C. or less in plastic containers (as a stock culture) and allowed to breed for one month after which they were collectively transferred into another container. New progenies were collected 7 days later and allowed to complete the development in several containers containing whole rice grains as food (Belloa *et al.* 2000). As a standard practice, individuals collected from infested rice grains were occasionally introduced into the culture in order to maintain the vigour of the colony. Adults of 2–3 weeks old were used for pathogenicity tests.

Production of Conidia

The original hosts and countries of origin for the fungal isolates used in this study are listed in Table 1. Twenty *S. oryzae* adults were placed in a vial (8 cm diameter and 4 cm long) and inoculated with 0.5 ml of 1 × 10⁸ conidia ml⁻¹ of the respective isolates using a Sigma hand atomizer, and then transferred to petri dishes consisting of rice as food. Fungus-killed cadavers were placed on moist filter

paper until sporulation. Conidia were scraped from the surface of sporulating cadavers with a sterile scalpel and placed on water agar in the plate. Upon germination the conidia were transferred by single spore isolation technique to slant PDA in test tubes and maintained at a room temperature of $28 \pm 2^\circ\text{C}$ in the dark for 1 week. The PDA had been sterilised for 20 minutes at 121°C and a pressure of 1.05 kg/cm^2 . Conidia from 15-day old sporulating cultures (slant PDA) were then transferred to a rice medium for mass culture.

Table 1: Isolates of *B. bassiana* and *M. anisopliae*, their original hosts and countries of origin.

Fungus	Code of isolates	Host	Country of origin
<i>B. bassiana</i>	BbGc	<i>Glenea celia</i> (Cerambycidae)	Malaysia
	BbPs	<i>Phyllotreta striolata</i> (Chrysomelidae)	Malaysia
<i>M. anisopliae</i>	MaPs	<i>P. striolata</i>	Malaysia

The culture medium consisting of not less than 90% whole grain rice in polyethylene bags was sterilised in an autoclave for 30 minutes and 121°C and a pressure of 1.05 kg/cm^2 . The bags were loosely sealed with a piece of PVC pipe (3 cm diameter) during autoclaving. After a 24-hour cooling period, each bag was inoculated with 5 ml of the respective conidial suspension (1×10^9 conidia ml^{-1}) using a micropipette and plugged with cotton. The bags were shaken vigorously for about 10 seconds every 24 hours for 3 days to distribute the inoculum evenly during incubation at $28 \pm 2^\circ\text{C}$ in the dark for 15 days. After incubation, the colonised substrate was then spread evenly, sandwiched between white paper towels to encourage full sporulation and then air-dried for 5–7 days in the laboratory. For the purpose of developing the formulations, the respective isolates of the dried conidia were then harvested and sieved through $125 \mu\text{m}$ particle size following the method of Daoust *et al.* (1983) and Belloa (2000).

Test for Pathogenicity

Conidial concentrations were prepared from an initial concentration of 1×10^9 conidia g^{-1} , as determined using a Neubauer haemocytometer, and serially diluted in a test tube with tapioca flour as the carrier to 1×10^8 , 1×10^7 , 1×10^6 , 1×10^5 and 1×10^4 conidia g^{-1} . The initial concentration was stored in the refrigerator at 4°C prior to use.

Twenty *S. oryzae* adults were placed in a 9 cm petri dish, each containing 0.1 g of the respective conidial concentrations. Control consisted of the carrier only. Each treatment was maintained at a room temperature of $28 \pm 2^\circ\text{C}$ and replicated five times. After 24 hours the weevils were transferred to a clean petri dish with only whole rice grains (14% M.C.) as food. Thereafter, mortality was recorded everyday for 15 days. Dead insects were removed and confirmed for fungal infection. Only those weevils that showed symptoms of fungal infection as manifested by sporulation of the fungus breaking through the cuticle were counted as a kill by the pathogen. The final proportions of diseased

insects were analysed by probit analysis (S103, Statistical Research Service, Canada DOA, unpublished) based on Finney (1971).

Effects of Admixtures of Conidial Dust Formulations with Rice Grain

Long grain rice with 5% broken grains was used in this study. The carriers were kaolin, talc and tapioca flour. Following the recommendation by Daoust *et al.* (1983), formulations were prepared by hand mixing in a test tube 20% w/w of the respective isolates or active ingredient (a.i.) of dried conidia with kaolin, talc or tapioca flour. The powdered formulation was applied at a dosage of 0.05 g a.i., 0.10 g a.i. and 0.15 g a.i. to 50 g a.i. rice grain (14% M.C.) and mixed evenly in a plastic cup. Twenty of 2–3 weeks old *S. oryzae* adults were placed in each cup (8 cm diameter by 5 cm high) 1 day after applying the respective treatments. The treatments were replicated four times with an unformulated control which consisted of the active conidial dust only. The experiment was run for 15 days with the mortality (fungus-killed) checked daily. Mortalities due to fungal infection were analysed using one-way ANOVA and the means were compared using least significant difference (LSD) (SAS Institute Inc. 1990). Prior to analysis data for ANOVA was transformed by arc sine (\sqrt{x}) to stabilise the variance. To determine percentage of grain weight loss, the experiment was extended up to 4 months.

RESULTS AND DISCUSSION

Test for Pathogenicity

All the test isolates were pathogenic against the rice weevil. Based on the comparative steepness of the gradients and supported by a lower median effective concentration (EC_{50} and EC_{95}) values, isolate BbGc was the most infective against *S. oryzae* adults (Table 2). The EC_{50} for isolate BbGc was the lowest with 9.491×10^6 conidia g^{-1} , while BbPs and MaPs were equally higher at 1.377×10^7 and 1.120×10^7 conidia g^{-1} , respectively. Isolate BbGc outperformed the other two isolates. Observations at the highest concentration of 1×10^9 conidia g^{-1} of these isolates, however, revealed that 50% of the weevils were infected by the 3rd day of exposure.

Table 2: Effective concentrations of three entomopathogenic fungal isolates against *S. oryzae* adults.

Isolates	A (intercept)	B ± SE (slope)	X^2	EC_{50}	95% FL	EC_{95}	95% FL
BbGc	-0.019	0.174 ± 0.060	2.034	9.491×10^6	6.083×10^6 $- 1.562 \times 10^7$	1.914×10^9	7.230×10^8 $- 7.173 \times 10^9$
BbPs	0.050	0.700 ± 0.061	3.091	1.377×10^7	8.645×10^6 $- 2.355 \times 10^7$	3.093×10^9	1.085×10^9 $- 1.311 \times 10^{10}$
MaPs	0.135	0.704 ± 0.060	0.921	1.120×10^7	7.115×10^6 $- 1.873 \times 10^7$	2.371×10^9	8.673×10^8 $- 9.367 \times 10^9$

20 adults per replicate, 5 replicates per dosage, 7 dosages per assay (N = 700).

Effects of Admixtures of Conidial Dust Formulations with Rice Grain

Table 3 shows the mortality of *S. oryzae* adults upon exposure to various admixtures of the selected fungal isolates with kaolin, talc or tapioca flour. In general, fungal formulations in kaolin and talc provided better kill compared to those in tapioca flour or unformulated control. It was suggested that the silicates in kaolin could induce mortality by causing desiccation (Varma & Siddiqui 1977; Golob 1997). However upon closer observation, it was revealed that whenever the insects move in the rice grains, some portion of the waxy layer of the integument was abraded and removed, and this had allowed the conidia to easily stick on and penetrated through the exoskeleton. Thus the physical abrasion by kaolin could have helped hasten the infection by the fungal admixtures. Mycelial growth was initiated especially from the tip of the rostrum and the ventral surface of the abdomen. Tapioca flour has no abrasive activity thus it has no added advantage against *S. oryzae*.

Table 3: Mean percent mortality of *S. oryzae* adults upon exposure to three isolates of entomopathogenic fungal formulations in 50 g rice grain 7 days after treatment.

Isolates	Carrier	Dosages (g. a.i.)		
		0.05	0.10	0.15
<i>B. bassiana</i> (BbGc)	Kaolin	88.75a	98.75a	98.75a
	Talc	90.0 a	91.25b	96.25a
	Tapioca flour	81.25ab	86.25b	88.75b
	Unformulated control	80.0b	88.75b	90.0b
<i>B. bassiana</i> (BbPs)	Kaolin	87.5a	96.25a	98.75a
	Talc	87.5a	90.0b	93.25b
	Tapioca flour	83.75a	85.0b	88.75b
	Unformulated control	82.5a	86.25b	88.75b
<i>M. anisopliae</i> (MaPs)	Kaolin	73.75a	96.25a	98.75a
	Talc	73.75a	97.5a	97.5a
	Tapioca flour	41.25b	77.5b	78.75b
	Unformulated control	50.0b	78.75b	77.5b

Means within column for each isolate followed by the same letter are not significantly different at P = 0.05 according to LSD.

Grain Weight Loss

Table 4 shows that there were significant differences in mean percent mortality of *S. oryzae* adults, and mean percent grain weight loss among the dust formulations of each fungal isolate. *B. bassiana* formulated in kaolin showed the highest mean percent mortality followed by talc, tapioca flour and the unformulated control. This is consistent with the lowest percentage grain weight loss in kaolin and talc compared to that in tapioca flour as the inert ingredient. Besides not being abrasive, tapioca flour became an alternative diet for *S. oryzae* adults, especially with MaPs formulation where the development of *S. oryzae* population was faster compared to those in kaolin or talc. Consequently, this had caused significantly higher grain weight loss compared to that treated with isolates BbGc and BbPs.

Table 4: Mean percent mortality of *S. oryzae* adult and mean percent grain weight loss upon exposure to three isolates of entomopathogenic fungal formulations in 50 g rice grain after four months of storage.

Isolates	Carrier	Dosages (g a.i.)	% mortality			% grain weight loss		
			0.05	0.10	0.15	0.05	0.10	0.15
BbGc	Kaolin		70.65a	91.30a	91.67a	1.29c	0.35b	0.33b
	Talc		32.23b	74.47a	80.0a	1.67bc	0.42b	0.34b
	Tapioca flour		36.55b	46.55b	54.09b	3.35ab	1.43a	1.29a
	Unformulated control		28.85b	31.11b	41.69b	3.99a	1.81a	1.40a
BbPs	Kaolin		66.44a	84.00a	86.67a	1.38b	0.51c	0.41b
	Talc		33.11b	61.18b	62.12b	2.17b	0.82bc	0.78b
	Tapioca flour		33.38b	44.58bc	45.13b	3.55a	1.80ab	1.67a
	Unformulated control		27.76b	30.49c	43.34b	4.11a	2.22a	1.98a
MaPs	Kaolin		29.86a	32.80b	39.05a	2.07b	1.31b	1.06b
	Talc		35.84a	38.43a	39.04a	2.66b	1.97b	1.87b
	Tapioca flour		20.19b	31.89b	31.38a	6.83a	5.16a	4.73a
	Unformulated control		17.66b	29.67b	31.74a	6.30a	4.91a	4.01a

Means within column for each isolate followed by the same letter are not significantly different at P = 0.05 according to LSD.

In this study the treated rice grains were still sustaining a number of progenies of *S. oryzae* even at the highest dosage of 0.15 g a.i. Detailed examination of the treated grains revealed that the weevils had deposited the eggs in the grains before death. Thus the juveniles were completely protected from fungal invasion. By the time the new adults emerged, the fungi were not at all pathogenic.

For commercial purposes the application of admixtures of dry conidia of *B. bassiana* in rice grain is more advantageous than *M. anisopliae*. This is because weevils infected with the admixtures of the cottony white *B. bassiana* did not contaminate the colour of the rice grains, while contamination of *M. anisopliae* infected weevil changed the colour of the rice grains to specks of green. For this reason admixtures of dry conidia of *M. anisopliae* is suggested only for application in non-milled paddy grains.

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