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Effectiveness of Selected Entomopathogenic Fungi in Packed Rice Grain at Room Temperature against *Corcyra cephalonica* Stainton

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EFFECTIVENESS OF SELECTED ENTOMOPATHOGENIC FUNGI IN PACKED RICE GRAIN AT ROOM TEMPERATURE AGAINST *CORCYRA CEPHALONICA* STAINTON

Hendrawan Samodra

Agricultural Quarantine Agency, Ministry of Agriculture,
JL. Harsono RM No. 3 Gd. E Lt. V, Ragunan, Jakarta Selatan 12550, Indonesia

Yusof Bin Ibrahim*

Department of Plant Protection, Faculty of Agriculture,
Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia

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ABSTRACT

Eight isolates of entomopathogenic fungi were evaluated as dried conidia against the rice moth, *Corcyra cephalonica.* In bioassays two isolates of *Beauveria bassiana* (BbGc and BbPs) and one isolate of *Metarhizium anisopliae* (MaPs) consistently gave high mortality to *C. cephalonica* larvae. Formulations in either kaolin, talc or tapioca flour (20 % w/w a.i.) thoroughly mixed with long grain rice in plastic cups (8 cm diameter by 5 cm) gave complete larval mortality by the 12th day of treatment. However, in general those formulated in kaolin and talc were more efficacious and faster to kill compared to those formulated in tapioca flour or the unformulated control. Even at the lowest rate of 0.05 g BbGc in kaolin provided 100% mortality 7 days after introduction compared with other dust formulations. Isolate BbGc in kaolin and talc administered at 0.4 g a.i. in 200 g rice packed in plastic kept at room temperature provided protection against the rice moth up to 4 months of storage. Larval mortality in excess of 90% was obtained 15 days after introduction. Formulations of MaPs was effective only within the first month of storage beyond which infectivity rapidly declined.

Keywords: *entomopathogenic fungi, dust formulation, rice moth, storage*

1. INTRODUCTION

The rice moth, *Corcyra cephalonica* Stn. is believed to be of eastern origin but has become a cosmopolitan species. It has spread through out the world with the transport of food and animal feeds. Beside rice, the rice moth is also a major pest of stored grains of pearl millet and sorghum. Rice packed in plastics are also known to get infested together with the rice weevils. It becomes established more readily in stored grains that are broken or seeds that have been damaged. For this reason the species is regarded as a secondary pest [10]. The larvae spin in the flour and broken seeds, silken tube in which it lives, and also spin fine threads wherever they crawl. Considerable amount of silk is produced until they become full grown. The broken seeds

* Corresponding author e-mail: yusofib@agri.upm.edu.my

have always been found to provide a more suitable medium than either whole seeds or flours. However, the favourability of whole seeds in comparison with flours depends upon the commodity under consideration. Its development in rice is both more rapid and successful on whole grains than on the flour.

Control has been primarily through the use of gaseous fumigants such as methyl bromide and pelletised phosphine and residual insecticides to augment the more obvious approach of hygiene [1, 9]. However, fumigation has many limitations among which the lack of penetration in sufficient concentration, undesirable residues and potential fire hazard [12]. In Malaysia methyl bromide is under threat of restricted use and possible withdrawal because it apparently depletes the ozone layer of the earth's atmosphere; the EPA (2001) has proposed elimination of this compound by 2005. The excessive use of conventional insecticides has resulted in a number of serious problems, among which is resistance to the insecticides, leading to a higher cost of crop production [11].

The use of entomopathogenic fungi is a novel approach for controlling insect pests of stored grains. Rice (1999) reported an isolate of *Beauveria bassiana* (Balsamo) Vuillemin to be pathogenic to adults of *Sitophilus oryzae*, *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst). A soil isolate of *B. bassiana* was also reported to be effective against adults of *Oryzaephilus surinamensis* (L.) [16]. *Beauveria bassiana* has also shown a considerable potential for the control of the larger grain borer, *Prostephanus truncatus* (Horn) of maize and cassava [15]. The other entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.) Sorokin was found to infect the groundnut bruchid *Carydon serratus* (Olivier) [4]. Mycoinsecticides have been shown to have considerable potential in insect pest management worldwide. However, their use as microbial control agents on stored grain insects has received little attention [2]. In this paper, we report on the effectiveness of selected entomopathogenic fungal isolates against the rice moth, *Corcyra cephalonica* Stn..

2. MATERIALS AND METHODS

2.1 Culture of insect

Plastic containers (40 × 30 × 20 cm) capped with muslin cloths were used to mass rear *C. cephalonica* on 50% w/w mixture of rice and maize [13] in an ambient environment of 28 ± 2ºC and 60 - 95% RH. This medium was cleaned and sterilised in an autoclave for 30 min and stored in the freezer prior to use. All utensils used were thoroughly cleaned and disinfected by storing in the oven. To obtain larvae of standardised age for treatment, 20 pairs of adults were placed in oviposition jars overnight and the eggs were collected the following morning. Only larvae of three and four weeks old were used in all treatments.

2.2 Production of dry conidia

The original hosts and countries of origin for the fungal isolates used in this study are listed in Table 1. These isolates were selected because they gave good sporulation as indicated by their full plate production of conidia on PDA. Conidia singlely isolated from reinfected cadaver of *C. cephalonica* larva were maintained at a room temperature of 28 ± 2ºC on PDA which had been sterilised for 20 min at 121ºC and a pressure of 1.05 kg/cm^2. These isolates were then used for the production of air-dried conidia in rice medium; only conidia from 15 d old sporulating cultures were transferred to the rice medium. The culture medium was consisted of 200 g whole grain rice (10% broken; 80% w/v distilled water) in a polyethylene bag sterilised in an autoclave for 30 min at 121ºC at a pressure of 1.05 kg/cm^2. The bags were loosely sealed with cotton plug in a PVC pipe (3 cm diameter) during autoclaving. After a 24 h cooling period, each bag was inoculated with 5 ml of the respective conidial suspension (1 × 10^9 conidia ml^{-1}) using a

micropipette and replugged with cotton. The bags were shaken vigorously every 24 h for 3 d to distribute the inoculum evenly during incubation at 28 ± 2ºC in the dark for 15 days. After incubation, the colonised substrate was then spread evenly, sandwiched between paper towels to further encourage sporulation and air-dried for 5 - 7 d in the laboratory. The dried conidia were then harvested by sieving through 125 µm particle size following the method of Daoust *et al*. [3] and Belloa *et al*. [2].

Table 1: *Origin of fungal isolates*

Species	Code	Insect host	Location
Beauveria bassiana	BbGc	*Glenea celia* (Cerambycidae; larva)	Malaysia (Tuaran, Sabah)
	BbPs	*Phyllotreta striolata* (Chrysomelidae; adult)	Malaysia (Serdang, Selangor)
	BbPc	*Phyllotreta cruciferae* (Chrysomelidae; adult)	Malaysia (Serdang, Selangor)
Metarhizium anisopliae	MaPs	*Phyllotreta striolata* (Chrysomelidae; adult)	Malaysia (Serdang, Selangor)
	MaORMaj	*Oryctes rhinoceros* (Scarabaeidae; larva)	Malaysia (Bukit Raja, Selangor)
	MaORMan	*Oryctes rhinoceros* (Scarabaeidae; larva)	Malaysia (Bukit Raja, Selangor)
	MaGmC	*Galleria melonella* (Pyralidae; larva)	Indonesia (Cilacap, Java)
	MaSc	*Scotinophara coarctata* (Pentatomidae; nymph)	Indonesia (Bogor, Java)

2.3 Test for pathogenicity

The conidial concentrations were prepared from an initial stock of 1×10^9 conidia g^{-1}, as determined using a Neubauer haemocytometer, and diluted with tapioca flour (as 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 overnight in the refrigerator at 4ºC prior to use. Twenty *C. cephalonica* larvae were placed in a 9 cm Petri dish containing 0.1 g of the respective conidial mixture. The control consisted of the carrier only. Each treatment was replicated 5 times. After 24 h the insects were transferred to a clean Petri dish with only rice grains as food. Thereafter, mortality was recorded everyday for 15 days. Dead insects were removed and confirmed for fungal infection. Only those insects 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 test was also repeated on the eggs; fifty 24 h old eggs were used per treatment. The final proportions of dead insects were analysed by probit analysis (S103, Statistical Research Service, Canada DOA, unpublished) based on Finney [6].

2.4 Effectiveness of conidial formulations in rice grain

Long grain rice (5% broken; 14% M.C.) was used in this study. The carriers were kaolin, talc and tapioca flour. Three most virulent isolates determined earlier from pathogenicity tests were used in this study. Formulations were prepared by mixing 20% w/w a.i. consisted of dried conidia with kaolin, talc or tapioca flour. The powdered formulation was applied at a dosage of

0.05, 0.10 and 0.15 g a.i. to 50 g rice grain in a plastic cup. Twenty 3 - 4 week old larvae were placed in each cup (8 cm diameter by 5 cm) 1 d after applying the respective treatments. The treatments were replicated 4 times with an unformulated control. The experiment was run for 15 d with the mortality checked daily. Mortalities were analysed using one-way ANOVA and the means were compared using least significant difference (LSD). The data were transformed arcsine square-root of the percent mortality of the initial number [7].

2.5 Persistency of virulence upon storage in packed rice

An amount of 0.4 g a.i. of *B. bassiana* and *M. anisopliae* formulated in kaolin, tapioca flour, talc and unformulated control were applied on 200 g rice grain (14% M.C.) packed in plastic. Twenty-five larvae were then introduced into each bag which had been stored at room temperature (28 ± 2ºC) and 60 ± 10% RH for 1, 2, 3, 4, and 6 months. The treatments were replicated 4 times. Percentage of larvae infected were recorded 4, 8, 12 and 15 d after introduction. The mortality responses were analysed using one-way ANOVA and the means were compared using least significant difference (LSD).

3. RESULTS AND DISCUSSION

3.1 Test for pathogenicity

Table 2 shows isolates BbGc, BbPs and MaPs were the three best isolates consistently giving high mortalities compared with the other isolates. It was apparent that 50% mortality was reached in less than four days and by the 7^{th} day of exposure to 1×10^8 conidia g^{-1} only these three isolates reached mortality level in excess of 80% (Fig. 1). The median effective concentration (EC_{50}) for these isolates against the larvae were 1.238×10^6 (BbGc), 2.072×10^6 (BbPs) and 1.775×10^6 (MaPs) conidia g^{-1} respectively (Table 3). These three isolates were selected for subsequent experiments.

Table 2: *Mean percent larval mortality of Corcyra cephalonica upon exposure to selected entomopathogenic fungal isolates 15 days after treatment*

Isolates	Concentrations					
	10^9	10^8	10^7	10^6	10^5	10^4
BbGc	100a	100a	80a	43a	17a	0a
BbPs	100a	98a	73a	35ab	13a	0a
BbPc	100a	85b	51b	30b	12a	2a
MaPs	100a	98a	72a	37ab	15a	2a
MaORMan	100a	73bc	36c	15c	2b	0a
MaORMaj	97a	63c	32c	15c	3b	0a
MaSc	97a	64c	35c	17c	9a	0a
MaGmC	84b	35d	15d	5d	0b	0a

Means within column followed by the same letter are not significantly different at p = 0.05 according to least significant difference (LSD). Control = zero mortality.

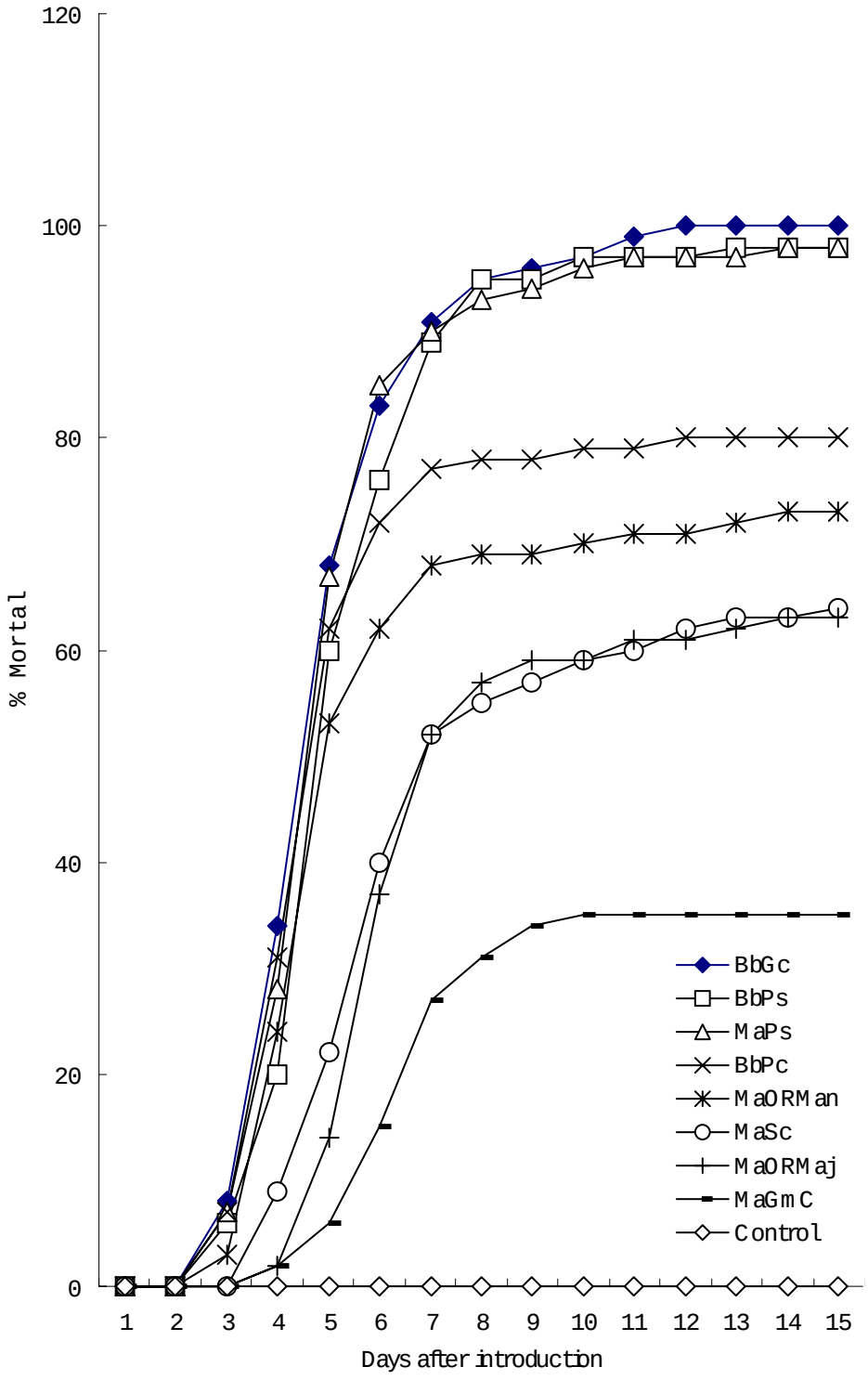

Fig. 1: *Mean percent cumulative mortality of C. cephalonica larvae upon exposure to eight entomopathogenic fungal isolates at concentration 1 × 10^8 conidia g^{-1} observed for 15 days after introduction*

Table 3: *Effective concentrations of selected entomopathogenic fungal isolates against Corcyra cephalonica larvae*

Isolate	**A (intercept)**	**b ± SE (slope)**	**X^2**	**EC_{50}**	**95%FL**	**EC_{95}**	**95%FL**
BbGc	-1.571	1.097 ± 0.079	1.747	1.238×10^6	8.916×10^5 -1.719×10^6	4.149×10^7	2.425×10^7 -8.224×10^7
BbPs	-1.893	1.091 ± 0.074	3.447	2.072×10^6	1.506×10^6 -2.855×10^6	6.669×10^7	4.007×10^7 -1.260×10^8
BbPc	0.109	0.724 ± 0.058	2.468	5.658×10^6	3.691×10^6 -8.991×10^6	1.057×10^9	4.303×10^8 -3.512×10^9
MaPs	-0.850	0.936 ± 0.062	4.125	1.775×10^6	1.246×10^6 -2.541×10^6	1.016×10^8	5.501×10^7 -2.226×10^8
MaORMan	-1.337	0.865 ± 0.078	0.687	2.104×10^7	1.398×10^7 -3.366×10^7	1.676×10^9	6.946×10^8 -5.703×10^9
MaORMaj	-0.551	0.735 ± 0.071	0.439	3.605×10^9	2.187×10^7 -1.977×10^9	6.262×10^9	1.977×10^9 -3.287×10^{10}
MaSc	0.272	0.631 ± 0.061	0.913	3.077×10^7	1.764×10^7 -6.144×10^7	1.241×10^{10}	3.293×10^9 -8.357×10^{10}
MaGmC	-1.022	0.708 ± 0.099	1.199	3.183×10^8	1.413×10^8 -1.159×10^9	6.696×10^{10}	1.047×10^{10} -1.659×10^{12}

20 larvae per replicate, 5 replicate per dosage, 7 dosages per assay (N = 700)

Table 4: *Mean percent larval mortality of Corcyra cephalonica upon exposure to dust formulations of selected entomopathogenic fungal isolates in 50 g rice grains seven days after treatment*

Fungi	**Carrier**	**Dosages (g a.i.)**		
		0.05	0.10	0.15
B. bassiana (BbGc)	Kaolin	100.0a	100.0a	100.0a
	Talc	95.0b	97.5b	97.5ab
	Tapioca Flourr	91.2b	92.5c	96.3b
	Unformulated	92.0b	96.4b	95.0b
B. bassiana (BbPs)	Kaolin	96.3a	97.5a	100.0a
	Talc	90.0b	93.8ab	96.3b
	Tapioca Flour	72.5c	81.3c	87.5c
	Unformulated	67.5c	88.3bc	91.3c
M. anisopliae (MaPs)	Kaolin	87.5b	96.3a	100.0a
	Talc	96.3a	96.3a	100.0a
	Tapioca Flour	81.3b	90.0b	97.5a
	Unformulatedr	78.8b	91.3b	95.0b

Means within column for each fungus followed by the same letter are not significantly different at p = 0.05 according to least significant difference (LSD).

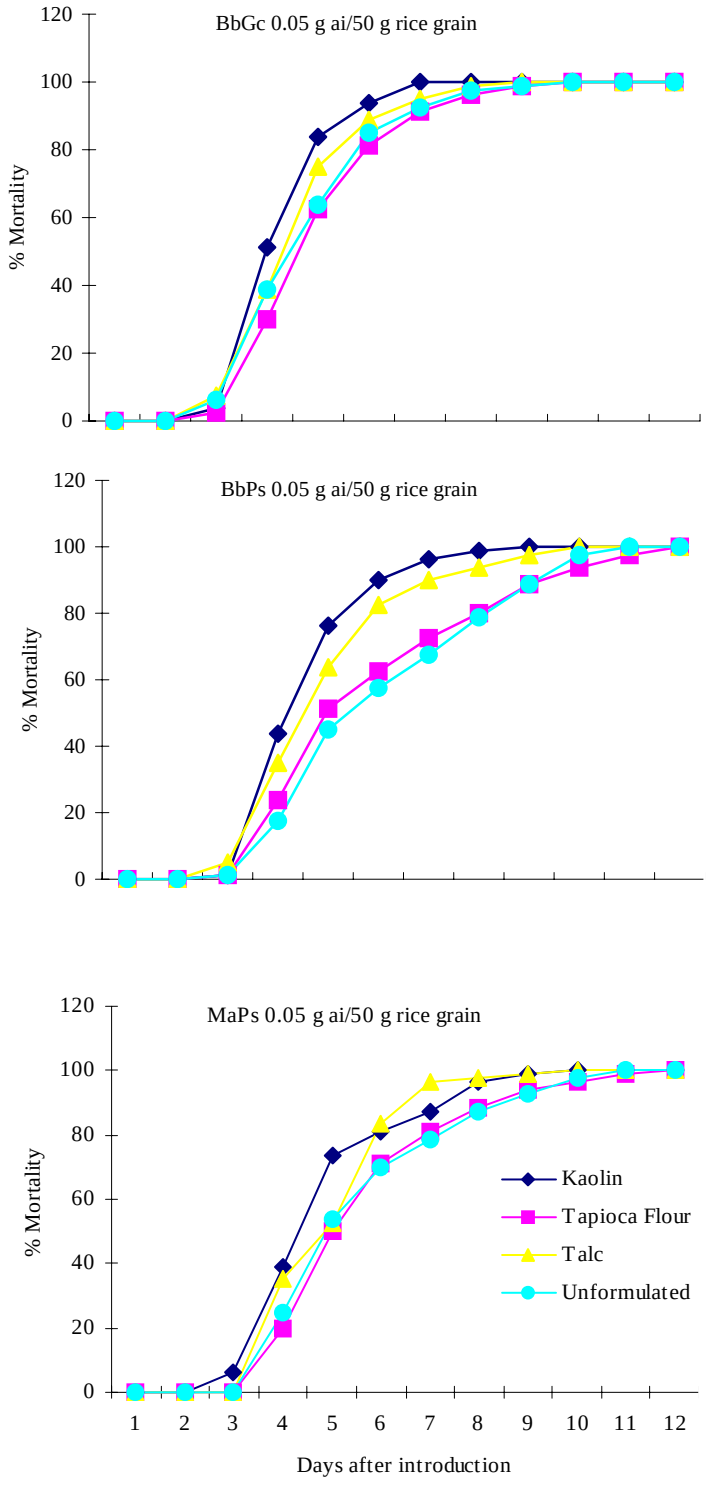

Fig. 2: *Comparative mean percent larval mortality of C. cephalonica upon exposure to conidial formulation of three entomopathogenic fungal isolates*

3.2 Effectiveness of conidial formulations in rice grain

The larval mortalities of *C. cephalonica* upon exposure to various formulations of the selected fungal isolates with kaolin, talc or tapioca flour are shown in Table 4. Isolate BbGc formulated in kaolin was significantly the most superior and gave 100% mortality 7 d after introduction compared with the other formulations. Apparently, the trend was similar with isolate BbPs, while formulations with kaolin was as effective as talc for isolate MaPs with mortalities recorded in excess of 90% (Fig. 2). Based on carrier types and classification of inertness, particles of kaolin which are derived from inorganic silicate [17] are harder than the tapioca flour which is an organic botanical. It was observed that when the larva crawls in the rice grains the waxy larval cuticle became abraded by this carrier. It is thus suggested that the abrasive activity by kaolin facilitates the penetration of conidial germ tube through the insect`s integument and hence leads to enhanced infection.

3.3 Persistency of virulence upon storage in packed rice

Table 5 shows that all the dust formulations and unformulated control of *B. bassiana* in the rice grains stored for 3 months at room temperature still provided 100% mortality to *C. cephalonica* larvae. Isolates of BbGc and BbPs formulated in kaolin and BbPs formulated in tapioca flour gave 100% larval mortality by the 15th day of introduction. Even those that had been stored for 4 months still provided effective control in excess of 90%. The pathogenicity of *M. anisopliae* against *C. cephalonica* larvae decreased rapidly upon storage at room temperature affording less than 60% control by the second month of storage; the unformulated control was significantly the least effective. All MaPs treatments were less than 50% effective by the third month of storage. A marked decrease in larval mortality was recorded after 6 months of storage; mean percent larval mortality against isolates BbGc and BbPs in all the treatments had dropped to less than 80%, ranging between 55 - 79%, while all formulations of MaPs were completely ineffective with no larval mortality 15 d after introduction.

Table 5: *Persistency of selected entomopathogenic fungal isolates in 200 g rice grains in plastic bags stored up to six months as indicated by mean percent larval mortality of Corcyra cephalonica 15 days after introduction*

Fungi	Carrier	Duration of storage (months)				
		1	2	3	4	6
B. bassiana (BbGc)	Kaolin	100a	100a	100a	100a	61a
	Talc	100a	100a	100a	96bc	66a
	Tapioca Flour	100a	100a	100a	96bc	67a
	Unformulated	100a	100a	100a	93c	58a
B.bassiana (BbPs)	Kaolin	100a	100a	100a	100a	55b
	Talc	100a	100a	100a	96ab	62b
	Tapioca Flour	100a	100a	100a	100a	79a
	Unformulated	100a	100a	100a	95b	60b
M. anisopliae (MaPs)	Kaolin	96a	52a	43a	15a	0a
	Talc	92ab	44b	42a	20a	0a
	Tapioca Flour	88b	56a	48a	27a	0a
	Unformulated	66c	35c	26b	5b	0a

Means within column for each fungus followed by the same letter are not significantly different at p = 0.05 according to least significant difference (LSD)

The results presented indicated that the persistency of virulence for *B. bassiana* in dust formulations was longer than *M. anisopliae* when tested against *C. cephalonica* larvae. *Beauveria bassiana* seemed to sustain pathogenicity better than *M. anisopliae* in a drier environmental condition, and this concurs with the report by Hallsworth and Magan (1999). We conclude that the approach using air-dry conidia of *B. bassiana* in dust formulation is an effective microbial control tactic against *C. cephalonica*. Isolates *Beauveria bassiana* (BbGc and BbPs) provided good protection against *C. cephalonica*, inflicting in excess of 90% larval mortality 15 d after introduction. Protection was effective up to 4 months of storage at room temperature; the effectiveness began to decrease after 6 months of storage. However, the pathogenicity of *M. anisopliae* against *C. cephalonica* larvae decreased rapidly by the second month of storage at room temperature. Since this study was limited to carriers easily available in Malaysia, further experimentation involving other carriers are necessary for the optimisation of dust formulations. This includes studying the persistency of virulence upon storage in materials other than the plastic bag and realistic large-scale application of these formulations for the commercial scale.

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