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Management of entomopathogenic fungi in cultures of *Tenebrio molitor* (Coleoptera: Tenebrionidae)

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Abstract

Larvae of mealworms *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) have been used as animal feed, but fungal pathogens rapidly downsize the populations, resulting in economic losses. In this work, we established an effective management strategy for fungal pathogens. An entomopathogenic fungus, *Beauveria bassiana*, was isolated from mealworm cadavers. The bioassay of some isolates of this species at >90% relative humidity revealed that the ERL1575 isolate had the highest virulence. At 20–30% RH, ERL1575 conidia when ingested produced 80% mortality but when sprayed topically produced only <10% mortality. Mealworms that had ingested conidia were exposed to 20, 25, 30 and 35°C and high humidity (>95%) for 5 days. This experiment produced about 90% mortality except at 35°C where mortality was <20%. When 40 fungicides were assayed against ERL1575, fluazinam (1000-fold) and mancozeb (667-fold) significantly inhibited conidial germination and/or hyphal growth. When fluazinam and mancozeb were added to the mealworm diet of conidia-inoculated wheat bran, most were alive 3 days post application. However, 100% mortality resulted 3 days post application in the conidia-inoculated wheat bran without any fungicides. In conclusion, *B. bassiana* isolates are pathogenic at <30°C when they are ingested by mealworms but fluazinam and mancozeb can be used for management to control the pathogen in their cultures.

Key words: *Beauveria bassiana*, entomopathogenic fungi, fluazinam, mancozeb, *Tenebrio molitor*.

Introduction

A large amount of biomass has been obtained from insects for industrial uses such as feed for animals (Huis 2013). Most insects are ranked as the first consumers of plants and they significantly contribute to the cycling of organic materials in the environment (Ramos-Elorduy *et al*. 2002). In regard to nutritional diversity, insects have high levels of proteins, fats, minerals and vitamins, and particularly amino acids, which are more prevalent than those found in cereals and beans (Chavunduka 1975). Crickets, soldier flies, grasshoppers and mealworms are well known to have large amounts of proteins that can be used as feed additives for animals (Bondari & Sheppard 1987; Sheppard *et al*. 1994; Ng *et al*. 2001; Newton *et al*. 2005; Hahn & Denlinger 2007; St-Hilaire *et al*. 2007; Anand *et al*. 2008; Njidda & Isidahomen 2010). For example, mealworm larvae *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) have been mass-reared worldwide for animal feed. Their protein content is as rich as those found in soybeans and casein, which are used as chicken feed (Gourlet *et al*. 1978; Ghaly & Alkoaik 2009).

In the past, emphasis has been given to larval population density and nutritional development in culture. However, some fungal pathogens are very virulent to mealworms and rapidly downsize populations resulting in huge economic losses. Plants or plant-oriented diets may be the sources of

these fungal contaminants. In this research, we isolated an entomopathogenic fungus *Beauveria bassiana* from the cadavers of *T. molitor* larvae. A more virulent isolate was found from the bioassay of several *B. bassiana* isolates in stock and used in the following work. We established an effective management strategy for fungal pathogens based on the relationship between abiotic conditions and their occurrence and the application of a fungicide library (40 fungicides). The result was effective in mass-rearing of mealworm larvae without fungal contaminants.

Materials and methods

Isolation of fungal pathogens from dead larvae

Dead *T. molitor* larvae were collected from a culture in an insect rearing room, and possible entomopathogenic fungi were isolated according to the Insect Pathology Manual (Humber 1997) and identified by sequencing the ITS regions of extracted gDNA (gDNA extraction kit; Qiagen, Valencia, CA, USA) using primers ITS1F (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4F (5′-TCC TCC GCT TAT TGA TAT GC-3′) and NCBI BLAST. To confirm the pathogenicity of the mealworm-originated isolates, 10 third instar larvae were placed on 7-day-old isolated fungal cultures on PDA (Difco, Sparks, MD, USA) and kept at room temperature for 10 days (Humber 1997).

Fungal pathogens in stocks

Beauveria bassiana isolates ERL836, 1050, 1051, 1575 and 1576 were provided by personnel at the Entomology Research Laboratory, University of Vermont, USA. *B. bassiana* were isolated from insect cadavers, which were collected from 2002 to 2008 and identified by sequencing the ITS region as described above. They were cultured on quarter-strength Sabouraud dextrose agar media (SDA/4) in darkness at 25°C (Kim *et al*. 2013). To produce conidia, the isolates were grown on SDA/4 for 10 days. Then the conidia were suspended in 0.03% (v/v) siloxane solution (Silwet L-77; Loveland Co, Greely, CA, USA) and adjusted to 1×10^7 conidia/mL in preparation for the following experiments.

Virulence assay

To choose the most virulent fungal pathogen, six *B. bassiana* isolates including the mealworm isolate were first subjected to a virulence assay against *T. molitor* larvae in Petri dishes (Butt & Goettel 2000). For testing, 10 third instar larvae were placed in a 60-mm diameter Petri dish, and a conidial suspension (1×10^7 conidia/mL) was sprayed using a microsprayer at rate of 3 mL per dish. Then Petri dishes were sealed with Para-film to maintain >90% relative humidity (RH) and incubated at 25 ± 1°C under conditions of 16 h light : 8 h dark (LD 16:8). The numbers of dead larvae were counted daily for 5 days. This assay was repeated three times, and each treatment was replicated three times in an experimental replicate.

Fungal infection pathway assay

To determine the pathways of fungal pathogens in the rearing of *T. molitor*, ERL1575, the most virulent isolate, was used. Third instar larvae were treated by spraying and by voluntary ingestion with three replicates (Butt & Goettel 2000). Spraying was done as described above. Treated Petri dishes were not covered, to simulate culturing procedures and kept at room temperature (~25°C) and ~30% RH for 24 h. For voluntary ingestion, three fungal agar sections (6 mm diameter) were taken from a 10-day-old culture plate and placed in a Petri dish with 10 third instar larvae and kept for 24 h for ingestion. After 24 h of incubation, all the larvae were transferred to a rearing dish (100 mm diameter × 41 mm height; SPL Life Sciences, Pocheon, Korea) containing 10 g wheat bran (<5% moisture). The numbers of dead larvae were counted daily for 5 days.

Abiotic factor assay

To investigate the relationship between abiotic factors and virulence of ERL1575, larvae were first allowed voluntary ingestion and then exposed to various temperature and humidity conditions. To vary RH, sterilized distilled water (1.05, 2.10 or 3.15 mL) was sprayed on 3 g aliquots of wheat bran as their diet in a 60-mm-diameter Petri dish. The larvae, which ingested conidia as above, were held in the dishes and kept at 20, 25, 30 or 35°C in an incubator with three replicates. The numbers of dead larvae were counted daily for 5 days. This experiment was repeated three times using different cultures of fungal conidia.

Fungal growth inhibition assay

To determine the inhibition of conidial germination and hyphal growth of ERL1575 by a variety of fungicides, a 50-μL conidial suspension (1×10^7 conidia/mL) was first spread on SDA/4 in Petri dishes. The 40 chemical fungicides (Table 1) used were obtained from the pesticide library of Dongbu Farm Hannong Agrochemical Company (Daejeon City, Korea). Each of the 40 fungicides was prepared at its standard application rate (SD), SD/2 and SD/5 according to the manufacturer's recommendation. Next, 5 μL of each fungicide preparation was dropped on the ERL1575 culture plate with three replicates. Two days later conidial germination was determined by randomly counting the number of germinated conidia per 100 spores under a light microscope

Table 1 Chemical fungicides used in this study

Group	Fungicide	Active ingredient (%)	Formulation type	Standard application dose (SD)
Guanidine	Imminoctadine (triacetate)	25	SL	500×
	Imminoctadine (tris albesilate)	30	SC	1000×
Dinitroaniline	Fluazinam	50	SC	500×
Dicarboximid	Iprodione	50	WP	1000×
	Procymidone	50	WP	1000×
Copper	Cooper hydroxide	52	WDG	200×
	Bordeaux mixture	76	WDG	500×
Benzophenol	Metrafenone	24	SC	2000×
Benzimidazole	Benomyl	50	WP	1000×
Sulfoamide	Amisulbrom	13	SC	1000×
Strobilurin	Kresoxim-methyl	47	WDG	2000×
	Trifloxystrobin	50	WDG	3000×
	Azoxystrobin	10	WP	1000×
	Orysastrobin	12	SC	2000×
Cyanoimidazole	Cyazofamid	10	SC	400×
Anilide	Thifluzamide	21	SC	5000×
Urea	Pencycuron	25	WP	1000×
Oxine-copper	Oxine copper	50	WP	500×
Dithiocarbamate	Mancozeb	75	WP	500×
	Etridiazole	25	EC	2000×
Imidazole	Prohloraz	25	EC	2000×
Carboxylicacidamide	Mandipropamid	22	SC	2000×
Carbamate	Carbendazim	60	WP	1000×
	Thiophanate-methyl	40	SC	1000×
Carboxyamide	Carpropamid	15	SC	1500×
Quinazolinetriazole	Fluquinconazole	10	SC	500×
Quinorinone	Oxolinic acid	20	WDG	1000×
Quinone	Dithianon	43	SC	1000×
Triazole	Tetraconazole	12	EC	1000×
	Tricyclazole	75	WP	2000×
	Bitertanol	25	WP	500×
	Hexaconazole	2	SC	2000×
	Triflumizole	30	WP	1000×
	Difenoconazole	10	WDG	2000×
Phenoxyamide	Fenoxanil	20	SC	1000×
Pyrimidine	Cypronidil	48	WDG	2000×
	Fenarimol	12	EC	3000×
Antibiotics	Streptomycin	20	WP	800×
	Polyoxin B	50	SP	5000×
	Validamycin-A	5	SL	1000×

EC, emulsion concentrate; SC, suspension concentrate; SL, soluble liquid; SP, soluble powder; WDG, water dispersible granule; WP, wettable powder.

at 100× magnification. To determine the inhibition of hyphal growth a 7-day-old 6-mm-diameter agar section of ERL1575 was placed on the center of SDA/4 in a Petri dish and a 6-mm-diameter paper disc with 5 μL of the diluted fungicide was placed at the border of the dish. Petri dishes were incubated at 25°C for 10 days. Inhibition of hyphal growth was determined by measuring the inhibition zone between the paper disc and the growing hyphal tip. This experiment was repeated three times using different cultures of fungal conidia.

Application of fungicides to rearing system

Fluazinam (CAS No. 79622-59-6) 50% suspension concentrate (500-fold dilution) and mancozeb (CAS No. 8018-01-7) 75% wettable powder (500-fold dilution), which were selected from the above experiments, were applied to wheat bran, the diet of mealworms, to manage ERL1575. In the rearing dish, 10 mL of ERL1575 suspension (1×10^7 conidia/mL) was evenly mixed with 10 g of sterilized wheat bran. Fungicides were sprayed using a micro-sprayer

Isolate (from cadavers)	Identification	Virulence against *T. molitor* larvae
M1	*Cladosporium sphaerospermum*	-
M2	*Alternaria oregonensis*	-
M3	*Alternaria infectoria*	-
M4	*Penicillium polonicum*	-
M5	*Penicillium sclerotiorum*	-
M6	*Penicillium citrinum*	-
M7	*Beauveria bassiana*	+
M8	*Leptosphaeria microscopica*	-

Figure 1 Isolation of fungi from cadavers of *T. molitor* larvae in cultures and their pathogenicity. Isolate M7 showed pathogenicity (+) against the larvae, but the other isolates did not (–) at 10 days after inoculation.

on the wheat bran at 1.05 mL of diluted suspension (recommended standard dose as above) per dish with three replicates. Dishes without fungicides or fungal inoculum served as controls. Three days post inoculation, 10 third instar larvae were placed in each dish. The numbers of dead mealworms were counted 1, 3, 5 and 7 days later. This experiment was repeated three times using different cultures of fungal conidia.

Data analysis

Data on the percentage of live mealworms were analyzed using the general linear model (GLM), followed by Tukey's honestly significant difference (HSD) for multiple comparisons. All analyses were conducted using SPSS v17.1 (SPSS Inc., Chicago, IL, USA) at the 0.05 (α) level of significance.

Results

Isolation of mealworm-pathogenic *B. bassiana* isolate

Eight fungi were isolated from the dead population, but the *B. bassiana* isolate M7 alone showed pathogenicity against the larvae (Fig. 1). The other fungal isolates, such as Cladosporium, Alternaria, Penicillium and Leptosphaeria did not show any significant pathogenicity.

Virulence of *B. bassiana* isolates against *T. molitor* larvae

Of the isolates tested, ERL1575 was most efficacious producing about 100% mortality 3 days post treatment ($F_{7,48} = 173.5$, $P < 0.001$) at high RH and ERL1576 was the least efficacious (Fig. 2). The other isolates produced 33% (ERL836) and <10% mortality (M7, ERL1050, ERL1051 and ERL1576) in 3 days, but ERL836, ERL1050 and M7

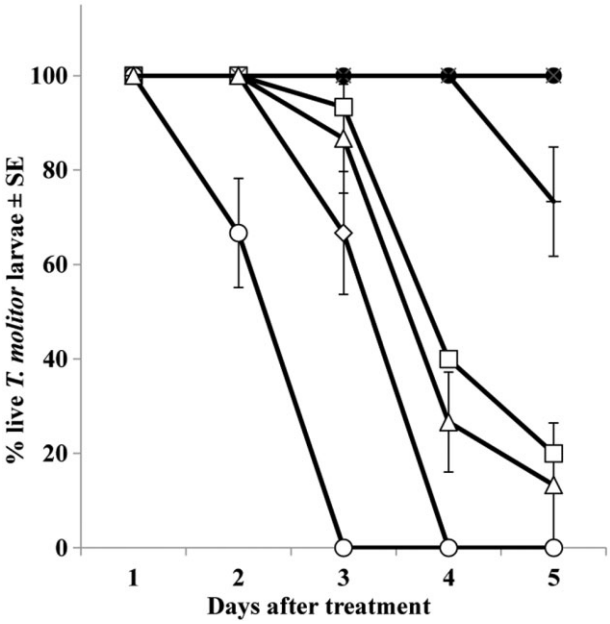

Figure 2 Virulence of several entomopathogenic *B. bassiana* isolates against third instar *T. molitor* larvae after spraying isolates (1×10^7 conidia/mL) under high RH (>90%) in laboratory conditions. Control; ERL836; ERL1050; ERL1051; ERL1575; ERL1576; M7 (mealworm-originated).

reached >80% of mortality in 5 days. Dead larvae turned dark brown and rapidly dried.

Relationship between environmental factors and fungal efficacy

When RH < 30%, the spray of ERL1575 was not significantly efficacious against the mealworms 5 days post treatment, but when it was ingested 80% of the larvae died ($F_{2,18} = 78.0$, $P < 0.001$) (Fig. 3). Virulence of ERL1575 was significantly related to temperature ($r^2 = 0.86$, $P < 0.001$) rather than RH ($r^2 = 0.02$, $P = 0.893$) (Fig. 4). At 30°C larval

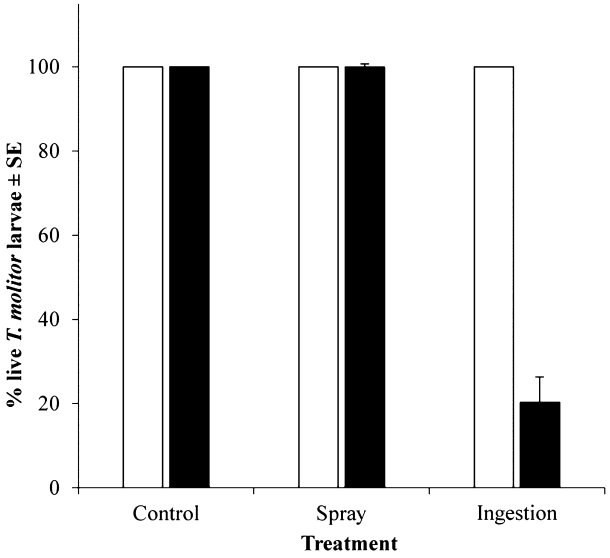

Figure 3 Comparison of the pathogenesis of *B. bassiana* ERL1575 against third instar *T. molitor* larvae between spray- and ingestion-mediated treatments under laboratory conditions 5 days after exposure to ERL1575 at about 30% RH. □ Infestation; ■ 5 days.

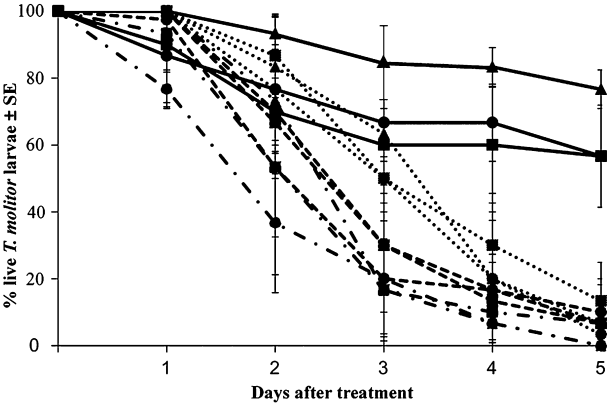

Figure 4 Effect of humidity (which was adjusted by spraying different volumes of water, mL, into the dish) and temperature on the virulence of ingested *B. bassiana* ERL1575 against third instar *T. molitor*. ···●··· 20°C 1.05 mL; ···■··· 20°C 2.10 mL; ···▲··· 20°C 3.15 mL; -●- 25°C 1.05 mL; -■- 25°C 2.10 mL; -▲- 25°C 3.15 mL; -●· 30°C 1.05 mL; -■· 30°C 2.10 mL; -▲· 30°C 3.15 mL; —●— 35°C 1.05 mL; —■— 35°C 2.10 mL; —▲— 35°C 3.15 mL.

mortality occurred sooner than at 25°C and 20°C treatments. At 35°C no significant mortality was observed.

Inhibition of conidial germination and hyphal growth

Of the chemical library tested, fluazinam showed the highest inhibition activity against the germination of ERL1575 conidia with a dose-dependence response, followed by mancozeb, oxine-copper, prochloraz, dithianon, benomyl, carbendazim and bitertanol (Table 2). Fluazinam also strongly inhibited ERL1575 hyphal growth, but the others did not have any significant inhibition activity except mancozeb at standard dose (Table 3).

Control of fungal pathogen in rearing of *T. molitor* larvae

Both fluazinam and mancozeb controlled ERL1575 without any significant adverse effect on the larvae (Fig. 5). Most larvae in the fungus-inoculated wheat bran without any fungicides were dead 7 days after exposure. In 3 days after the introduction of the larvae, about 80% mortality was observed in the non-fungicide treatment. Fluazinam and mancozeb treatments with fungal inoculum showed <5% mortality, similar to the non-treated control.

Discussion

Most fungal isolates from the cadavers of *T. molitor* were saprophytic fungi, but still we had little information what were the main factors for insect deaths. Bacterial and viral pathogens might be involved in the death of the insects. However, *B. bassiana* isolate was collected from many cadavers of this insect in the larval stages, and consideration should be given to this genus to prevent rapid decrease in population numbers.

It was confirmed that several *B. bassiana* isolates had pathogenicity against *T. molitor* larvae and the virulence was different among those tested. The level of mechanical penetration and the production of cuticle-degrading enzymes such as lipases, proteases, esterases and chitinase (Pedrini *et al*. 2007) are possibly involved. Given the high virulence of this genus, other entomopathogenic fungal genera such as *Metarhizium*, *Lecanicillium*, *Isaria* and *Nomuraea* need to be considered as possible fungal pathogens to mealworms.

Consideration should be given to the different virulence levels between the spray and ingestion methods. In the spray (Fig. 3), the low virulence might be due to the dry conditions of the wheat bran (<5% moisture) used as the culturing diet. Relative humidity is a key factor for high virulence. In routine mealworm rearing, humidity is relatively low because only dried wheat bran, small amounts of vegetables, and small amounts of minerals and vitamins were used. Therefore, contact of virulent conidia on the mealworms maybe insufficient for causing disease; the possibility of fungal infection in dry conditions is quite low.

Relatively high virulence was observed in the ingestion compared to the spray method. Once the conidia entered the insect gut, the outer environment would not influence virulence. In mass culturing, contact of conidia to mealworm cuticle does not usually lead to pathogenesis, which

Table 2 Germination (mean) of *B. bassiana* ERL1575 conidia after exposure to chemical fungicides on SDA/4 at room temperature (about 25°C)

	Germination (%)					Germination (%)			
		Dilution†					Dilution†		
Fungicide	None	SD	SD/2	SD/5	Fungicide	None	SD	SD/2	SD/5
Iminoctadine tris (ace)	98	97	98	96	Prochloraz*	97	11	31	90
Iminoctadine tris (alb)	99	95	98	98	Mandipropamid	98	98	98	97
Fluazinam*	98	3	4	8	Carbendazim*	98	21	87	98
Iprodione	98	97	96	97	Thiophanate-methyl	97	97	98	98
Procymidone	99	95	96	98	Carpropamid	100	94	97	97
Copper hydroxide	98	95	96	97	Fluquinconazole	98	98	98	100
Bordeaux mixture	99	94	94	97	Oxolinic acid	97	97	97	99
Metrafenone	98	95	94	95	Dithianon*	98	5	44	95
Benomyl*	99	8	67	91	Tetraconazole	99	94	94	95
Amisulbrom	100	97	97	99	Tricyclazole	99	96	95	99
Kresoxim-methyl	98	95	97	95	Bitertanol*	98	52	97	99
Trifloxystrobin	99	97	97	97	Hexaconazole	100	97	97	98
Azoxystrobin	97	96	95	96	Triflumizole	100	95	97	98
Orysastrobin	98	98	98	99	Difenoconazole	98	93	94	95
Cyazofamid	97	98	96	94	Fenoxanil	99	95	95	98
Thifluzamide	98	95	94	97	Cyprodinil	97	98	97	99
Pencycuron	99	96	98	98	Fenarimol	98	100	98	100
Oxine-copper*	98	5	18	84	Streptomycin	97	97	97	99
Mancozeb*	100	3	7	29	Polyoxin B	99	95	100	98
Etridiazole	98	95	97	97	Validamycin-A	97	94	96	98

*Chemical treatment showed a significant decrease ($P < 0.001$) in conidial germination with a dose-dependent manner.
†Each of the 40 fungicides was prepared at its standard application rate (SD), SD/2 and SD/5 according to the manufacturer's recommendation (Table 1).

definitely depends on abiotic conditions such as high relative humidity (Vega & Kaya 2012). Additionally, cuticle composition may retard conidial germination. Hydrocarbon and lipids in the cuticles may serve as inhibitors (Smith & Grula 1982). However, at higher humidity, spraying of fungal pathogens may induce strong virulence. So, keeping the environment as dry as possible is one solution for managing insect-pathogenic fungi in mealworm cultures.

ERL1575 showed lower virulence at 35°C than at other temperatures. Outer temperature is a key factor on pathogenicity. Previous research reported that *B. bassiana* did not grow well at 35°C (Ekesi *et al*. 1999); therefore, it is not unusual that low virulence was observed in this research. The larvae survived at this higher temperature. If there were highly thermotolerant insect pathogenic fungi, they would likely be serious pathogens to the mealworms at high temperatures.

Fluazinam and mancozeb showed different inhibition activities against fungal growth. Fluazinam is dinitroaniline fungicide (Anema *et al*. 1992), a multispectral agent that targets fungal respiration and disrupts the energy production system (Gasztonyi & Lyr 1995). It is effective against Oomycota, Ascomycota, Basidiomycota and mitosponic fungi and against both conidial germination and hyphal growth, thereby giving it protective action with a good residual effect. The active ingredient, mancozeb, is a fungicide in the dithiocarbamate subclass of the carbamate group. It is used to control a number of fungal diseases such as anthracnose, pythium blight, leaf spot, downy mildew and rust (Berg 1988; Meister 1992). Mancozeb is a non-systemic fungicide with protective and contact action. Mancozeb acts on multiple sites in fungal cells by disrupting lipid metabolism, respiration and production. It has inhibition activity against fungal conidia, but little control activity against hyphal growth. Given the mode of action, fluazinam and mancozeb inhibit conidial germination and are proven safe for the development of *T. molitor* larvae. Based on the results of this work, fluazinam rather than mancozeb may be recommended to control the pathogen.

We isolated *B. bassiana* M7 from the cadavers of *T. molitor* larvae in this work, which may support our idea that a group of *B. bassiana* could be pathogenic to larvae during mass rearing. Thus, we tested the group of *B. bassiana* ERL isolates, which were previously obtained as described above, and found that ERL1575 was more virulent than the M7 isolate. One reason why we used

Table 3 Inhibition activity of fungicides against hyphal growth of *B. bassiana* ERL1575 on SDA/4 after 10 days incubation at room temperature

	Inhibition zone (mm)†			
		Dilution of fungicide‡		
Fungicide	None	SD	SD/2	SD/5
Fluazinam*	0.0c	5.7a	5.5a	3.7b
Oxine-copper	0.0	0.0	0.0	0.0
Mancozeb*	0.0b	2.1a	0.0b	0.0b
Prochloraz	0.0	0.0	0.0	0.0
Carbendazim	0.0	0.0	0.0	0.0
Dithianon	0.0	0.0	0.0	0.0
Bitertanol	0.0	0.0	0.0	0.0
Benomyl	0.0	0.0	0.0	0.0

*Chemical treatment showed a significant decrease ($P < 0.001$) in the inhibition of hyphal growth with a dose-dependent manner.
†Distance (mean) between growing hyphal tip of *B. bassiana* ERL1575 and fungicide-treated paper disc on SDA/4 after 10 days incubation at room temperature (about 25°C).
‡Each of the 40 fungicides was prepared at its standard application rate (SD), SD/2 and SD/5 according to the manufacture's recommendation (Table 1).
Means with the same lower case letter in each row are not significantly different according to Tukey's HSD test ($P > 0.05$).

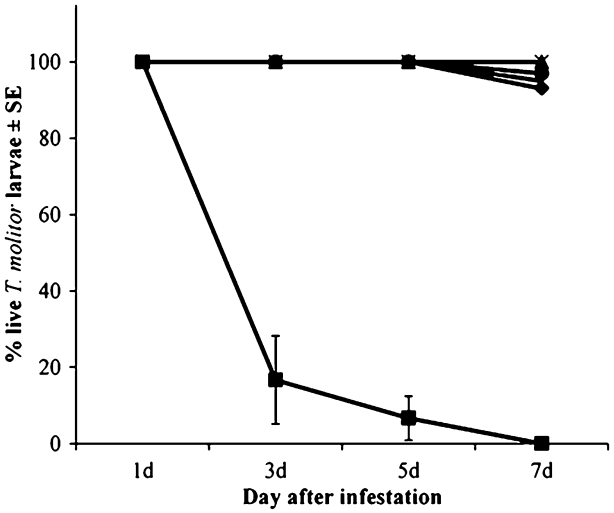

Figure 5 Chemical-based control of *B. bassiana* ERL1575 pathogen in the culturing of *T. molitor*. Fluazinam and mancozeb were diluted and sprayed on the fungus-inoculated wheat bran at 1.05 mL per 60-mm-diameter Petri dish, and in 3 days third instar *T. molitor* larvae were placed in a dish. ◆ Control; ■ ERL1575; ▲ Fluazinam; ✕ Mancozeb; ✱ ERL1575+Fluazinam; ● ERL1575+Mancozeb.

ERL1575 rather than M7 in the fungicide assay was to select more effective fungicides that could work against the group with high virulence.

In conclusion, contamination of wheat bran can be controlled by spraying fluazinam either before or during rearing of *T. molitor* larvae. Our findings can be used in the mass rearing of insects for animal feed. Careful management of the rearing environment and disinfection of the area with fluazinam should be seriously considered.

Acknowledgments

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References

Anand H, Ganguly A, Haldar P (2008) Potential value of acridids as high protein supplement for poultry feed. *International Journal of Poultry Science* **7**: 722–725.

Anema PE, Bouwman JJ, Komyoji T, Suzuki K (1992) Fluazinam: a novel fungicide for use against *Phytophthora infestans* (Cap P) in potatoes. Proceedings of Brighton Crop Protection Conference of Pests and Diseases, Farnham.

Berg GL (1988) *Farm Chemicals Handbook*. Meister Publishing Co., Willoughby.

Bondari K, Sheppard DC (1987) Soldier fly, *Hermetia illucens* L., larvae as feed for channel catfish, *Ictalurus punctatus* (Rafinesque), and blue tilapia, *Oreochromis aureus* (Steindachner). *Aquaculture and Fishery Management* **18**: 209–220.

Butt TM, Goettel MS (2000) Bioassay of entomopathogenic fungi. In: Navon A, Ascher KRS (eds) *Bioassays of Entomopathogenic Microbes and Nematodes*, pp 141–196. CABI Publishing, Wallingford.

Chavunduka DM (1975) Insects as a source of food to the African. *Rhode Island Science News* **9**: 217–220.

Ekesi S, Maniania NK, Ampong-Nyarko K (1999) Effect of temperature on germination, radial growth and virulence of *Metarhizium anisopliae* and *Beauveria bassiana* on *Megalurothrips sjostedti*. *Biocontrol Science and Technology* **9**: 177–185.

Gasztonyi M, Lyr H (1995) Miscellaneous fungicides. In: Lyr H (ed.) *Modern Selective Fungicides*, pp 389–414. Gustav Fischer, Jena.

Ghaly AE, Alkoaik FN (2009) The yellow mealworm as a novel source of protein. *American Journal of Agricultural and Biological Sciences* **4**: 319–331.

Gourlet G, Mullier P, Sinave P, Brisson GJ (1978) Nutritional evaluation of dried *Tenebrio molitor* L. larvae in the rat. *Nutrition Reports International* **18**: 11–15.

Hahn DA, Denlinger DL (2007) Meeting the energetic demands of insect diapause: nutrient storage and utilization. *Journal of Insect Physiology* **53**: 760–773.

Huis A (2013) Potential of insects as food and feed in assuring food Security. *Annual Review of Entomology* **58**: 563–583.

Humber RA (1997) Fungi: preservation of cultures. In: Lacey LA (ed.) *Manual of Techniques in Insect Pathology*, pp 269–279. Academic Press, San Diego.

Kim JS, Choi JY, Lee SJ *et al*. (2013) Transformation of *Beauveria bassiana* to produce EGFP in *Tenebrio molitor* for use as animal feed additives. *FEMS Microbiology Letters* **344**: 173–178.

Meister RT (1992) *Farm Chemicals Handbook*. Meister Publishing Co., Willoughby.

Newton L, Sheppard C, Watson DW, Burtle G, Dove R (2005) *Using the Black Soldier Fly,* Hermetia Illucens*, as a Value-Added Tool for the Management of Swine Manure*. North Carolina State University, Raleigh.

Ng WK, Liew FL, Ang LP, Won KW (2001) Potential of mealworm (*Tenebrio molitor*) as an alternative protein source in practical diets for African catfish, *Clarias gariepinus*. *Aquaculture Research* **32**: 273–280.

Njidda AA, Isidahomen CE (2010) Haematology, blood chemistry and carcass characteristics of growing rabbits fed grasshopper meal as a substitute for fish meal. *Pakistan Veterinary Journal* **30**: 7–12.

Pedrini N, Crespo R, Juárez MP (2007) Biochemistry of insect epicuticle degradation by entomopathogenic fungi. *Comparative Biochemistry and Physiology Part C* **146**: 124–137.

Ramos-Elorduy J, Gonzales EA, Hernandez AR, Pino JM (2002) Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. *Journal of Economic Entomology* **95**: 214–220.

Sheppard DC, Newton GL, Thompson SA, Savage S (1994) A value added manure management system using the black soldier fly. *Bioresource Technology* **50**: 275–279.

Smith RJ, Grula EA (1982) Toxic components on the larval surface of the corn earworm (*Heliothis zea*) and their effects on germination and growth of *Beauveria bassiana*. *Journal of Invertebrate Pathology* **37**: 222–230.

St-Hilaire S, Sheppard C, Tomberlin JK *et al*. (2007) Fly prepupae as a feedstuff for rainbow trout (*Oncorhynchus mykiss*). *Journal of the World Aquaculture Society* **38**: 59–67.

Vega FE, Kaya HK (2012) Fungal entomopathogens. In: Vega FE, Kaya HK (eds) *Insect Pathology*, pp 171–220. Academic Press, Wallingford.