



Laboratory and field efficacy of entomopathogenic fungi for the management of the sweetpotato weevil, *Cylas formicarius* (Coleoptera: Brentidae)



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ABSTRACT

The sweetpotato weevil, *Cylas formicarius* (F.) (Coleoptera: Brentidae), is one of the most important pests of sweet potatoes in the world. With free trade between the United States and the U.S.-controlled Mariana Islands, *C. formicarius* has spread along with this commodity. Because of the cryptic nature of the larvae and nocturnal activity of the adults, and the cancellation of long-residual pesticides, this pest has become increasingly difficult to control. Therefore, the present study sought to explore and to compare the effectiveness of *Metarhizium brunneum* F52 (90 ml a.i./ha), *Beauveria bassiana* GHA (40 ml a.i./ha), spinosad (90 g a.i./ha), azadirachtin (1484 ml a.i./ha), *B. bassiana* + *M. brunneum* (20 ml a.i./ha + 45 ml a.i./ha), *B. bassiana* + azadirachtin (20 ml a.i./ha + 742 ml a.i./ha), *B. bassiana* + spinosad (20 ml a.i./ha + 45 ml a.i./ha), *M. brunneum* + azadirachtin (45 ml a.i./ha + 742 ml a.i./ha) and *M. brunneum* + spinosad (45 ml a.i./ha + 45 grams a.i./ha) in controlling this pest in both the laboratory and the field. The treatment with *B. bassiana* + *M. brunneum* was the most effective in reducing tuber damage by *C. formicarius*, producing the highest yields. The most adult cadavers were found in plots treated with the combination of two fungi. This combined fungal formulation appears to be appropriate for the practical control of *C. formicarius* on sweet potatoes.

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1. Introduction

The sweetpotato weevil, *Cylas formicarius* (F.) (Coleoptera: Brentidae), is the most destructive insect affecting tropical and subtropical production of sweet potato (*Ipomoea batatas* (L.) Lam., Convolvulaceae) (Chalfant et al., 1990), attacking sweet potatoes both in the field and in storage (Sherman and Tamashiro, 1954). The production of terpene in the stored roots in response to tunneling by *C. formicarius* larvae imparts a bad odor, a bitter taste and leaves the sweet potatoes ranging from unpalatable to inedible (Ray and Ravi, 2005; Uritani et al., 1975). The infestation normally spreads from old sweet potato gardens, through the cuttings used for planting (Sutherland, 1986). The weevil population is greatest at the start of the dry season as high temperatures crack the surface of the soil, thereby exposing the tubers (Talekar, 1982). Larvae generally cannot move through the soil but can

easily enter into the soil cracks to reach the tubers (Cockerham et al., 1954).

In addition to *I. batatas*, the major host plant of *C. formicarius* (Chalfant et al., 1990), at least 49 other members of the Convolvulaceae have been recorded as hosts for *C. formicarius*, which has been recorded feeding on seven genera in six tribes within this plant family (Austin et al., 1991). In Guam and other Micronesian Islands, the Aiea Morning Glory, *Ipomoea triloba* L. (Convolvulaceae), is widespread and serves as an alternative host for *C. formicarius* (Reddy et al., 2012b). Because of the cryptic nature of the larvae and the nocturnal activity of the *C. formicarius* adults, it is becoming difficult to control this pest using chemicals. Additionally, the life history of *C. formicarius* make the pest easiest to control with long residual pesticides that are now out of favor and often unavailable. Recently, Leng and Reddy (2012) reported several low-risk insecticides such as spinosad and azadirachtin to be effective against *C. formicarius* in a laboratory study, but their effectiveness was not tested in the field. Our previous studies dealing with pheromone-baited traps have also shown promise for monitoring this pest (Reddy et al., 2012a), and mass trapping techniques

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have been shown to reduce damage caused by *C. formicarius* (Reddy et al., 2014). Sweet potatoes are mainly grown on the island of Rota and exported to other neighboring islands. Since there are no quarantine restrictions to the movements of sweet potatoes among the Mariana Islands (Guam and Northern Mariana Islands of Saipan, Rota and Saipan), *C. formicarius* is spreading to new areas.

The larvae and adults of *C. formicarius* are susceptible to many natural enemies such as parasitoids, predators, and pathogens (Jansson, 1991). In particular, the fungal pathogens *Beauveria bassiana* and *Metarhizium brunneum* (a taxon in the *Metarhizium anisopliae* species complex) (Ascomycota: Hypocreales) have commonly been observed to attack *C. formicarius* (Jansson, 1991) and other *Cylas* species (Ondiaka et al., 2008). Entomopathogenic fungi such as those from the *M. anisopliae* and *B. bassiana* species complexes are currently being used to control agricultural and forest pests worldwide (Butt et al., 2001). These fungi are registered in the USA, as well as in many other countries, as biopesticides (Kabaluk et al., 2010). Such microbial biopesticides are sustainable in IPM programs because of their active relationship with insects. In some cases, compatible products may be combined with entomopathogenic fungi to increase control, to decrease the amount of insecticides required, and to minimize the risks of environmental pollution and pest resistance (Quintela and McCoy, 1998). Nonetheless, the efficacy of some fungi as a biological control agents can be reduced by unfavorable temperature and humidity (Yasuda et al., 1997). However, the hot and humid conditions of sweet potato fields in Guam and other Micronesian Islands are favorable for the use of *B. bassiana* and *M. anisopliae*.

In this study, various fungal entomopathogens were tested individually and in combination along with several effective, low risk insecticides such as azadirachtin and spinosad (Leng and Reddy, 2012), for their laboratory and field efficacy against *C. formicarius*.

2. Materials and methods

2.1. Insect rearing

Pheromone lures consisting of rubber septa loaded with Z3-dodecenyyl-E2-butenate, sealed in an impermeable bag for shipping and storage, were obtained from Chem Tica Internacional S.A. (San José, Costa Rica). Pherocon unitraps (Trécé Incorporated, Adair, Oklahoma, USA) baited with these lures were used to trap adult *C. formicarius* in sweet potato fields in Latte Heights (Guam, USA) during 2010. The trapped adults were taken to the laboratory, placed in batches in collapsible cages (12 × 10 × 10 cm), fed leaves and pieces of the sweet potato, and maintained at 22 ± 2 °C, 70–80% relative humidity and a 16:8 h L:D photoperiod. Approximately 5–6 generations were completed before using the offspring for experiments. For all experiments, 3–4 week old adults were obtained from these laboratory colonies (Gadi and Reddy, 2014).

2.2. Fungi and other chemicals

Conidia of *B. bassiana* strain GHA were supplied as an unformulated technical grade powder by Laverlam International (Butte, Montana, USA). The conidial titer was 1.6×10^{11} conidia/g and viability was 98%, based on conidial germination in the laboratory on potato dextrose yeast extract agar after incubation for 18 h at 27 °C. Cultures of *M. brunneum* F52 (a commercialized isolate previously identified as *M. anisopliae*) were obtained from Novozymes Biologicals Inc. (Salem, Virginia, USA). Conidial powders were stored dry at 4–5 °C until formulation and use. The chemicals used in the present study – azadirachtin (Aza-Direct) and spinosad – were obtained as shown in Table 1.

2.3. Laboratory tests

Laboratory tests were carried out from 12 September to 15 October 2013 with the hypothesis that the chemicals we tested, when topically applied, would exhibit contact toxicity to *C. formicarius* adults (Table 1). For each replicate, 10 adults were transferred to a disk of Whatman No. 1 filter paper (9 cm diam, Whatman® quantitative filter paper, ashless, Sigma–Aldrich, St. Louis, Missouri, USA) in a 9 cm disposable Petri dish.

Each dish received a 10-g piece of sweet potato and two 7 cm sweet potato branches with leaves (4–8) as food for the insects. Five replicate (prepared at separate times using different cultures and batches of insects) Petri dishes of 10 adults were sprayed (Household Sprayer, Do It Best Corp., Ft. Wayne, Indiana, USA) with 0.5 mL of its assigned treatment (Leng and Reddy, 2012). Two control treatments were maintained; in one, the dishes were sprayed with 0.5 mL of tap water, and in the other, no treatment was applied. Following applications, dishes were maintained under laboratory conditions (previously described), and adult mortality was assessed at 24, 48, 72–96, 120–144, and 168–192 h after treatment.

2.4. Field experiments

Identical trials were conducted at the University of Guam Agricultural Experiment Stations at Yigo (N13°31.930' E144°52.351') in northern Guam and at the Inarajan Experiment Station (N13°61.963' E144°45.353') in southern Guam from October 01, 2013 to January 30, 2014.

2.4.1. Plot design and treatment procedures

Treatment plots measuring 6 × 6 m were arranged in a randomized block design and separated from other plots by 1 m buffer zones to prevent any treatment effect. Sweet potato cuttings of the variety IB 195 (Kuma 2) known to be highly susceptible to *C. formicarius* damage (Nandawani and Tudela, 2010) were transplanted into rows 80 cm apart with 30 cm between plants within each row. Each treatment was replicated three times, for a total of 33 individual plots. Each plot consisted of 12 rows of 15 sweet

Table 1
Material and rate of application in each treatment. Spray volume 94 L/ha.

Treatment	Material	Rate (active ingredient)	Source
C	Control (water spray)	–	–
T1	<i>Metarhizium brunneum</i> F52 emulsifiable concentrate (Met 52 EC)	90 ml/ha	Novozymes Biologicals (Salem, VA).
T2	<i>Beauveria bassiana</i> GHA emulsifiable concentrate (BotaniGard ES)	40 ml/ha	Laverlam International Corporation, Butte, MT
T3	spinosad (Conserve SC®)	90 g/ha	Dow Agro Science LLC, Indianapolis, IN
T4	azadirachtin/Aza-Direct®	1484 ml/ha	Gowan Company, Yuma, AZ
T5	<i>B. bassiana</i> + <i>M. brunneum</i>	20 ml/ha + 45 ml /ha	As stated above
T6	<i>B. bassiana</i> + azadirachtin	20 ml/ha + 742 ml/ha	As stated above
T7	<i>B. bassiana</i> + spinosad	20 ml/ha + 45 g/ha	As stated above
T8	<i>M. brunneum</i> + azadirachtin	45 ml/ha + 742 ml/ha	As stated above
T9	<i>M. brunneum</i> + spinosad	45 ml/ha + 45 g/ha	As stated above

potato plantings, for a total of 180 plants per plot. Fertilizer in the form of N, P, K, and S was applied at the actual time of planting according to published recommendations (Nandawani and Tudela, 2010).

Since plants require thirty days to form tubers, at which time *C. formicarius* infestation starts, the first treatment applications (Table 1) were made on October 1, 2013.

2.4.2. Observation

A pretreatment count of *C. formicarius* damage was taken on September 30, 2013, and subsequent counts were made on October 14, November 4 and 18, and December 02 and 16. The damage to roots (tubers) in each plot was evaluated by randomly selecting eight roots from each treatment plot and counting the number of feeding holes. The yield of sweet potato as measured by tuber weight was recorded for each plot. Damage levels and yields from the treatment and control plots were compared, relative to controls, to evaluate the effectiveness of entomopathogens and low risk insecticides in reducing damage from *C. formicarius*.

2.4.3. Number of cadavers

Adult weevils were collected from each plot in randomly selected 1 m² quadrats (Reddy, 2011) searched the surface of the ground at the same time intervals as mentioned above. Sampled insects were then incubated in the laboratory for up to two weeks and observed for mortality. Any adults failing to move when probed with a dissecting needle were recorded as dead and removed from the boxes. These dead adults were surface-sterilized and incubated separately in Petri dishes containing moist filter paper. The cadavers were inspected for the presence of fungal mycelium (mycosis) after 7–14 days.

2.5. Statistical analyses

All mortality in each treatment was transformed to adjusted mortality (AM) according to the control (water spray). The AM was calculated as the following equation:

$$AM = \frac{Mortality_{treatment} - Mortality_{control}}{1 - Mortality_{control}}$$

where $Mortality_{treatment}$ was the mortality of adult (*C. formicarius*) in each treatment while $Mortality_{control}$ was the mortality of adults in the control treatment.

The data of AM were log-transformed to meet the normal distribution requirement, with homogeneous variance among different treatments. Then, repeated measures ANOVA was used to examine the effects of different treatments (T1: *M. brunneum*, T2: *B. bassiana*, T3: spinosad, T4: azadirachtin, T5: *B. bassiana* + *M. brunneum*, T6: *B. bassiana* + azadirachtin, T7: *B. bassiana* + spinosad, T8: *M. brunneum* + azadirachtin, T9: *M. brunneum* + spinosad) on the adjusted mortality of adult sweetpotato weevils *C. formicarius*.

To estimate degree of damage caused by *C. formicarius* in different treatments, damage reduction rates (DRR) were established. First, holes/tuber was used to indicate the damage degree (DD). Then, the DRR of *C. formicarius* was calculated as the following equation:

$$DRR = \frac{DD_{treatment} - DD_{control}}{1 - DD_{control}}$$

where $DD_{treatment}$ was the holes/tuber caused by *C. formicarius* in each treatment while $DD_{control}$ was the holes/tuber caused by *C. formicarius* in the control treatment (water spray).

Repeated measures ANOVA was also used to examine the effects of different treatments on DRR. In addition, the numbers of cadavers in each plot, evaluated by counting in randomly

selected 1 m² quadrats in each plot, were examined to detect differences at different sampling dates with repeated measures ANOVA. Multiple Comparison method (LSD) was then used to test the differences in yield of different treatments.

All analyses were conducted using SAS version 9.3 (SAS Institute, 2011).

3. Results

3.1. Laboratory tests

Adult mortality tests (Fig. 1, presented as adjusted percentage mortality) found that all treatments caused significant adult mortality compared to the water control treatment ($F_{9,441} = 10.37$, $P = 0.001$; Fig. 1). Spinosad, *B. bassiana* + spinosad, and *M. brunneum* + spinosad each caused 100% mortality at 48 h post-treatment. Azadirachtin, *B. bassiana* + *M. brunneum*, *B. bassiana* + azadirachtin, and *M. brunneum* + azadirachtin caused 100% mortality but not until 72–144 h after the treatment. Treatments with either *M. brunneum* or *B. bassiana* alone required 168–192 h post-treatment to reach 100% mortality.

3.2. Field studies

3.2.1. Effect of treatments on reduction in percent damage

All the biorational and low risk chemical treatments significantly (both Yigo and Inarajan sites; see Fig. 2; Table 2) reduced the level of tuber damage by *C. formicarius*. However, the treatment with *B. bassiana* + *M. brunneum* was significantly superior (Yigo, $F_{8,153} = 8.62$, $P = 0.001$; Inarajan, $F_{8,153} = 15.62$, $P = 0.001$) to all other treatments as it eliminated all damage to sweet potato tubers, something no other treatment achieved.

3.2.2. Number of cadavers recorded in the treatment plots

The treatment with *B. bassiana* + *M. brunneum* produced an average of 42.7 cadavers/m² compared to 0.0 adult cadavers/m² in the control plots. Plots treated with *B. bassiana* or *M. brunneum*, either alone or in combination, produced an average of 0.7–16.7 cadavers/m², which was significantly different (Yigo,

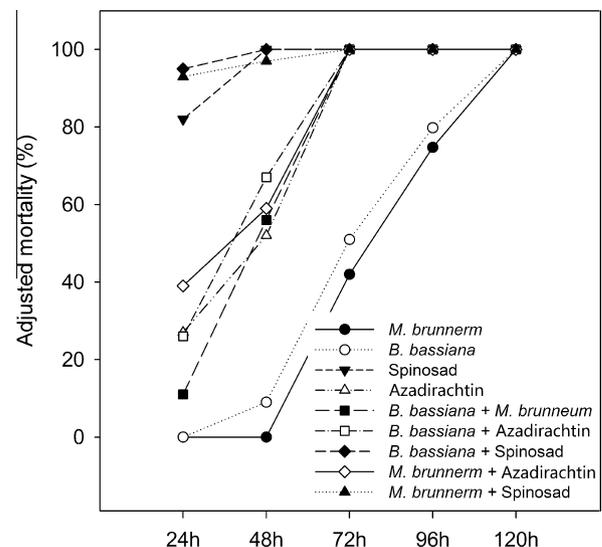


Fig. 1. The effects of nine treatments in a laboratory study (T1: *M. brunneum*, T2: *B. bassiana*, T3: spinosad, T4: azadirachtin, T5: *B. bassiana* + *M. brunneum*, T6: *B. bassiana* + azadirachtin, T7: *B. bassiana* + spinosad, T8: *M. brunneum* + azadirachtin, T9: *M. brunneum* + spinosad) on adjusted mortality of the sweetpotato weevil, *Cylas formicarius* under laboratory conditions.

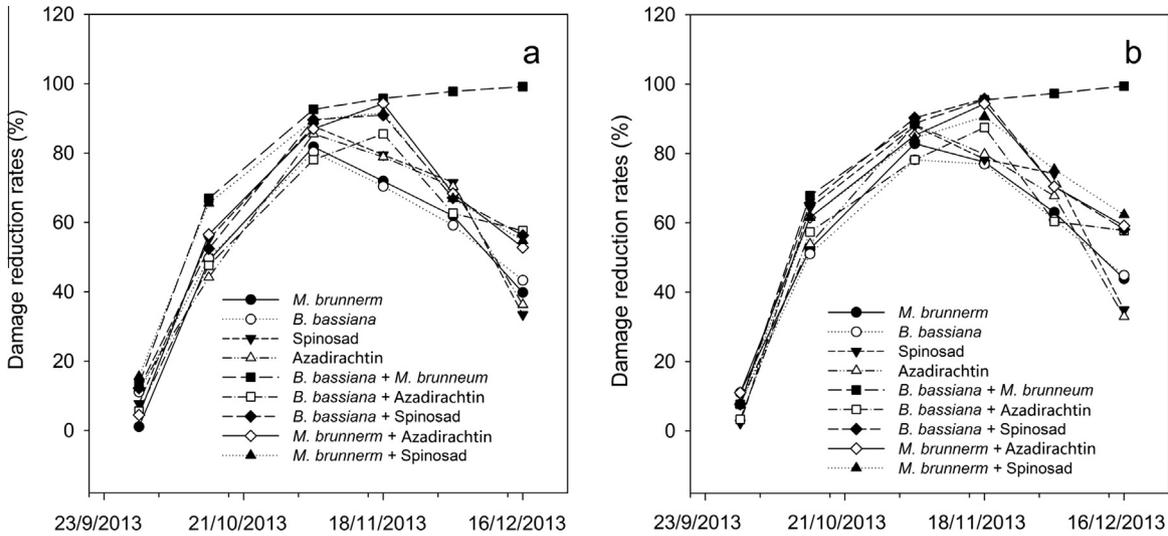


Fig. 2. The effects of nine treatments on damage reduction rates (relative to control damage) as%, of sweetpotato weevil, *Cylas formicarius*, in crop fields in the Mariana Islands (a, Yigo; b, Inarajan) in 2013–2014.

Table 2

Results of repeated measures ANOVA for the effect of different treatments on damage reduction rates, number of cadavers, and yield in trials of biorational pesticides for control of *Cylas formicarius* in the Mariana Islands in 2013–2014.

Variables	Yigo		Inarajan	
	F values	P values	F values	P values
Damage reduction rates	8.62	0.001	15.62	0.001
Number of adult cadavers (1 m ² quadrats in each plot)	16.15	0.001	39.74	0.001
Yield	217.30	0.001	535.56	0.001

$F_{4,85} = 15.07, P = 0.001$; Inarajan, $F_{4,85} = 9.89, P = 0.001$; Fig. 3) from *B. bassiana* + *M. brunnerm*.

3.2.3. Yield levels

All treatments with low-risk insecticides had significantly higher yields than the control treatments (Yigo, $F_{9,20} = 217.30,$

$P = 0.001$; Inarajan, $F_{9,20} = 535.56, P = 0.001$; Fig. 4). However, the treatment with *B. bassiana* + *M. brunnerm* was significantly superior (Yigo, $F_{4,10} = 45.46, P = 0.001$; Inarajan, $F_{4,10} = 164.26, P = 0.001$) to *B. bassiana* + azadirachtin, *B. bassiana* + spinosad, *M. brunnerm* + azadirachtin, or *M. brunnerm* + spinosad. That being said, the yield levels of these combination treatments was significantly higher than for treatments with a single chemical application (Yigo, $F_{5,12} = 66.56, P = 0.001$; Inarajan, $F_{5,12} = 289.00, P = 0.001$).

4. Discussion

Environmentally friendly microbial pesticides can play a significant role in sustainable crop production by providing successful pest management. The current study indicated that the combination of the pathogenic fungi *B. bassiana* + *M. anisopliae* significantly reduced the damage levels and increased the sweet potato yields in comparison to individual applications of single pathogenic fungal species, low-risk insecticides, or the control treatments. We have

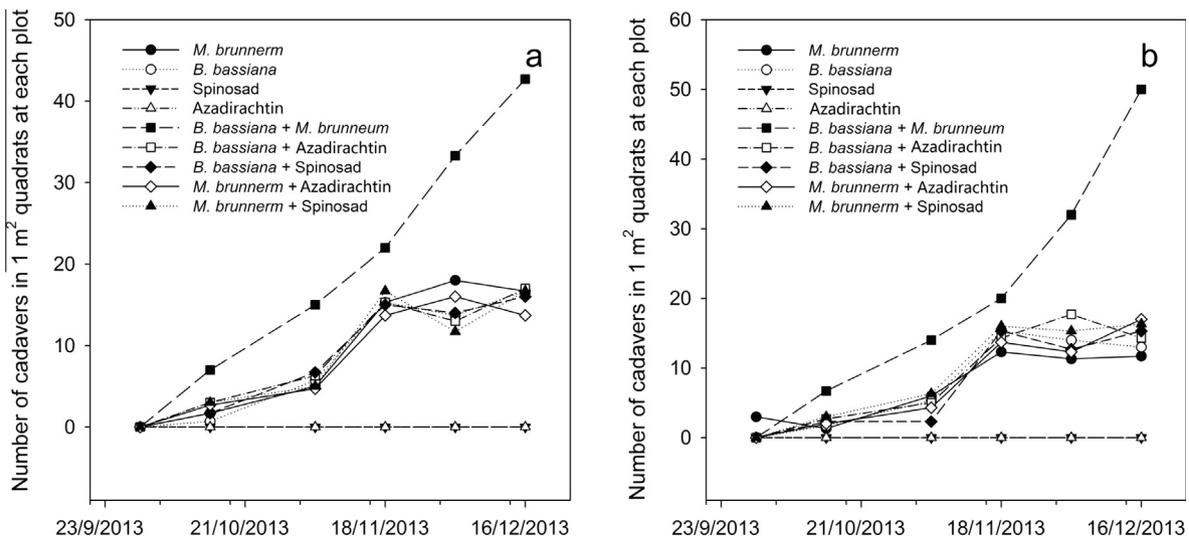


Fig. 3. The effects of nine treatments on number of adult cadavers of sweetpotato weevil, *Cylas formicarius* in 1 m² quadrats in sweet potato fields in the Mariana Islands (a, Yigo; b, Inarajan) in 2013–2014.

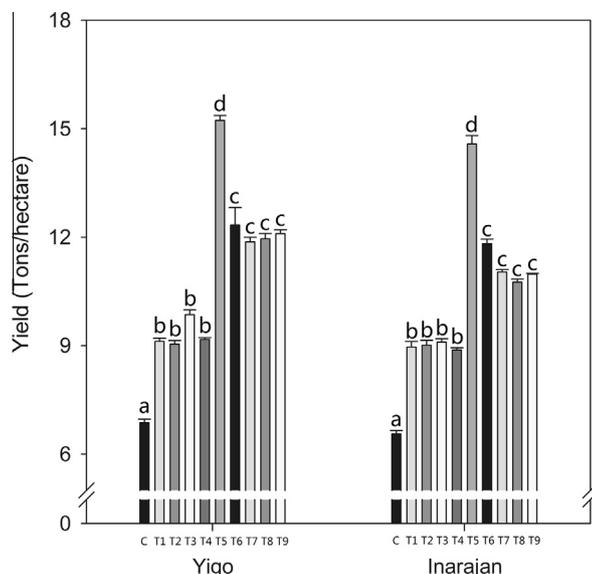


Fig. 4. The effects of nine biorational pesticide or entomopathogen treatments on yield of sweet potatoes in crop fields in the Mariana Islands in 2013–2014, where C = control with water spray, T1: *M. brunneum*, T2: *B. bassiana*, T3: spinosad, T4: azadirachtin, T5: *B. bassiana* + *M. brunneum*, T6: *B. bassiana* + azadirachtin, T7: *B. bassiana* + spinosad, T8: *M. brunneum* + azadirachtin, and T9: *M. brunneum* + spinosad.

demonstrated the additive effect of these two pathogenic fungi on control of *C. formicarius*. The reason for using the combination of the two entomopathogenic fungi at 50% reduced application rates compared to the full rate of individual compounds is that these pathogenic fungi have different optimum temperatures ranges, which could affect conidial germination. Tests with *B. bassiana* and *M. anisopliae* have given promising results for the control of *C. formicarius* in India (Tarafdar and Sarkar, 2006), Kenya (Ondiaka et al., 2008), Taiwan (Su et al., 1988), and the Philippines (Burdeos and Villacarlos, 1989). While adult weevils are the only noticeable stage, infected adults can transmit infections to other individuals in the field. This study clearly found that the number of cadavers of adults in the field increased after the application of entomopathogenic fungi.

The field efficacy of entomopathogenic fungi toward various pests depends on many factors, some of which are related to the behavior of the insect host in its natural habitat (Gindin et al., 2006). As soil is the natural habitat of these fungi, and since larvae and pupae dwell in the soil, it can be inferred from this study that the applied fungal formulations caused the observed infection. Although the adults feed on plant foliage, they can be seen crawling on the soil where it is possible that they become contaminated by the fungal spores.

Conidial survival is known to be affected by agrochemicals, environmental factors (Benz, 1987) or by bio-pesticides or other chemical products used to protect plants (Anderson and Roberts, 1983). Both *B. bassiana* and *M. anisopliae* applied in combination with azadirachtin or spinosad were less effective than the combination of the two entomopathogens, possibly due to fungicidal effects of the azadirachtin or spinosad. There have been some reports on neem-based products possessing fungicidal properties applied at certain doses, such as a significant inhibitory effect on vegetative growth and conidiogenesis of *B. bassiana* spores caused by the commercial formulation of neem leaves in concentrations of 5% a.i. or greater (Castiglioni et al., 2003). A 1% aqueous neem extract caused significant inhibition of mycelial growth of *B. bassiana* (Castiglioni et al., 2003). Conidiogenesis of *B. bassiana* was

reduced among the highest neem concentrations. Amutha et al. (2010) reported that 3% azadirachtin was slightly harmful to *B. bassiana*. This may explain why azadirachtin plus *B. bassiana* was less effective than the combination of the two species of fungi in our study. Ericsson et al. (2007) reported that the combination of spinosad and *M. anisopliae* caused significantly higher mortality of *Agriotes lineatus* (L.) and *Agriotes obscurus* (Coleoptera: Elateridae) than either treatment alone, suggesting that low levels of a reduced-risk pesticide can be combined with a biological agent to reduce wireworm populations in lieu of traditional pesticide strategies. But in our case, this sort of combination was less effective than combining two entomopathogens.

5. Conclusions

This is the first time that a combination of two entomopathogenic fungi has been tested against *C. formicarius*. This study showed the potential of entomopathogens as an alternative to the currently employed traditional insecticides or two combinations of entomopathogens and biorational chemical insecticides. As an alternative to individual applications of low-risk insecticides, we suggest that *C. formicarius* could be controlled by surface applications of the *M. anisopliae* + *B. bassiana* combination to reduce damage levels and to increase sweet potato yields. Moreover, the potential for using a fungal delivery system through synthetic pheromone-baited traps (Lopes et al., 2014) could be useful in managing the population of insect pests with such cryptic habits as *C. formicarius*.

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