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Effects of UV-B and Solar Radiation on the Efficacy of *Isaria fumosorosea* and *Metarhizium anisopliae* (Deuteromycetes: Hyphomycetes) for Controlling Bagworm, *Pteroma pendula* (Lepidoptera: Psychidae)

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ABSTRACT

One of the impediments to the success of entomopathogenic fungi for controlling insect pests in the field is their sensitivity to solar radiation, UV-B in particular. Their sensitivity to UV, however, can be minimized by adding materials that can block the radiation from reaching the conidia. In this study, *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Isaria fumosorosea* (Wize) isolated from field collected bagworms, *Pteroma pendula* (Joannis) (Lepidoptera: Psychidae) were formulated with UV protectants and tested for their pathogenicity on their original host. Both fungi were infective to the bagworms. The median effective concentrations (EC_{50}) were $2\times10^{5.10}$ and $2\times10^{5.17}$ conidia mL^{-1} for *I. fumosorosea* and *M. anisopliae*, respectively. At concentration of 2×10^{9} conidia mL^{-1} of either *I. fumosorosea* or *M. anisopliae* recorded the lowest LT_{50} values at 5.72 and 5.40 days, respectively. Less than 10% of the conidia germinated after 12 h of exposure to UV-B and solar radiation. When the conidia were formulated as a wettable powder in kaolin, with or without Tinopal LPW, a significant sunlight radiation and UV-B protection was achieved up to 12 h of exposure. More than 80% of the conidia germinated. Tinopal LPW, however, did not significantly improve efficacy of the formulation, although recorded a better conidia protection than those without Tinopal LPW. A field trial using *M. anisopliae* and *I. fumosorosea* conidia without Tinopal LPW achieved 58 and 68% control, respectively, while Dipel®, a *Bacillus thuringiensis* product, exceeded 80% control. Even though both isolates were less effective than Dipel® but these indigenous pathogens could effectively reduced the pest population to less than 50%. They need to be conserved and/or augmented so that bagworms can be suppressed with minimal disruption to the ecological balance.

Key words: Bagworms, *Pteroma pendula*, *Metarhizium anisopliae*, *Isaria fumosorosea*, ultraviolet radiation, UV-B

INTRODUCTION

Pteroma pendula (Joannis) (Lepidoptera: Psychidae), an economically important pest of plantation crops, fruit and landscape trees in Malaysia, is a polyphagous bagworm with a high

record of host plants (Norman *et al.*, 1994). The species also thrives well on an invasive species, *Acacia mangium* Wild. (Cheong *et al.*, 2010a). In Peninsular Malaysia, *P. pendula*, recognized earlier as the second most economically important bagworm (Basri *et al.*, 1998), has now become the most dominant species among other bagworm species such as *Metisa plana* (Walker) and *Mahasena corbetti* Tams on oil palm (Ho. 2002). Lately, recurring outbreaks of this bagworm occurred in oil palm, *Elaeis guineenis* (Jacquin) plantations at Lower Perak, Malaysia (Cheong *et al.*, 2010b). Continuous infestation of bagworms was reported to cause a yield loss of 10.9% in the first year and 30.19% in the subsequent year, amounting to FFB (Fresh Fruit Bunch) yield loss of 20.5% per year. To date trunk injection using highly toxic chemicals like Monocrotophos (Azodrin) or Methamidophos are still the most widely method used to control bagworms in oil palm and coconut plantations as recommended by Wood *et al.* (1974) since several decades ago. In recent years, however, consumers have expressed concern over pesticides and their health and environmental effects. Consumer action groups for a sustainable agriculture, including palm oil production, demand growers to be more stringent in their use of chemical pesticides for pest control. Growers are expected to improve their agronomic operations through good agricultural practices that require an adoption of an Integrated Pest Management (IPM) which essentially involves the use of biological control agents. A number of biological control agents of bagworms such as predators, parasitoids and pathogens have been documented in Malaysia by Sajap and Siburat (1992), Basri *et al.* (1995), Norman *et al.* (1998) and Cheong *et al.* (2010a). Among the entomopathogens, *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Isaria fumosorosea* (Wize) contributed 6.6 and 23.9% of *P. pendula* mortality in the field, respectively (Cheong *et al.*, 2010b). The infection levels, however, were still ineffective to bring the bagworm population below the economic threshold level. Thus, efforts to improve their effectiveness to an economic level, including inundating the agro ecosystem with the entomopathogens, are warranted. The success of such an augmentation program, however, depends upon the inherited characteristics of the target pests; virulence of the pathogens and the environment. One of the environmental factors that influences performance of entomopathogens for controlling insect pests in an open field of tropical regions is their susceptibility to sunlight. Ultraviolet component of sunlight, particularly UV-B with wavelengths from 280 to 320 nm is very damaging to *Beauveria bassiana* (Balsamo) Vuillemin and *M. anisopliae* (Basri *et al.*, 1995; Alves *et al.*, 1998; Braga *et al.*, 2001). Apart UV radiation, temperature also has been shown to influence pathogenicity *of Paecilomyces tenuipes* to Diamond Back Moth (Baksh and Khan, 2012). Nevertheless, *M. anisopliae* formulated in a wettable powder could still maintained its effectiveness on cotton jassid, *Amrasca bigutta bigutta*, in a field trial with temperature ranging from 26-36°C (Maketon *et al.*, 2008). In this study the effects of UV-B and natural sunlight on infectivity of formulated *M. anisopliae* and *I. fumosorosea* on *P. pendula* larvae were evaluated in a laboratory bioassay and in a field trial.

MATERIALS AND METHODS

Bagworm rearing: This study commenced on February 2009 for a duration of one year. Female bagworm pupae were collected from infested *Acacia mangium* trees. These pupae were kept in plastic cups for neonate emergence. The neonates were transferred to a covered plastic cup (3 cm diameter width×4 cm height) containing a fresh *A. mangium* leaf that had been rinsed in 5% sodium hypochlorite and washed in running tap water. The larvae were transferred daily to a new plastic cup containing fresh *A. mangium* leaf. These larvae were used for the subsequent experiments.

Fungal isolation: Bagworms were collected during outbreaks occurring in an oil palm plantation at Hutan Melintang, Perak, Malaysia. The bags were dissected and larvae were removed from the bags. They were examined for fungal infection. Cadavers were surface-sterilized by immersing in 0.5% sodium hypochlorite for 3 sec and rinsed in three changes of sterilized distilled water. Infected specimens were cut with a clean dissecting knife into 10 parts and cultured separately on Potato Dextrose Agar (PDA) plus 0.5% yeast extract in 10 different Petri dishes. The Petri dishes were incubated at 25±2°C, 59±10% RH and examined for fungal sporulation daily.

Conidia production: Conidia of both fungi were produced on cooked rice. The cooked rice, about 250 g, packed in an autoclavable polypropylene plastic bag (18×25 cm), was autoclaved for 20 min at 121°C. The bags were cooled and inoculated with 10 mL of 1×10^7 conidial suspension of each fungus. The bags were kept at 25±2°C in the dark and agitated manually every other day to prevent aggregation of rice grains and to improve aeration. After 14 days, the rice cultures were air-dried at 30°C under a laminar flow for 24 h and sieved through a 300 μm sieve. Harvested conidia, stored in plastic containers, were kept in desiccators with silica gels. The desiccators were kept in a cool incubator at 4 to 5°C.

Preparation of conidial suspensions: About 0.3 g of conidia was placed in a 50 mL test tube. Ten milliliter of distilled sterilized water with 0.05% Tween® 20 was added and mixed thoroughly using a vortex mixture. The concentration of conidia suspension was estimated by using an improved Neubauer Haemocytometer. This suspension was diluted to concentrations of 2×10^9, 2×10^7, 2×10^5, 2×10^3 and 2×10^1 conidia mL^{-1}.

Pathogenicity test: Third instars of *P. pendula* were immersed in 5% sodium hypochlorite for 10 sec and rinsed in three change of sterilized water. The bagworms were dipped in the conidial suspension and were then placed individually in a clean plastic bottle (6.5×10 cm), containing a piece of fresh *A. mangium* leaf of with its stalk submerged in water. The bottles were kept in a room temperature (28±2°C) with photoperiod 12:12 (L:D) hours and 80±10% RH. Mortality of *P. pendula* was monitored daily for 14 days. Fungal infected larvae were removed and cultured again on PDA to confirm the fungus infecting the larvae. The experiment was conducted in a randomized block design in five replicates with ten larvae per treatment per replicate. Mortality rates of treated bagworms were adjusted following the Abbott's formula.

Formulation of conidia: Conidia from *I. fumosorosea* and *M. anisopliae* were formulated in a wettable powder formulation using wheat fiber as wetting agent; while, gum Arabic and kaolin were used as dispersant and diluent, respectively. The proportion of materials used in the formulation was calculated from results of several preliminary tests. Thus a formulation consisting 5% w/w active ingredient, 15% w/w wetting agent, 70% w/w dispersant and 10% w/w diluent was prepared for the experiment. The wheat fiber, gum Arabic and kaolin were weighed and adjusted according to the ratio. They were mixed and blended in a Waring® Blender for 5 min at low speed and 5 min at high speed. Five grams of the conidia and 95 g the mixture were mixed thoroughly in a 250 mL beaker for 10 min. The formulated conidia powder was then kept in a plastic container and placed in a cool incubator at 4 to 5°C before use.

Exposure of formulated conidia to ultraviolet-B (UV-B): Suspensions of *I. fumorososea* and *M. anisopliae* from previously formulated conidia, wettable powder (F), F+10% Tinopal LPW (FTL),

conidia+palm oil (O) and conidia in 0.05% Tween® 20 (TO) as the control, were prepared for the experiment. The oil formulation was prepared according to Moslim *et al.* (2004), with 0.3 g conidia were added into 10 mL palm oil. The suspension was standardized into 1×10^5 conidia mL^{-1}. Approximately 2 mL of conidial suspension was spread over a 9.0 cm glass Petri dish. The Petri dishes containing conidial suspensions were placed into a plastic box with UV-B lamb light (UV-B lamb, 302 nm, 8 w, UVM-18 Ultra-Lum Inc., California) that was set 15 cm above the Petri dishes. The UV-B lamb light source was calibrated using Lutron uv-340® UV light meter. The Petri dishes were exposed to the UV-B for durations of 1, 4, 7, 12 and 24 h. The controls, consisting conidial suspension in 0.05% Tween® 20 covered with aluminum foil and conidial suspension in 0.05% Tween® 20, were not exposed to the UV-B. After exposing to UV-B, the Petri dishes were removed from the box and the volume of the solution was adjusted to 2 mL with sterile distilled water and sealed with parafilm (American National Can™). The Petri dishes were kept in a cool incubator at 4 to 5°C and their germination rate was determined within one day. The experiment was conducted in three replicates per treatment of ten experimental units.

Exposure of formulated conidia to natural sunlight: Similar formulations and treatment units used in the UV-B test were prepared for the sunlight test. The test was conducted in an open space on the roof of the Faculty of Forestry building. Conidial suspension was exposed to bright sunlight on a cloudless day from 1200 to 1500 (GMT+8). After exposing to the sunlight, the Petri dishes were removed and the volume of the solution was adjusted to 20 mL with sterile distilled water and sealed with parafilm (American National Can™). The Petri dishes were kept in a cool incubator at 4-5°C before the germination test. UV intensity, temperature and relative humidity were measured every one hour during the experiment. UV intensity was measured using Pyranometer (Model LI-189, LI-COR Inc, USA). Temperature and relative humidity were recorded using the Thermo, clock and humidity monitor (model 4040, Control Company, USA). The test was conducted in three replicates per treatment of ten experimental units.

Percentage germination of conidia after exposure to UV-B and sunlight: About 0.1 mL of conidial suspension that had been exposed to UV-B and sunlight was evenly spread on PDA in 9.0 cm diameter Petri dishes. The Petri dishes were then sealed with parafilm and incubated at 25±2°C for 24 h. After 24 h, conidial germination rates were estimated. Germinating and non-germinating conidia were counted from approximately 300 conidia in a culture plate. A conidium with a germ tube was considered germinated (Milner *et al.*, 1991). The experiment was conducted with three replicates per treatment.

Efficacy of formulated conidia on *P. pendula* larvae in a laboratory bioassay: Formulations of *I. fumosoroseas* and *M. anisopliae* were prepared separately as formulated wettable powder (F), conidia with palm oil (O) prepared according to Moslim *et al.* (2004) and conidial suspension in 0.05% Tween® 20 (T20). All conidial suspensions were adjusted to 1×10^5 conidia mL^{-1}. Conidia suspended in 0.05% Tween® 20 were the control. First, third and fifth instars *P. pendula* were used as the test insects. The bagworms were surface-sterilized by immersing them in 5% sodium hypochlorite for 10 sec and rinsed in three changes of sterilized water. They were placed on a paper towel in a tray and sprayed using a hand sprayer, Preval® spray gun, with 15 mL of conidial suspension for 10 sec at a distance of 20 cm from the bagworms. Treated larvae were then placed individually in a covered plastic cup (3 cm diameter×4 cm high)

containing a piece of *A. mangium* leaf and maintained at 26±3°C, humidity 45-60% and 12:12 (L:D) photoperiod. The leaves were changed daily and mortality recorded. Dead bagworms were removed from the test units and placed on a moist filter paper in a 9 cm Petri dish. The dishes were incubated at 25±2°C in the dark for pathogen sporulation. The experiment was replicated five times with 10 larvae per treatment per replicate. Mortality rates were corrected following the Abbott's formula.

Efficacy of formulated conidia on *P. pendula* larvae in a field trial: In the absence of accessible and easily monitored bagworm-infested oil palms in the vicinity of University Putra Malaysia campus, a small-scale field trial was conducted on three highly *P. pendula*-infested mango trees, *Mangifera indicia* in the campus orchard. The experiment was conducted in a randomized block design. Each tree, representing a block, was divided arbitrarily around the tree into five sections and marked as replicates. Within each replicate, three branches with at least 30 active larvae per branch were selected. Thus a total of 15 branches were selected from each tree and a treatment was randomly assigned to each branch. The treatments were formulated fungal preparations, *I. fumosorosea* and *M. anisopliae*, *Bacillus thuringiensis* (Dipel®) as comparison and distilled water with Tween® 20 as the control. Each branch was sprayed with 30 mL of conidial suspension at 1×10^5 conidia mL^{-1} of each treatment was sprayed using a hand sprayer. Dipel® was used at the recommended concentration of 2.6 mL 1 per L Treated branches with bagworms were enclosed in bags, 50 cm length×30 cm diameter, of 300 μm netting. One treated branch, randomly selected from each tree, was cut on day 3, 5 and 7 after treatment. In the laboratory, bagworms were dissected, examined for fungal infection and the number of infected bagworms was recorded. The cadavers were placed on a moist filter paper in a Petri dish and kept at 25±2°C in the dark for fungal sporulation. All mortality rates were corrected following the Abbott's formula.

Data analysis: Data from laboratory and field experiments were analyzed using ANOVA and the mean significant differences were determined by Tukey HSD test ($p<0.05$) using Statistical Analysis System (SAS) program (SAS, 2000). EC_{50} and LT_{50} values were calculated using probit analysis (Finny, 1971).

RESULTS

Infection of *I. fumosorosea* and *M. anisopliae* on *P. pendula*: The field isolated entomopathogenic fungi, *M. anisopliae* and *I. fumosorosea* were pathogenic to its host, *P. pendula* (Fig. 1). Bagworms that were treated with 2×10^3-2×10^9 conidia mL^{-1} *I. fumosorosea* achieved 100% mortality between 9 to 14 days after treatment while those with 2×10^1 conidia mL^{-1} treatment only achieved 80% mortality by day 14. *Metarhizium anisopliae* caused 100% mortality on day 13, when the bagworms were treated with 2×10^5 and 2×10^7 conidia mL^{-1}. At 2×10^3 mL and 2×10^1 conidia mL^{-1}, mortality of 97 and 89%, respectively, were achieved on day 14. The LT_{50} values, ranging from 5.72 to 9.68 days, were recorded from *I. fumosorosea* in 2×10^9 mL to 2×10^1 conidia mL^{-1}. All *M. anisopliae* preparations achieved LT_{50} values in less than 9 days, with shortest LT_{50} value of 5.40±0.18 and longest LT_{50} value of 8.13±0.21 days recorded from concentrations of 2×10^9 and 2×10^1, respectively (Table 1). The median effective concentration (EC_{50}) values, $2\times10^{5.10}$ and $2\times10^{5.17}$ conidia mL^{-1} for *M. anisopliae* and *I. fumosorosea*, respectively, were not significantly different from each other.

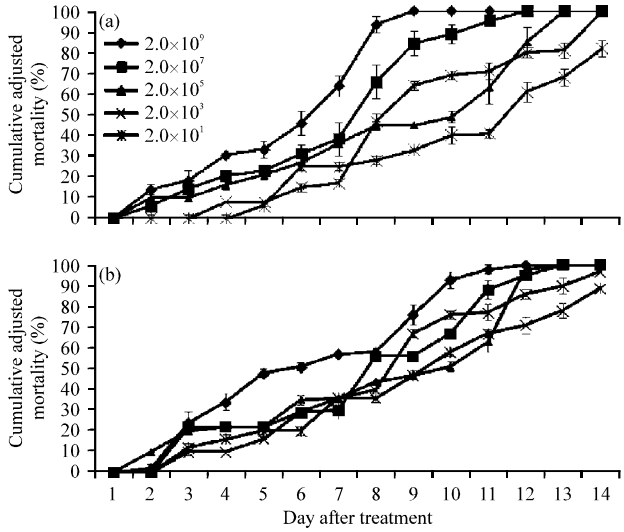

Fig. 1(a-b): Mortality trend of *Pteroma pendula* after being treated with (a) *Isaria fumosorosea* and (b) *Metarhizium anisopliae*

Table 1: Median lethal time (LT_{50}) values of *Pteroma pendula* treated with different concentrations of *Metarhizium anisopliae* and *Isaria fumosorosea*

	M. anisopliae			*I. fumosoroseus*		
		95% CI			95% CI	
Concentration (conidia mL^{-1})	Days (LT_{50}±SE)	Lower	Upper	Days (LT_{50}±SE)	Lower	Upper
2×10^{9}	5.40±0.18	5.20	5.59	5.72±0.24	5.52	5.95
2×10^{7}	7.28±0.18	7.09	7.49	6.74±0.19	6.55	6.94
2×10^{5}	7.55±0.17	7.35	7.76	8.02±0.18	7.82	8.23
2×10^{3}	7.59±0.30	7.44	7.73	8.29±0.38	8.14	8.43
2×10^{1}	8.13±0.21	7.95	8.31	9.68±0.30	9.47	9.88

CI: Confidence interval

Effect of UV-B radiation on germination of *M. anisopliae* conidia: The germination percentages of formulated conidia of *M. anisopliae* were not significantly affected by UV-B within 1 h of exposure, while those in Tween® 20 suspension (T20) had less than 50% germination. After 12 h of exposure, only 3% conidia in Tween® 20 (T20) remained viable while the formulated wettable conidia (F), including in oil (O) and Tinopal LPW (FTL), maintained more than 80% germination, ranging from 79.56% in oil (O) to 94.56.44% in Tinopal LPW (FTL). These germination, however, significantly dropped to less than 4%, ranging from highest effect of 2.33% occurred in oil to 3.89% occurred in formulated wettable conidia (F) (Table 2). In comparison, conidia that were not exposed to UV-B (WUVB) or covered with aluminum foil (Control) maintained 94.78 and 95.67% germination, respectively, of while those in Tween® 20 remained at 3.44% germination.

Effect of UV-B radiation on germination of *I. fumosorosea* conidia: Germination rates of formulated *I. fumosorosea* conidia except those in Tween® 20 were not significantly different after 1 h of exposure to UV-B. The germination of conidia in Tween® 20 dropped to 50% while those

formulated as a wettable powder (F), in oil (O) and Tinopal (FTL) recorded more than 84% with 83.56% in oil (O) and 90.67% in Tinopal LPW (FTL. After 12 h of exposure, the germination in Tween® 20 (T20) dropped to about 40% and those in oil had 77% germination. Conidia in other formulations, in a wettable powder (F) and Tinopal LPW (FTL), maintained more than 80%. After 24 h exposure, only 10% of conidia in all formulations were viable while those in Tween® 20 were non-viable. Conidia that were not exposed to UV-B and those in the control maintained more than 90% germination (Table 3).

Effect of solar radiation on germination of *M. anisopliae* conidia: Germination percentages of *M. anisopliae* in all formulations were not significantly affected when they were exposed to sunlight for 1 h. More than 88% of the conidia germinated. After 4-12 h of exposure, the germination of conidia in Tween® 20 (T20) dropped from 88.22% in the first hour to 6% in the 12 h, while conidia in other formulations still had more than 80% germination, a reduction of about 10% from the first hour. After 24 h, all conidia were non-viable, except those in the controls, covered with an aluminum foil and those that were not exposed to sunlight (WUVB) where more than 93% germinated (Table 4).

Effect solar radiation on germination of *I. fumosorosea* conidia: Germination percentages of *I. fumosorosea* in all formulations were not significantly affected when they were exposed to sunlight for 1 h. The germination exceeded 82%. However, those in Tween® 20 recorded less than 72% while conidia in other formulations maintained germination of more than 80% after 4 to 7 h

Table 2: Mean conidia germination rate of *Metarhizium anisopliae* after exposure to UV-B

	Time (h)				
Treatment*	1	4	7	12	24
T20	46.78±1.98^{bA}	46.33±2.01^{bA}	10.78±3.18^{bB}	3.33±0.51^{cB}	3.44±0.48^{bB}
F	90.44±0.95^{aA}	90.67±2.52^{aA}	90.89±3.35^{aA}	92.00±3.46^{aA}	3.89±1.16^{bB}
FTL	95.33±1.20^{aA}	88.67±5.98^{aA}	91.89±2.70^{aA}	94.56±1.06^{aA}	2.22±0.59^{bB}
O	81.11±8.48^{aA}	83.56±4.30^{aA}	86.33±3.01^{aA}	79.56±3.63^{bA}	2.33±0.88^{bB}
Control	89.33±2.31^{aA}	92.44±3.56^{aA}	87.22±5.46^{aA}	87.45±2.99^{abA}	94.78±0.91^{aA}
WUVB	97.67±0.84^{aA}	95.00±0.19^{aA}	93.89±1.24^{aA}	94.47±0.97^{aA}	95.67±1.02^{aA}

Means with the same letters in the same column (lower case) or in the same row (upper case) are not significant at $p<0.05$, Tukey's test, *T20: Tween®20, F: Formulated wettable powder, FTL: Formulated wettable powder+Tinopal, O: Oil formulation, Control: Covered with aluminium foil, WUVB: Without UVB exposure

Table 3: Mean conidia germination rate of *Isaria fumosorosea* after exposure to UV-B

	Time (h)				
Treatment*	1	4	7	12	24
T20	50.00±3.48^{bA}	44.56±2.54^{cA}	47.11±1.57^{cA}	40.67±3.20^{cA}	0^{cB}
F	84.11±6.78^{aA}	79.67±2.67^{abA}	81.67±6.96^{aA}	83.00±3.37^{abA}	4.11±1.13^{bcB}
FTL	91.89±1.90^{aA}	91.89±3.86^{aA}	89.44±1.93^{abA}	92.56±2.04^{aA}	11.00±1.07^{bB}
O	89.22±2.13^{aA}	68.00±5.48^{bB}	74.89±1.44^{bAB}	77.44±3.27^{bAB}	1.89±0.29^{cC}
Control	92.67±3.24^{aA}	94.44±2.51^{aA}	90.00±5.67^{abA}	86.56±3.23^{abA}	90.44±3.88^{aA}
WUVB	96.22±1.42^{aA}	92.56±1.56^{aA}	98.11±1.06^{aA}	94.77±1.07^{aA}	96.00±1.35^{aA}

Means with the same letters in the same column (lower case) or in the same row (upper case) are not significant at $p<0.05$, Tukey's test, *T20: Tween®20, F: Formulated wettable powder, FTL: Formulated wettable powder +Tinopal, O: Oil formulation, Control: Covered with aluminium foil, WUVB: Without UVB exposure

of exposure. After 12 h, the germination of conidia in Tween® 20 (TO) and oil (O) dropped 20.33% and 30.33%, respectively, a reduction of 70 to 50% from the first hour of exposure. Other formulations maintained germination of more than 85%, with wettable powder with Tinopal LPW (FTL) maintained 91.89% germination. After 24 h of exposure to sunlight, conidia in all formulations were non-viable. Those conidia covered with an aluminum foil and unexposed conidia (WUVB) maintained more than 90% germination (Table 5).

UV intensity, temperature and relative humidity during solar radiation test: The UV intensities, temperatures and Relative Humidity (RH) recorded during the experiment are shown in Table 6. The recorded mean UV intensity ranged from 509.33 to 605.05 w m^{-2}, the mean temperature ranged from 35.75 to 51.75°C and the mean RH ranged 34.45 to 41.95%. These values varied with exposure duration.

Efficacy of formulated conidia for controlling *P. pendula* in laboratory: The efficacy of formulated *M. anisopliae* and *I. fumosorosea* at 1×10^5 conidia mL^{-1} on *P. pendula* larvae was compared. Formulation with added Tinopal LPW (FTL) was not included in this test because its performance was not significantly different from that of formulation without Tinopal LPW (F). In general, both fungi formulated as a wettable powder without Tinopal LPW were more effective than those in oil and also early instars were more susceptible to fungal infection than

Table 4: Mean conidia germination rate of *Metarhizium anisopliae* after exposure to sunlight

	Time (h)				
Treatment*	1	4	7	12	24
T20	88.22±5.38^{aA}	54.11±2.21^{bB}	23.78±2.23^{bC}	6.11±1.18^{cD}	0^{bD}
F	95.45±1.28^{aA}	90.78±2.12^{aA}	85.44±3.09^{aA}	85.33±4.22^{abA}	0^{bB}
FTL	93.22±0.48^{aA}	85.45±1.46^{aB}	88.67±2.91^{aAB}	84.78±1.46^{abB}	0^{bC}
O	88.67±1.68^{aA}	84.11±2.33^{aA}	82.33±2.46^{aA}	81.33±2.19^{bA}	0^{bB}
Control	88.89±1.98^{aA}	86.89±5.34^{aA}	90.56±5.13^{aA}	89.89±4.08^{abA}	93.11±1.94^{aA}
WUVB	97.67±0.84^{aA}	95.00±0.19^{aA}	93.87±1.24^{aA}	94.47±0.97^{aA}	95.67±1.02^{aA}

Means with the same letters in the same column (lower case) or in the same row (upper case) are not significant at $p<0.05$, Tukey's test, *T20: Tween®20, F: Formulated wettable powder, FTL: Formulated wettable powder+Tinopal, O: Oil formulation, Control: Covered with aluminium foil, WUVB: Without UVB exposure

Table 5: Mean conidia germination rate of *Isaria fumosorosea* after exposure to sunlight

	Time (h)				
Treatment*	1	4	7	12	24
T20	92.33±2.60^{aA}	71.33±4.53^{bB}	70.11±4.65^{cB}	20.22±3.93^{bC}	0^{bD}
F	88.22±2.48^{aA}	92.45±2.79^{aA}	88.67±1.68^{abA}	86.56±1.74^{aA}	0^{bB}
FTL	89.22±7.79^{aA}	95.56±0.78^{aA}	93.89±0.80^{abA}	91.89±1.54^{aA}	0^{bB}
O	82.67±1.17^{aA}	89.22±3.46^{aA}	85.67±2.99^{bA}	30.33±2.84^{bB}	0^{bC}
Control	84.00±5.16^{aA}	83.00±3.29^{abA}	94.78±2.11^{abA}	89.11±3.77^{aA}	90.11±4.38^{aA}
WUVB	96.22±1.42^{aA}	92.56±1.56^{aA}	98.11±1.06^{aA}	94.77±1.07^{aA}	96.00±1.35^{aA}

Means with the same letters in the same column (lower case) or in the same row (upper case) are not significant at $p<0.05$, Tukey's test, *T20: Tween®20, F: Formulated wettable powder, FTL: Formulated wettable powder+Tinopal, O: Oil formulation, Control: Covered with aluminium foil, WUVB: Without UVB exposure

later instars. Mortality rates ranging from 70.0% on the fifth to 80% on the first instars were achieved from infection of *I. fumosorosea* conidia in the wettable powder while conidia in oil caused 66% mortality on the first and 40% on the fifth instars. Mortality rates of different instars treated with *M. anisopliae* in a wettable powder were significant, with first instars recorded 92% as compared to less than 52% mortality on fifth instars. *Metarhizium anisopliae* in oil (O) recorded mortality rates ranging from 80% on the first to less than 28% on the fifth instars (Table 7).

Efficacy of formulated conidia for controlling *P. pendula* in the field: The relative efficacies of fungal and Dipel® spraying for controlling *P. pendula* in the field is shown in Table 8. All the three different formulations caused significant mortalities on day 3 and 7 after Days after Treatment (DAT). On 3 DAT, Dipel® recorded more than 52% mortality while *M. anisopliae* and *I. fumosorosea* recorded 42 and 44% mortality, respectively. On 5 DAT, *I. fumosorosea* and Dipel® treatments recorded more than 50% while *M. anisopliae* caused 43% mortality. The mortality rates increased by more than 15% on 7 DAT, with 58 and 68% occurred on *M. anisopliae* and *I. fumosorosea* treated branches, respectively, while bagworms on Dipel®-treated branches exceeded 80% mortality.

Table 6: UV intensity, temperature and relative humidity during sunlight exposure periods

Sunlight exposure period (h)	UV intensity* (w m^{-2})*(Mean±SE)	Temperature** (°C)**(Mean±SE)	Relative humidity*** (%)***(Mean±SE)
1	509.33±4.16	53.50±0.65	32.68±0.22
4	605.05±4.79	51.00±0.82	34.50±0.39
7	599.03±6.18	48.75±0.48	36.93±0.18
12	606.75±0.62	51.75±2.50	33.25±0.75
24	572.63±5.62	35.75±0.25	41.95±0.05
24 h (Shade)	572.63±5.62	35.75±0.25	41.95±0.05

*UV intensity: mean: 578.56±8.63 (SE) (w m^{-2}), K-S distance: 0.24, p = 0.01, mean of four replicates, **Temperature: mean: 35.86±0.79 (SE)°C, K-S distance: 0.16, p = 0.15, mean of four replicates, ***Relative humidity: mean: 48.15±1.55 (SE)%, K-S distance: 0.24, p = 0.01, mean of four replicates

Table 7: Adjusted mean percent mortality of *Pteroma pendula* treated with oil and wettable powder formulations of *Metarhizium anisopliae* and *Isaria fumosorosea*

	M. anisopliae		*I. fumosorosea*	
Instar	Wettable powder	Oil	Wettable powder	Oil
1st	92.00±4.900^{aA}	80.00±4.470^{aA}	80.00±8.94^{aA}	66.00±9.80^{aA}
3rd	64.00±10.30^{aAB}	58.00±13.57^{aAB}	76.00±8.12^{aA}	62.00±12.93^{aA}
5th	52.00±10.20^{abB}	28.00±11.58^{bB}	70.00±6.32^{aA}	40.00±5.48^{abA}

Means with the same letter in the same row (lower case) or the same column (upper case) are not significance at p<0.05, Tukey's test

Table 8: Adjusted mean percent mortality of *Pteroma pendula* treated with *Isaria fumosorosea*, *Metarhizium anisopliae* and Dipel® in a field trial

Days after treatment	*I. fumosorosea*	*M. anisopliae*	Dipel®
3	44.45±2.74^{abB}	42.31±0.91^{bA}	52.47±2.51^{aB}
5	51.98±4.45^{aAB}	43.87±5.23^{aA}	51.44±8.34^{aB}
7	68.30±7.03^{bA}	57.83±8.52^{bA}	80.86±5.30^{aA}

Means with the same letter in the same row (lower case) and the same column (upper case) are not significance at p<0.05, Tukey's test

DISCUSSION

Even though, both field isolated entomopathogenic fungi, *M. anisopliae* and *I. fumosorosea* were pathogenic to its host, *P. pendula,* their virulence varied with the species. This study shows that *I. fumosorosea* was more virulent on *P. pendula* larvae, although not significantly different than *M. anisopliae* in the laboratory bioassay. Since both fungi were isolated from the same host species, the differences in their virulence may be due to their inherent characteristics of the species. *Isaraia fumosorosea* was more prevalent than that of *M. anisopliae* in the bagworm population in the field (Cheong *et al*., 2010a). Like other microorganisms, these fungi were sensitive to sunlight and UV-B and deleteriously affected upon prolonged exposure to sunlight and UV-B component of sunlight (Alves *et al*., 1998; Braga *et al*., 2001, 2002; Fernandes *et al*., 2007). The detrimental effect was detected when unprotected conidia of both *M. anisopliae* and *I. fumosorosea* were exposed to UV-B and natural sunlight. Their germination rates dropped significantly to less than 50% within 1 h of exposure and declined further with an increasing exposure time. Although, both fungi were significantly affected by UV-B and sunlight, *I. fumosorosea* was relatively more tolerant to UV-B than *M. anisopliae*. This factor together with its tolerance to higher temperature (Vidal *et al*., 1997) could possibly contribute to its prevalence on arboreal insects, such as bagworms in the field (Cheong *et al*., 2010a). Even though *I. fumosorosea*, to a certain extent, was able to withstand environmental stresses from high temperature and sunlight, the conidia still require protection if they were to be sprayed in an open field. The addition of wheat fiber, gum Arabic and kaolin in the formulation did improve the tolerance of the conidia to detrimental effects of UV-B and natural sunlight. Both fungi formulated as a wettable powder with or without Tinopal LPW maintained more than 80% conidia viability even after they had been exposed to sunlight or UV-B for 12 h. Although Tinopal LPW, a good sunlight protectant that has been used in viral and fungal-based biopesticides (Shapiro and Robertson, 1992; Basri *et al*., 1995), generally provided a better conidia protection but the improvement was not significantly different from those without Tinopal LPW. A similar situation was reported where *Spodoptera litura* nucleopolyhedrovirus (SpltNPV) formulated with Tinopal LPW did not significantly enhance efficacy of the virus against early instars of *S. litura* in the field (Sajap *et al*., 2009). There is a possibility that interactions between components, wheat fiber, gum Arabic and kaolin, may have protected the conidia as well as Tinopal LPW. This result corroborates with a report by Basri *et al*. (1995). Kaolin or clay, a common inert ingredient and diluent has also been shown to provide good sunlight and UV light protectant in viral (Shapiro and Shepard, 2007) and fungal-based biopesticide formulations (Studdert *et al*., 1990). With possible interference of optical brighteners on insect pollinators particularly bees (Goulson *et al*., 2000) and on plant growth (Goulson *et al*., 2003), clays serve a better alternative to Tinopal LPW as UV protectants. Clays offer UV protection through their UV-visible light scattering property which attenuates the intensity of the irradiation (Cohen *et al*., 2003) thereby reduce its impact on DNA of the affected microbes. Both wettable powder formulations of *I. fumosorosea* and *M. anisopliae* conidia could effectively killed the bagworms regardless their stages under laboratory conditions. However, they were more effective in killing early than later instar larvae. A similar situation was also observed by Sajap *et al*. (2009) where first instars were very susceptible to *Paecilomyces* infection. Even though both fungi could significantly reduced field population of *P. pedulla* larvae to more than 57%, one week after spraying, they were less effective than Dipel®. Unlike, *B. thuringiensis* as in Dipel® which requires ingestion to cause an infection in the bagworms, fungi require germination of conidia on the cuticle and then penetration into the haemocoel. Some factors may have influenced these initial stages of

infection. Apart from the limiting environmental factors, one inherent factor is the presence of a protective silken bag surrounding their body. These protective bags of neatly woven silk, covered externally with plant materials, could have prevented direct contact of the conidia with the insect cuticle. The bags as shown by Reddy and Yang (2010) in *Theriodopteryx ephemeraeformis* (Haworth) consist of silk fibers with uniformly distributed acidic and basic groups of amino acids that are rather hydrophilic in nature. This though fabric-like of the inside surface of the bag, in addition to its chemistry of the silk fibers, could have delayed or hindered germinating hyphae from reaching the cuticle in a short time. Most of the conidia possibly remained on the bags rather than germinating on the host cuticle. The effectiveness of these fungi may be improved by applying a higher conidia concentration as suggested by Jaronski (2009) so as to provide sufficient number of conidia for infection to occur on the bagworms in addition to improving their stability in the environment. Spraying may be performed in the evening so as to avoid intense solar irradiation of the day and to increase the humid conditions directly after spraying. In one study, fungal infections were found to be positively correlated with rainfall and about 90% of the infected *P. pendula* were located in middle or bottom of *A. mangium* canopies where the relative humidity is much higher than the relatively dry upper canopy (Sajap and Siburat, 1992). With many reports of possible risk of lepidopteran insects becoming resistant to *B. thuringiensis* toxin now emerging (Andow *et al*., 2000; Herrero *et al*., 2001; Gonzalez-Cabrera *et al*., 2003; Heckel *et al*., 2007), fungal entomopathogens can be a potential microbial tool in managing insect resistance, including *B. thuringiensis* resistance, for insect pests such as the bagworms.

CONCLUSION

The study proves that the entomopathogenic fungi, *I. fumosorosea* and *M. anisopliae*, formulated as wettable powder in kaolin, with or without Tinopal LPW, were effective in controlling the bagworm larvae both in laboratory and field trials. Even though *I. fumosorosea* and *M. anisopliae* were less effective in controlling the bagworms in the field as compared with that of B. *thuringiensis* but these formulated indigenous fungi could effectively reduced the bagworm population to less than 43%. Thus the entomopathogenic fungi have the potential to be part of an integrated pest management in an oil palm plantation. Hence the fungi need to be conserved and/or augmented so that bagworms can be suppressed with minimal disruption to the ecological balance, an effort of maintaining ecological balance in the oil palm ecosystem, as prescribed in a sustainable agriculture system.

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