THE EFFECTS OF OILS ON GERMINATION OF *Beauveria bassiana* (Balsamo) VUILLEMIN AND ITS INFECTION AGAINST THE OIL PALM BAGWORM, *Metisa plana* (Walker)

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ABSTRACT

This study reports the effects of oils on the conidial germination of four strains of Beauveria bassiana *(Balsamo) Vuillemin (F1, F5, F8 and F10) and their infectivity against the larvae of the oil palm bagworm,* Metisa plana *Walker. The effects of the oils and age of the conidia on the germination of the conidia were examined in the first experiment. Of the five oils tested, soyabean oil and paraffin gave the highest germination for both two- and four-week-old conidia. Palm and corn oils completely inhibited the conidial germination. Germination was influenced by the age of conidia with the mature conidia germinating better than the younger conidia. The pathogenicity of all the four strains of* B. bassiana *conidia formulated in soyabean oil against the larvae of* M. plana *revealed that more than 95% mortality at 10 days after treatment. Although Strain F5 produced the lowest* LT_{50} *(2.6 days), based on the mortality rate and percentage infection, Strain F10 was the more pathogenic. The short (254 nm) ultraviolet radiation was more detrimental to the conidia compared to the long (365 nm) ultraviolet radiation. Soyabean and paraffin oils gave a similar level of protection to the conidia but oil with 1% (w/v) sunscreen gave significantly better protection. Strain F10 was more stable than Strain F5 to both wavelengths of ultraviolet radiation.* B. bassiana *conidia formulated in oil plus 1% sunscreen and oil alone caused about 12 and 15 times higher mortality against the larvae of* M. plana *than the water formulation. The advantages of using oil over water for the formulation of* B. bassiana *to control* M. plana *in the field are discussed.*

Keywords: *Beauveria bassiana*, bagworm, *Metisa plana,* oil formulation, ultraviolet radiation.

Date received: 4 March 2003; **Sent for revision:** 5 March 2003; **Received in final form:** 26 July 2004; **Accepted:** 29 July 2004.

INTRODUCTION

In mid 1970s to late 1980s, the bagworm, *Metisa plana* Walker (Lepidoptera: Pscyhidae), was a major pest of oil palm (*Elaeis guineensis* Jaquin) in Malaysia (Wood, 1982; Basri *et al.*, 1988). The pest was controlled with broad and selective insecticides and currently by trunk injection of a systemic insecticide. Over use of insecticides led to increased problems of insecticide resistance, destruction of beneficial insects and non-target organisms, toxic residues and human poisoning (Haris and Dent, 2000). Concern over these problems has initiated the Malaysian Palm Oil Board (MPOB) to develop safer control measures. These include the use of a fungus (Ramlah Ali *et al.*, 1993), virus (Ramlah Ali *et al.*, 1996) and bacteria (Ramlah Ali and Mohd Basri, 1997).

Using fungus as a control measure in oil palm has focussed on the entomopathogenic *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomyctetes). Laboratory studies using water formulation had shown *B. bassiana* to be effective in controlling the second and fourth instars larvae of *M. plana* (Ramlah Ali *et al.*, 1993; Ramle *et al.*, 1993; Ramle *et al.*, 1996). In the field, however, the higher

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temperature, lower humidity and exposure to ultraviolet (UV) radiation could be detrimental to the conidia. As such, field application of *B. bassiana* in water has failed to suppress the pest (unpublished data). Attention has therefore shifted to oil for formulating the fungus. Studies on other entomopathogenic fungi have shown oil formulation to have increased their infectivity to insects, even at low humidity and high temperatures (Bateman *et al*., 1993). Furthermore, oil could protect the fungal conidia from the UV of sunlight (Moore *et al*., 1993).

This paper reports on the germination of selected strains of *B. bassiana* (Balsamo) Vuillemin (Strains F1, F5, F8 and F10) and their infectivity in various oil formulations on the larvae of *M. plana* were studied.

MATERIALS AND METHODS

Experiment 1: Effects of Oils and Age of Conidia on Conidial Germination

***Source of* B. bassiana.** Four strains of *B. bassiana* were used (*Table 1*). Strains F1, F5 and F8 were obtained from Universiti Kebangsaan Malaysia, Bangi, Selangor. Strain F10 was isolated from diseased adults of *M. plana* collected at Kapar, Selangor (Ramlah *et al.*, 1994). All strains were maintained in malt extract agar (MEA) at 28 ± 1°C in the dark.

TABLE 1. SOURCES OF THE *B. bassiana* STRAINS

Strain	Host	Origin
F1	Unknown	Japan
F5	Unknown	Japan
F8	Unknown	Japan
F10	*M. plana*	Malaysia

Types of oils. Four plant-based oils, one plant-based mixed oil and a mineral oil were used. The plant-based oils were palm oil (Pl), soyabean oil (Sy) and corn oil (Cn). The mixed oil (Mx) was a mixture of palm, cottonseed and rapeseed oils. The plant-based and mixed oils were refined commercial products easily available in Malaysia. Paraffin (Pf) represented the mineral oil and was purchased from Sigma Chemicals Co., USA.

Germination test. Conidia from two- and four-week-old sporulating MEA cultures were harvested by adding 10 ml oil and scraping the conidia from the surface of the cultures using a L-shaped sterile inoculating needle. Suspensions of the conidia were thoroughly mixed and placed in screw-capped universal bottles. The conidia were then separated from the debris by filtering through glass wool. The concentrations of conidia were estimated using an improved Neubauer haemocytometer (Germany) and adjusted to 10^7 conidia/ml. After pipetting 0.3 ml of each adjusted suspension onto sterile potato dextrose agar (PDA) supplemented with 0.02% chloramphinecol (Sigma Chemical Co., USA), the suspension was streaked evenly across the surface of the plate. Germination of conidia was assessed after 20–24 hr of incubation at 28 ± 1°C. The percentage of germination was estimated by dividing the number of germinated conidia with the total number of conidia counted. A conidium was considered germinated when its germ tube was longer than its diameter. In the control, the same procedures were applied using distilled water plus 0.2% Tween 80.

Experiment 2: Pathogenicity of *B. bassiana* Formulated in Oils Against the Larvae of *M. plana*

***Culture of* M. plana.** The *M. plana* used was obtained from Teluk Merbau Estate, Sepang, Selangor and cultured in the laboratory by the method of Mohd Basri and Kevan (1995). Only the fourth instar larvae (L4) were used.

Bioassay procedures. The conidia were formulated in Sy. This oil was used because results from the first experiment revealed that it was found to be the least detrimental to conidial germination. Active L4 larvae were placed on segments of oil palm leaflets prepared as in the bagworm cultures. The leaflets were sprayed with 2.0 ml oil conidial suspension at 10^7 conidia/ml using a controlled-droplet applicator (CDA) (ULVA+, Micron, UK). This conidial level was the LC_{50} value for L4 in ambient conditions (Ramlah *et al.*, 1993). Both sides of the leaflets were sprayed to ensure that the conidia were evenly distributed. The control leaflets were sprayed only with Sy. Bioassay was conducted in five replicates. Each replicate contained 10 larvae (50 larvae/strain). Larval mortality was recorded every two days for 10 days after treatment (DAT). Only cadavers with fungal growth were considered as a successful infection.

Experiment 3: Protection Afforded by Oils and Sunscreen from UV Radiation of Short and Long Wavelengths

Test procedures. Only the two strains of *B. bassiana* representing the extremes in LT_{50} were used. Strain F5 represented the lowest ethal time (LT_{50}) with 2.6 days and F10 the highest with 3.6 days. The conidial suspensions were prepared from the four-week-old cultures in Sy and Pf. For both oils, the conidia were prepared in oil alone and oil containing 1% w/v sunscreen, Oxybenzone (Sigma). Oxybenzone has been proven to give significant protection to conidia

from UV radiation (Moore *et al*., 1993). For the control, the conidia were prepared in distilled water plus 0.2% v/v Tween 80.

Harvesting the conidia followed the procedure described previously. A total of 250 µl oil conidial suspension were pipetted into a 50 mm diameter petri dish and evenly streaked across the surface to give an oil depth of 100 µm (Moore *et al*., 1993). This depth was approximately the diameter of a large droplet in an ultra-low volume spaying by the ULVA+ CDA as applied in the second experiment. Nine of the dishes were exposed to long (365 nm) wave UV radiation produced by 15 Watt tubes (7 mW cm^{-2} intensity) (VL-215LC, Vible Lourmat, France). At every 1, 2 and 3 hr after exposure (HAE), three dishes were taken out for germination test. The exposure of the dishes was conducted in a hood (IRYAS Inc., Malaysia) at 28 ± 3°C with the ventilation blower on. The window of the hood was positioned at the lowest level, which allowed air to enter at 150 ft^3 ml^{-1}. This reduced the heat generated by the UV radiation from around the plates. The same preparations were repeated when the dishes exposed to short (254 nm) wave UV.

Before the germination test, the conidia were washed free from oil by the addition of 5 ml soapy water containing 1% v/v detergent (Colgate Palmolive, Malaysia) to the dishes. The soapy water was swirled gently to remove the oil. The wash (which contained the conidia) was transferred into a 10-ml bottle and thoroughly vortexed to separate out the conidia. The conidia were collected by passing the suspension through a cellulose nitrate membrane filter (pore 0.2 µm) under vacuum. The filter was washed twice with distilled water, then inverted onto a PDA plate with 0.2% chloramphenicol. A few drops of water placed on the PDA surface helped to loosen and transfer some of the conidia onto the medium surface. The same washing procedure was used for the control plates to ensure that all the conidia received the same treatment. Conidial germination was estimated following procedure described earlier in the germination test.

Experiment 4: Effectiveness of Oil Formulated Conidia in Controlling *M. plana* in the Glasshouse

Mated females of *M. plana* collected from FELDA Gunung Besout, Perak were transferred to two-year-old DxP oil palm seedlings in a glasshouse. Eggs laid were allowed to hatch and the culture was maintained until the second generation L4 larvae were available. Two palms were used in each treatment. Fifty larvae were placed on the first palm and the 75 on the second palm. The palms were then treated with three formulations of *B. bassiana*, *i.e*. with Sy alone, Sy with 1% sunscreen and water containing 0.2% Tween 80. The control palms were untreated. All the larvae were marked with different colours on the bags according to the treatment given. This ensured easy identification of the larvae that fell off the palms because of death or movement. The oil and water conidial suspensions were prepared following the procedure described in the first experiment. The amount of conidia used in each treatment was 8.0×10^8 conidia per palm. For the oil formulations, each palm was treated with 20 ml conidial suspension using the ULVA+ CDA. The water formulation was applied at 100 ml per palm by a hand sprayer. Larval mortality was recorded every alternate day for 14 days after which further recording was terminated as all the larvae had pupated.

All the larval cadavers were surface sterilized with 0.3% sodium hyphoclorite, rinsed twice in distilled water, air dried for 5 min and placed on PDA plates with 0.02% chloramphenical. Fungal infection was confirmed if the cadavers developed mycosis two-three days after incubation at 28 ± 1°C.

Statistical Analysis

The percentage germination of conidia and percentage larval mortality of *M. plana* in Experiments 2 and 4 were angularly transformed prior to analysis of variance (ANOVA) and the means were separated by Duncan's multiple range test (DMRT) at P=0.05 (SAS Institute, 1990). To assess the influence of age of conidia on germination, the mean percent germinations for the two- and four-week-old conidia were compared by the T-test at P=0.01 and 0.05 (SAS Institute, 1990). The LT_{50} values at 95% fiducial limits and mortality rate (regression slope) of *B. bassiana* were estimated according to Finney (1971) and Wigley and Kalmakoff (1977). To estimate the effect of UV radiation, ANOVA was performed on the angular transformed percentage corrected germination (PCG). The PCG was calculated by subtracting from 100 the percentage reduction of conidial germination (PRG) obtained as follows:

$$PRG = \frac{(\%\ \text{germination of unexposed conidia} - \%\ \text{germination of exposed conidia}) \times 100}{\%\ \text{germination of unexposed conidia}}$$

RESULTS

Experiment 1: Effects of Oils and Age of Conidia on Conidial Germination

The germinations of the two- and four-week-old conidia of *B. bassiana* in plant-based and mineral oils are shown in *Figure 1*. Conidial germination in water excess of 95% was observed with all the strains tested

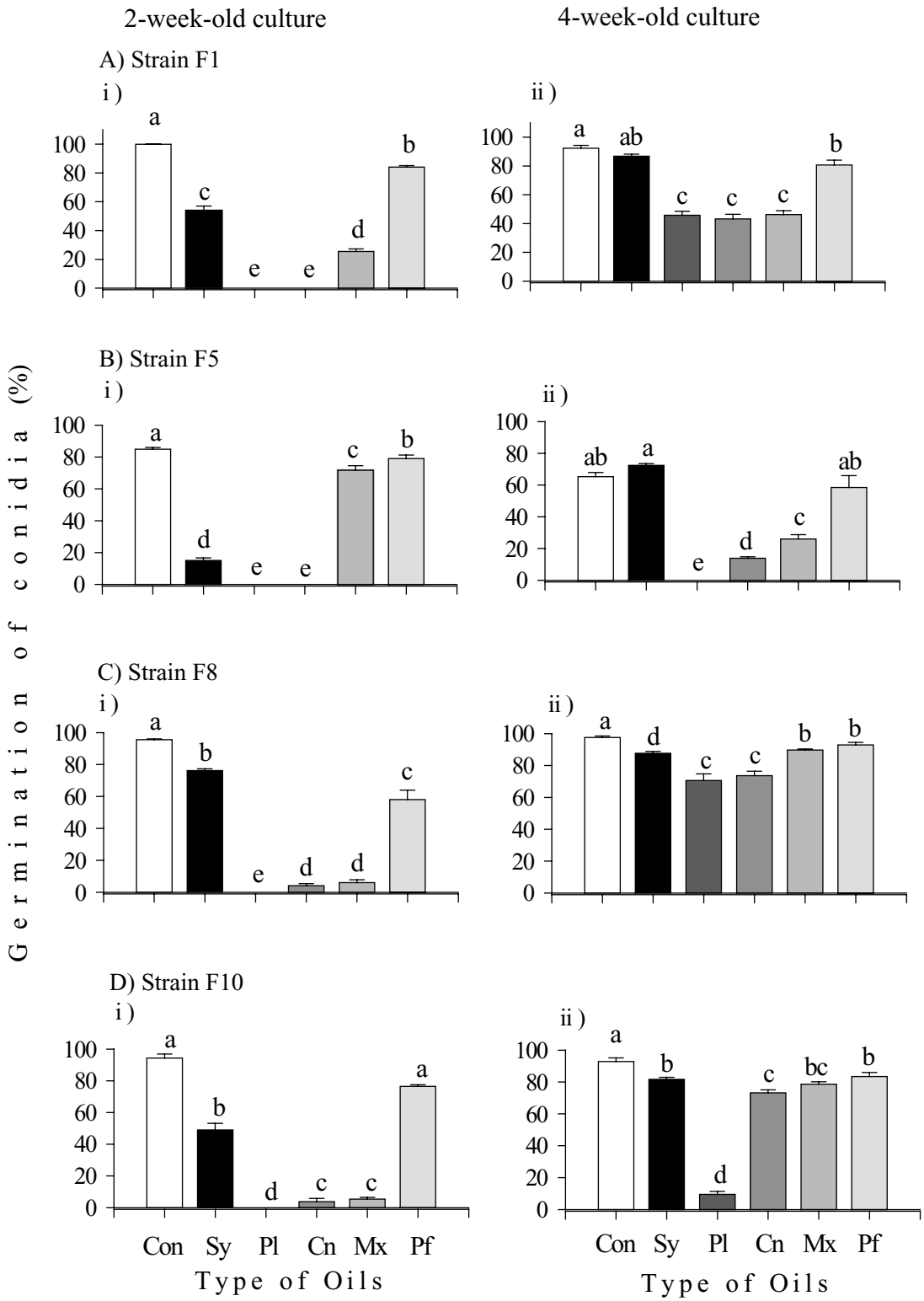

Figure 1. The germination of two- and four-week-old cultures of four strains of B. bassiana *after submerged in water and oils. Bars with the same letter are not significantly different (P>0.05) according to Duncan's multiple range test. Co-control; Sy-soyabean oil; Pl-palm oil; Cn-corn oil; Mx-mixed oil and Pf-paraffin oil.*

at both ages. Although these germinations were significantly higher (P<0.05) than those in oil, in three cases the germinations in oil were not significantly (P>0.05) lower. The first case was the two-week conidia from Strain F10 [*Figure 1d(i)]*. The second case was the four-week conidia from Strain F1 where the germination in water was no different (P>0.05) from the germination in Sy [*Figure 1a(ii)*]. In the third case, the germination of Strain F5 at four weeks was not significantly different (P>0.05) in water, Sy and Pf [*Figure 1b(ii)*].

At two-week, the conidia from all the strains of *B. bassiana* did not germinate in P1. Similar results were found for Cn notwithstanding the fact that Strains F8 and F10 managed very low germinations of <4.5% in it [*Figures 1c(i)* and *1d(i)*]. In Mx, only Strain F5 showed a reasonable germination of up to 71%, while the other strains showed very low germinations (below 25%) [*Figure 1b(i)*]. It was obvious that better germination of *B. bassiana* was to be from the four-week oil conidia although it varied between the strains and oils. P1 again inhibited conidial germination, especially for Strains F5 and F10 – the F5 conidia did not germinate at all [*Figure 1b(ii)*] while F10 had only 9.5% germination [*Figure 1d(ii)*].

Among the oils, Sy and Pf produced the highest conidial germination. All the strains, except for the younger conidia of F5 [*Figure 1b(i)*], germinated significantly better (P<0.05) in them, suggesting that they are the oils to use in formulations.

Table 2 estimates the influence of age on the germination of *B. bassiana* conidia. In most cases, the four-week-old conidia geminated significantly better (P<0.05) than the two-week-old except in Pf. In this oil, significantly higher (P<0.05) percentage germination for strain F8 was observed with the mature conidia (93%) compared with the young (58%), however, no difference was recorded for the remaining strains F1, F5 and F10.

TABLE 2. INFLUENCE OF AGE OF CONIDIA ON THE GERMINATION OF *Beauveria bassiana* FORMULATED IN WATER AND OILS

Strain	Treatment	Conidial germination (%) (mean ± SE)	
		2-week-old	4-week-old
F1	Water + 0.2% Tween 80	99.8 ± 0.2	92.3 ± 2.0 *
	Soyabean oil	53.7 ± 3.4	86.8 ± 1.5 **
	Palm oil	0 ± 0	51.2 ± 4.9 **
	Corn oil	0 ± 0	43.2 ± 3.1 **
	Mixed oil	25.4 ± 1.8	46.2 ± 2.8 **
	Paraffin	84.0 ± 1.0	80.6 ± 3.4 ns
F5	Water + 0.2% Tween 80	84.9 ± 1.1	65.3 ± 2.0 **
	Soyabean oil	15.0 ± 1.6	72.4 ± 1.0 **
	Palm oil	0 ± 0	0 ± 0
	Corn oil	0 ± 0	13.9 ± 1.0 **
	Mixed oil	71.7 ± 2.8	26.1 ± 2.7 **
	Paraffin	79.0 ± 2.2	58.5 ± 7.5 ns
F8	Water + 0.2% Tween 80	95.6 ± 0.4	97.6 ± 0.9 ns
	Soyabean oil	76.2 ± 1.1	87.6 ± 1.2 **
	Palm oil	0 ± 0	70.7 ± 4.1 **
	Corn oil	4.1 ± 1.2	73.7 ± 2.8 **
	Mixed oil	6.1 ± 1.8	89.7 ± 0.8 **
	Paraffin	58.1 ± 5.9	92.9 ± 1.7 **
F10	Water + 0.2% Tween 80	98.7 ± 0.4	92.9 ± 5.3 *
	Soyabean oil	48.9 ± 4.2	81.6 ± 1.4 **
	Palm oil	0 ± 0	9.5 ± 1.9 *
	Corn oil	3.8 ± 2.0	73.2 ± 1.9 **
	Mixed oil	5.7 ± 1.1	78.7 ± 1.5 **
	Paraffin	76.4 ± 1.0	83.5 ± 2.5 ns

Note: Means were significantly different (* at P = 0.05) or highly significantly different (** at P = 0.01) or not significantly different (ns at P = 0.05) as determined by T-test. No F value was obtained for F5 (palm oil).

Experiment 2: Pathogenicity of *B. bassiana* Formulated in Oils Against the Larvae of *M. plana*

Table 3 shows the percentage cumulative mortality of *M. plana* larvae treated with 10^7 conidia/ml of *B. bassiana* formulated in Sy. At 10 DAT, all the strains had killed in excess of 95% of the larvae, highly significant (P<0.01) than the mortality in the control. The estimated LT_{50} values for each strain are shown in *Table 4*. Strain F5 produced the lowest LT_{50} (2.6 days). Strains F1 and F8 shared the same LT_{50} of 3.1 days. While Strain F10 produced the longest LT_{50} of 3.9 days.

Experiment 3: Protection Afforded by Oils and Sunscreen from UV Radiation of Short and Long Wavelengths

Estimates of the effects of long (365 nm) and short (254 nm) wave UV radiation on the conidial germination of Strains F5 and F10 in Sy and Pf are shown in *Tables 5* and *6*. In general, the percentage conidial germination declined with the time of exposure to UV radiation. The short wave was more detrimental to germination as shown by the total inhibition of conidia in water for all the strains even after only 1 hr exposure (*Table 6*). With the long wavelength, the conidial germination for Strain F5 reached 9% and Strain F10 40%, respectively in water even after three hours of irradiation *(Table 5*).

Sy and Pf accorded the same level of protection to the conidia of *B. bassiana* from the short wave radiation (*Table 6*). Incorporating 1% UV sunscreen into the oils significantly improved the protection (P<0.05). Almost with all the exposure times, the conidial germination in oils with 1% UV sunscreen was significantly higher (P<0.05) than those in oil or water alone.

Experiment 4: Effectiveness of Oil Formulated Conidia in Controlling *M. plana* in the Glasshouse

Estimates of the effectiveness of the Sy formulation of *B. bassiana* against *M. plana* in the glasshouse are shown in *Table 7*. At 12 DAT, the larval mortality by the oil with sunscreen (58.4%) was not significantly different (P>0.05) from those with oil alone (46.7%). Larval mortality with the water treatment (3.7%) was significantly lower (P>0.05) than those for oil alone and oil plus sunscreen. Based on the infection, it was obvious that formulation of oil or oil with sunscreen killed significantly higher (P<0.05) the larvae of *M. plana* as compared to water.

TABLE 3. PERCENTAGE CUMULATIVE MORTALITY AND INFECTION RATE OF *Metisa plana* LARVAE AFTER TREATMENT WITH FOUR STRAINS OF *Beauveria bassiana* FORMULATED IN SOYABEAN OIL

Strain	Cumulative mortality (%) (mean ± SE)			Infection rate (%)
	6 DAT	8 DAT	10 DAT	
Control	18.0 ± 3.7a	26.0 ± 5.1a	32.0 ± 3.7a	0 ± 0a
F1	82.0 ± 6.6b	94.0 ± 4.0b	98.0 ± 2.0c	57.0 ± 2.0b
F5	90.0 ± 5.5b	98.0 ± 2.0b	100.0 ± 0.0c	59.0 ± 3.4b
F8	78.0 ± 3.7b	92.0 ± 5.8b	96.0 ± 2.4b	54.0 ± 4.3b
F10	76.0 ± 5.1b	92.0 ± 3.7b	98.0 ± 2.0b	74.0 ± 3.7c

Note: Means within the same column followed by the same letter are not significantly different (P>0.05) according to Duncan's multiple range test.

TABLE 4. PATHOGENICITY OF THE FOUR STRAINS OF *Beauveria bassiana* FORMULATED IN OIL AGAINST THE LARVAE OF *Metisa plana*

Strain	LT_{50} (95% fiducial limits) (days)	χ^2 value	Intercept (a)	Slope (b)
F1	3.1 (3.6 – 2.6)	1.36**	3.4	3.1
F5	2.6 (3.2 – 2.1)	3.36**	3.7	3.1
F8	3.1 (3.1 – 3.0)	6.4*	4.0	2.1
F10	3.9 (4.4 – 3.4)	5.6*	2.9	3.5

Notes: ** Significant at P = 0.01, df = 3 (χ^2_{Table} = 6.25).
* Significant at P = 0.05, df = 3 (χ^2_{Table} = 7.8).

TABLE 5. EFFECT OF 365 nm ULTRAVIOLET (UV) RADIATION ON THE GERMINATION OF *Beauveria bassiana* FORMULATED IN WATER AND OILS WITH AND WITHOUT ULTRAVIOLET SUNSCREEN

Treatment	Corrected germination (%) (mean ± SE)		
	1 HAE	2 HAE	3 HAE
a) Strain F5			
Water + 0.2%Tween 80	74.9 ± 2.9 a	28.9 ± 4.3 b	9.0 ± 2.6 c
Soyabean	33.7 ± 8.8 b	15.5 ± 5.1 c	6.0 ± 4.0 d
Soyabean + 1% UV sunscreen	38.8 ± 2.6 b	15.7 ± 3.6 c	6.4 ± 2.4 cd
Paraffin	87.1 ± 2.2 a	74.9 ± 4.5 a	37.4 ± 1.9 b
Paraffin + 1% UV sunscreen	85.3 ± 4.3 a	75.4 ± 2.5 a	50.4 ± 4.4 a
b) Strain F10			
Water + 0.2%Tween 80	73.8 ± 0.8 b	70.4 ± 2.6 b	39.9 ± 3.2 b
Soyabean	29.4 ± 9.3 c	26.1 ± 7.1 d	16.6 ± 2.4 c
Soyabean + 1% UV sunscreen	73.7 ± 2.0 b	50.1 ± 5.5 c	18.7 ± 4.2 c
Paraffin	95.5 ± 0.7 a	79.2 ± 1.7 ab	75.3 ± 4.1 a
Paraffin + 1% UV sunscreen	88.4 ± 2.1 a	83.8 ± 0.8 a	78.9 ± 1.9 a

Notes: Means within the same columns for each strain followed by the same letter are not significantly different (P>0.05) according to Duncan's multiple range test.
UV: Ultraviolet.

TABLE 6. EFFECTS OF 254 nm ULTRAVIOLET RADIATION ON THE GERMINATION OF *Beauveria bassiana* FORMULATED IN WATER AND OILS WITH AND WITHOUT ULTRAVIOLET SUNSCREEN

Treatment	Corrected germination (%) (mean ± SE)		
	1 HAE	2 HAE	3 HAE
a) Strain F5			
Water + 0.2%Tween	0 ± 0d	0 ± 0c	0 ± 0c
Soyabean	11.9 ± 3.1c	0.3 ± 0.3c	0 ± 0c
Soyabean + 1% UV sunscreen	69.1 ± 8.8a	59.7 ± 7.9a	35.3 ± 8.4a
Paraffin	7.14 ± 3.1cd	3.1 ± 1.8c	2.2 ± 1.2c
Paraffin + 1% UV sunscreen	50.4 ± 10.9b	38.4 ± 8.4b	28.7 ± 5.3a
b) Strain F10			
Water + 0.2%Tween	0 ± 0d	0 ± 0d	0 ± 0e
Soyabean	55.7 ± 3.1c	6.6 ± 1.3c	6.5 ± 1.0c
Soyabean + 1% UV sunscreen	59.2 ± 3.6c	43.3 ± 3.5b	38.3 ± 3.4b
Paraffin	70.6 ± 3.0b	8.4 ± 3.1c	3.89 ± 0.8d
Paraffin + 1% UV sunscreen	86.4 ± 4.2a	59.9 ± 1.7a	47.4 ± 4.8a

Notes: Means within the same column for each strain followed by the same letter are not significantly different (P>0.05) according to Duncan's multiple range test.
UV: Ultraviolet.

TABLE 7. EFFECTIVENESS OF *Beauveria bassiana* FORMULATED IN SOYABEAN OIL AGAINST THE LARVAE OF *METISA PLANA* IN THE GLASSHOUSE

Formulation	Cumulative mortality (%) (mean ± SE)		Infection (%)
	6 DAT	12 DAT	
Untreated control	0.7 ± 0.7a	0.7 ± 0.7a	0 ± 0a
Water + 0.2% Tween 80	3.0 ± 3.0b	3.7 ± 2.4b	0.7 ± 0.3a
Oil	40.0 ± 4.0c	46.7 ± 6.7c	39.5 ± 6.7b
Oil + UV sunscreen	50.0 ± 10.0c	58.4 ± 7.7c	51.0 ± 8.8b

Notes: Means within the same column followed by the same latter are not significantly different (P>0.05) according to Duncan's multiple range test.
UV: Ultraviolet.

DISCUSSION

Although it is well documented that the conidia of entomopathogenic fungi can germinate in oils (Daoust *et al.,* 1983), the germination of the conidia is also influenced by the oil type and the age of the conidia. This study has shown that not only the oil itself but the age of the conidia also affected the conidial germination (*Figure 1*). The younger conidia (two-week) of all the strains were unable to germinate in P1. Although some strains (F8 and F10) germinated in Cn and Mx, the rates were very low [*Figures 1c(i)* and *1d(i)*]. All the strains germinated better in Sy and Pf, suggesting that these are the oils to be used for formulating *B. bassiana* conidia for field application against *M. plana*.

An understanding of why P1, Cn and Mx inhibited the germination of conidia is still lacking and further research is needed. The same results were reported by Stathers *et al.* (1993) who found that P1 totally inhibited conidial germination after only two weeks' storage, whereas Sy allowed a germination of up to 22.7% even after 18 weeks of storage. Although the age of the conidia affected their germination, this effect can easily be obviated by the use of older conidia which, in most cases, germinated better (*Table 2*).

An isolated strain from the host or locality of the host would normally possess a higher pathogenicity against it (the host) (Johnson *et al.,* 1992). In this study, although Strains F1, F5 and F8 came from different ecological and geographical origins as the target pest, their pathogenicities on *M. plana* were slightly better than that of the local strain, F10 (*Table 4*). This finding, however, is not unusual because it has also been reported for other insects. Prior (1990) gave several examples where foreign strains were successfully used to control some insects. These included the control of *Hyphothenemus hampei* (Coleoptera: Scolytidae) in Colombia using the Italian strain of *B. bassiana* isolated from *Cossus cossus* (Lepidoptera: Cossidae). In USA, the USSR strain of *B. bassiana* was used to control *Diuraphis noxia* (Homoptera: Aphididae). In Brazil, a strain isolated from the family Vespidae (Hymenoptera) was used to control the curculionid pest, *Chalcodermus bimaculatus* (Coleoptera).

Ramlah *et al.* (1993) has shown that with water formulation, the infection by *B. bassiana* of the L2 and L4 larvae of *M. plana* was influenced by humidity. The LT_{50}s by *B. bassiana* in normal conditions were 6.9 days (L2) and 13.3 days (L4), but in especially humid conditions, 3.8 days (L2) and 9.0 days (L4). Based on the LT_{50} values from the present study, the infectivity of *B. bassiana* in oil formulation against the larvae of *M. plana* was increased by one to three times compared to that in water. This study concurs with the finding of Prior *et al.* (1988) that the oil formulation of *B. bassiana* was 39 times better for controlling *Pantorhytes plutus* than the water-formulated conidia. Another example is from Bateman *et al.* (1993) who reported that at as low as 35% humidity, the infectivity of *Metarhizium flavoviride* in oil on the desert locust, *Schistocerca gregaria*, was 76% better than in water.

The effects of UV-A at 365 nm wavelength on the conidial germination of *B. bassiana* formulated in water and oils were less detrimental than UV-B at 254 nm wavelength. This effect was more obvious when the conidia were prepared in water. None of the *B. bassiana* conidia germinated after only 1 hr exposure to the short UV (*Table 6*). But at the same exposure period, 74% of the fungus still germinated after exposure to long UV, although the germination rates declined with the time of exposure (*Table 5*). The findings in this study supported those of Moore *et al.* (1993) on *M. flavoviride.*

There was variability in the conidial strains in their susceptibility to UV radiation. Strain F10 was more stable than Strain F5 especially to the shorter wavelength (*Table 6*). Variability in the susceptibility of conidia to UV radiation was also observed in other species of the entomopathogenic Hyphomycetes, such as *M. anisopliae*, *M. flavoviride* and *Paecilomyces fumosoroseus* (Fargues *et al*., 1996).

The UV radiation in sunlight comprises UV-A (320- 400 nm), UV-B (280-320 nm) and UV-C (180-280 nm). The greatest damage is potentially caused by UV-C but most of it is screened out by the atmosphere, leaving mainly UV-A and UV-B to reach the earth (Moore *et al.,* 1993). UV-B is undoubtedly one of the more important factors affecting the survival of fungal inoculum in the field. A possible strategy to counteract its deleterious effects is to use an oil formulation or incorporate an UV screen in the formulation. Both strategies were evaluated. Incorporating a screen in oil provided more protection to the conidia than the oil alone. The germination of *B. bassiana* conidia in oil added with 1% (w/v) UV sunscreen increased significantly ($P<0.05$) from the germination in oil alone even after exposure for 3 hr (*Table 6*).

Results from the third experiment were consistent with the findings from the fourth (*Table 7*). As expected, in the glasshouse, conidia formulated in oil with and without UV sunscreen killed more larvae of *M. plana* ($P<0.05$) than the water formulation. Oil with UV sunscreen killed 58.4% of the larvae, slightly more than for oil alone (46.7%) however the difference was not significant ($P>0.05$). It was clear that the increase in mortality was due to the increase in conidial infectivity of *B. bassiana*. Other factors that enhance the infectivity of oil formulations on insects were described by Prior *et al.* (1988). An oil drift evaporates more slowly than water, thus giving the conidia more time to germinate and infect. Oil formulation also enhances adhesion of the conidia to the insect cuticle. The other advantages of oil over water formulation include the ready suspension of the lipophilic conidia of *B. bassiana* in oil. Oil can be sprayed in ultra-low volume by a CDA unit which makes the application simpler than for water formulation (Prior *et al.,* 1988). In addition, oil can give some protection to the conidia from heat (Scherer *et al*., 1992).

CONCLUSION

This study showed the variation in conidial germination in different oils. P1 and Cn totally inhibited conidial germination, ruling out their use in formulation. All the strains of *B. bassiana* germinated better in Sy and Pf, suggesting their potential in formulations. The age of conidia influenced the germination of the cultures with mature conidia germinating better than the younger ones.

Conidia formulated in Sy had their infectivity against the larvae of *M. plana* enhanced. In oil, the fungus killed *M. plana* one to three times better than in water. Among the strains of *B. bassiana*, on the basis of mortality rate and percentage infection, Strain F10 was the most pathogenic against *M. plana*.

Short UV radiation was more detrimental to the conidia of *B. bassiana* than the long UV radiation. Sy and Pf gave the same level of protection to the conidia from UV radiation. Incorporating 1% UV sunscreen, oxybenzone, in oil provided more protection to the conidia than the oil alone. For all the exposure times, the germination of conidia in oils that contained the UV sunscreen was significantly higher than those for the conidia formulated in oil alone. Besides being the most pathogenic against *M. plana*, Strain F10 was also more stable than Strain F5 against UV radiation. In the glasshouse experiment, oil with sunscreen and oil alone were equally effective to control the larvae of *M. plana*. Both formulation killed 58.4% and 46.7% of larvae, highly significant ($P<0.05$) than the 32% mortality in the control. Although this laboratory study has been promising, the results should still be verified in the field especially with higher conidial concentrations.

In addition, before any field application, the effect of *B. bassiana* on the oil palm pollinating weevil, *Elaeidobius kamerunicus* (Faust), must first be studied, since the infection of this pathogen on curculionid insects has been reported (Prior, 1990).

ACKNOWLEDGMENT

The authors would like to thank the Director-General and Deputy Director-General (R&D) of MPOB, for their support and permission to publish this paper. Some of the data in this study are part of a M.Sc. thesis submitted to Universiti Kebangsaan Malaysia. The authors also acknowledge all the staff in the Entomology and Mammalia Section for their valuable assistance in conducting this study.

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