ISARIA FUMOSOROSEA AND *METARHIZIUM ANISOPLIAE* FOR CONTROLLING *ATTEVA SCIODOXA* (LEPIDOPTERA: YPONOMEUTIDAE), A PEST OF *EURYCOMA LONGIFOLIA*

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Keywords: Biological control, tiger moth, indigenous entomopathogens

SAJAP AS, ROZIHAWATI Z, OMAR D & LAU WH. 2014. *Isaria fumosorosea* dan *Metarhizium anisopliae* untuk mengawal *Atteva sciodoxa* (Lepidoptera: Yponomeutidae), sejenis perosak *Eurycoma longifolia*. Ulat harimau *Atteva sciodoxa* (Lepidoptera: Yponomeutidae) ialah perosak utama tongkat ali (*Eurycoma longifolia,* Simaroubaceae). Untuk mencari kaedah yang selamat dan berkesan bagi mengawal perosak ini, dua kulat entomopatogen asli *Isaria fumosorosea* dan *Metarhizium anisopliae* yang diasingkan daripada ulat bungkus *Pteroma pendula* (Lepidoptera: Psychidae) diuji ke atas perosak ini. Ulat disembur secara berasingan dengan kepekatan 1×10^2 konidia mL^{-1} hingga 1×10^5 konidia mL^{-1} setiap kulat. Kedua-dua kulat bersifat patogen terhadap larva instar ketiga *A. sciodoxa*. Bagaimanapun, *M. anisopliae* didapati lebih virulen daripada *I. fumosorosea*. Kepekatan berkesan median bagi *M. anisopliae* dan *I. fumosorosea* ialah masing-masing 4.23×10^3 konidia mL^{-1} dan 8.24×10^4 konidia mL^{-1}. Masa serangan median berjulat dari 4.3 hari hingga 10.3 hari untuk *M. anisopliae* dan dari 7.6 hari hingga 16.3 hari bagi *I. fumosorosea*. Sepuluh hari selepas rawatan, *M. anisopliae* membunuh 48% hingga 88% larva manakala *I. fumosorosea* membunuh 26% hingga 62% larva, masing-masing untuk kepekatan terendah dan tertinggi. Semburan ampaian konidia *M. anisopliae* sebanyak 2×10^7 konidia mL^{-1} dapat menurunkan populasi *A. sciodoxa* yang menyerang anak pokok berusia 2 tahun sehingga 89%, 7 hari selepas rawatan. Kajian menunjukkan yang *M. anisopliae* berpotensi untuk mengawal *A. sciodoxa*.

INTRODUCTION

Eurycoma longifolia locally known as tongkat ali is one of the popular ingredients in a libido-enhancing supplement for men. High demand coupled with rapidly decreasing resources from the natural forest has created interest among farmers and investors to grow the plant ex-situ as a plantation crop. To date, the Federal Land Development Authority (FELDA), one of the major plantation establishments in Malaysia, has planted more than 300,000 *E. longifolia* trees in several land schemes in the country. Since its establishment as a plantation crop, a number of pests and diseases have been reported. One of the most important pests is the tiger moth *Atteva sciodoxa* (Lepidoptera: Yponomeutidae) (Abood et al. 2009). The larvae feed gregariously

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on apical shoots, young leaves and flowers, often leading to stunted growth and death of plants. The absence of natural enemies associated with this pest may pose problems when plants are grown as monoculture.

To date, no specific method of controlling the pest has been recommended. Often the pest is regularly sprayed with synthetic systemic insecticides to keep population down. These insecticides may have adverse effects especially on plants that are intended for pharmaceutical preparation. Even though no study on the accumulation of pesticides in *E. longifolia* has been carried out, Ang and Lee (2006) have detected mercury in tongkat ali hitam herbal preparations. However, there are reports indicating the ability of pesticides to modulate phenolic compounds in plants (Daniel et al. 1999). The synthesised phenolic compounds may have subtle effects on physiological processes pertinent to human health. Thus, a pest control strategy with minimal impact on product contamination and is safe to the environment and human is highly recommended. One of the strategies involves using entomopathogenic fungi such as *Metarhizium anisopliae, Beauveria bassiana* and *Isaria fumosorosea* for controlling lepidopteran pests. However, their effectiveness varies with fungal isolates and target pests. Thus, screening of different indigenous fungal isolates against insect pest such as *A. sciodoxa* is necessary for the development of an effective biological control agent. So far, only isolates of *B. bassiana* were reported to be pathogenic to the larvae of *A. sciodoxa* (Abood et al. 2010). However, they have yet to be tested in the field. *Metarhizium anisopliae* and *I. fumosorosea* have been shown to cause high mortalities to larvae of diamondback moth *(Plutella xylostella)*, also a yponomeutid moth (Maketon et al. 2008, Godonou et al. 2009). In this study, the virulence of the entomopathogenic fungi, *M. anisopliae* and *I. fumosorosea*, was evaluated against the larvae *A. sciodoxa*. The effectiveness of the most virulent fungus against a natural population of *A. sciodoxa* was evaluated in the field.

MATERIALS AND METHODS

Source of insects

Atteva sciodoxa larvae were collected from infested *E. longifolia* saplings in a field in Bukit Badong, Selangor, Malaysia. The larvae were maintained in cages on fresh cuttings of *E. longifolia*. Early third instar larvae were used in the bioassay.

Fungal isolates

Entomopathogenic fungi *I. fumosorosea* and *M. anisopliae* originally isolated from bagworms *Pteroma pendula* (Lepidoptera: Psychidae) (Cheong et al. 2010) were maintained on Sabouraud's dextrose agar with 1% yeast (SDAY). The isolates were passed through *A. scodioxa* larvae, re-isolated, cultured on SDAY and kept in an incubator at 27 ± 2 °C. These isolates were maintained as stock cultures. Isolates from stock cultures were mass produced on rice as described by Soper and Ward (1981). After 15 days of incubation, bags containing conidiated rice had their contents sieved through a 74 µm mesh and conidia were collected in a receiving tray at the bottom. The conidia were suspended in sterile distilled water containing 0.05% Tween 80 and homogenised in a vortex mixture for 1 min. Conidia were counted under compound microscope using an improved haemocytometer and the required concentrations were serially diluted. Four concentrations, namely, 1×10^5, 1×10^4, 1×10^3 and 1×10^2 conidia mL^{-1} were prepared from each fungus and tested on the larvae. Viability of the conidia was determined using a method described by Milner et al. (1991). All isolates yielded germination of over 90%.

Bioassay

Early third instar of larvae *A. sciodoxa* were placed on filter paper in a Petri dish. Larvae were sprayed with 5 mL of fungal concentration. Controls received distilled water with 0.05% Tween 80. Treated larvae were allowed to dry for 10 min. Ten larvae were randomly picked using a pair of sterilised soft forceps and individually placed in a Petri dish containing a fresh pinnacle of *E. longifolia* leaf with its stalk wrapped in wet cotton. Leaves were changed every 3 days. With limited field-collected larvae, five replicates of 10 larvae per treatment were carried out over time. Larval mortality was recorded daily for 14 days. Dead larvae were removed, kept on moist filter paper in a Petri dish and maintained at 27 ± 2 °C for sporulation.

Field trial with *Metarhizium anisopliae*

Site

The field trial was conducted at Bukit Badong, Selangor, Malaysia. The area has an average daily rainfall of 8.9 mm, temperature of 21–32 °C and relative humidity of 82%. The site had 50 saplings aged 2 years. They were about 1.5 m tall and planted at a distance of 3 × 3 m. Even though the site was surrounded by oil palms, saplings were frequently infested with *A. sciodoxa*. No chemical was used to control the pest.

Field trial

Infested saplings were numbered and randomly selected for treatment. Before spraying, the number of larvae on each sapling was counted. Saplings with larvae between second and third instars were chosen. One day before spraying, 1 L of conidial suspension of 2×10^7 conidia mL^{-1} was prepared in distilled water containing 0.05% Tween 80. About 50 mL of fungal suspension were sprayed using a hand-held compression sprayer onto each sapling. The control saplings received only distilled water with 0.05% Tween 80. Spraying was carried out before 9 a.m. One hour after treatment, one larva each from treated and untreated saplings was randomly picked using a pair of sterilised forceps and kept individually in a Petri dish containing fresh leaves and a moist filter paper. Infectivity of the fungus on the larvae was monitored daily in the laboratory. On day 7 after treatment, the number of living larvae on treated saplings was recorded. A similar procedure was applied for control saplings. The field trial was repeated three times at the same site at an interval of at least 2 months between each trial. A total of 5, 8 and 10 saplings were treated for the first, second and third trials respectively. An equal number of saplings was correspondingly assigned as controls.

Data analysis

The data were subjected to analysis of variance and means separated using Tukey's test at 0.05 significance. Median effective times (ET_{50}) and median effective concentrations (EC_{50}) for the fungi were obtained 7 days after inoculation using POLO (Probit Or Logit) Version 1.5. Data from field trials were subjected to t test.

RESULTS

Laboratory bioassay

Both fungi were pathogenic to third instar larvae of *A. sciodoxa* (Figures 1 and 2). Both fungi caused larval mortality as early as day 2 after treatment. By day 10, larvae that were treated with *M. anisopliae* at concentrations of 1×10^4 and 1×10^5 conidia mL^{-1} had cumulative

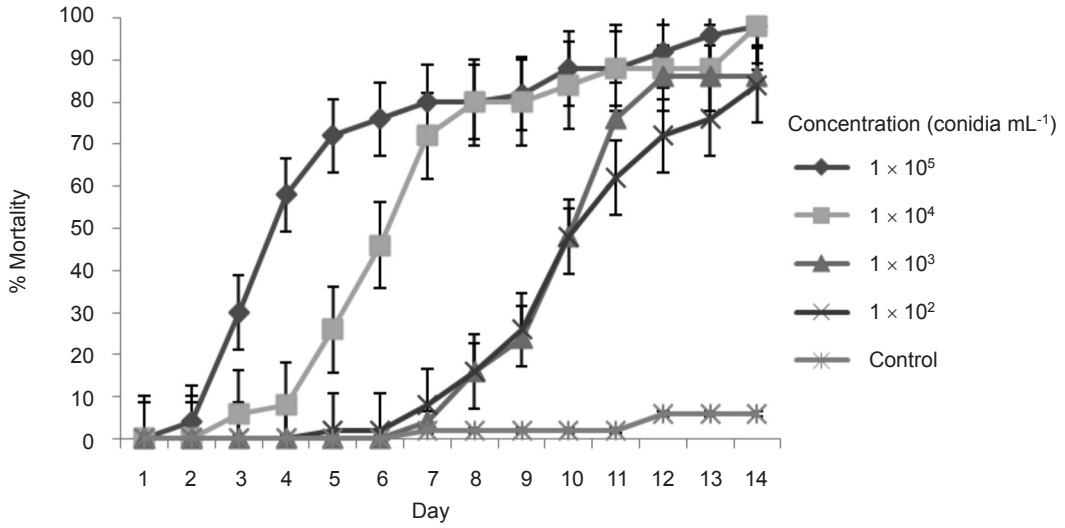

Figure 1 Cumulative per cent mortality of third instar *Atteva sciodoxa* larvae infected with various concentrations of *Metarhizium anisopliae*

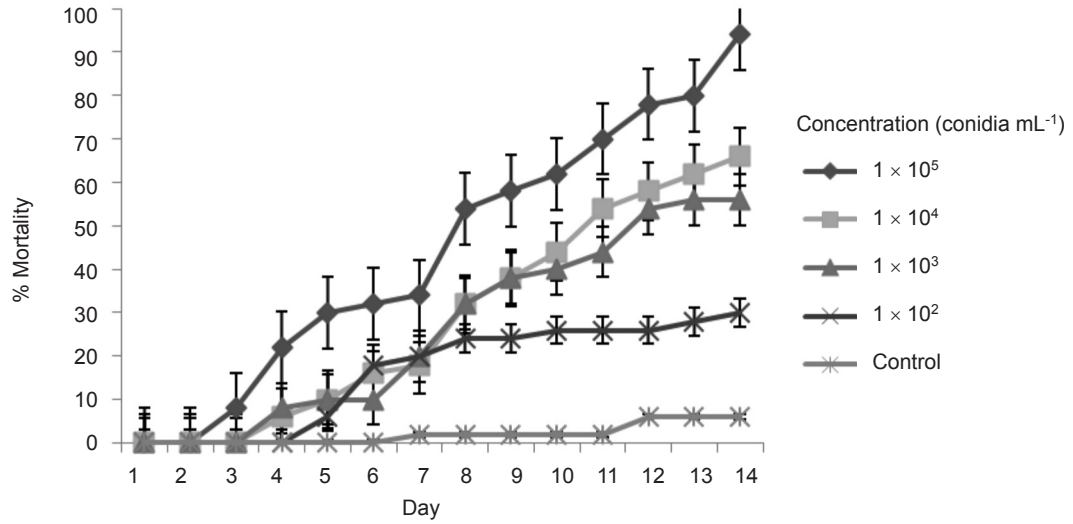

Figure 2 Cumulative per cent mortality of third instar *Atteva sciodoxa* larvae infected with various concentrations of *Isaria fumosorosea*

mortalities of above 80%. Larvae treated with concentration 1×10^3 conidia mL^{-1} had about 50% mortality. At the end of the test, all larvae treated with *M. anisopliae* exceeded 70% mortality.

Larvae treated with *I. fumosorosea* had mortality ranging from 26 to 62% on day 10 after treatment. With the exception of the highest concentration, 1×10^5 conidia mL^{-1}, none of the treatment exceeded 70% mortality at the end of the test. Mortality in the control was less than 10%. The concentration–mortality response curve indicated that there was significant relationship between fungal concentration and larval mortality.

The estimated ET_{50} values of larvae decreased with increase in concentration of both fungi (Table 1). The estimated ET_{50} values for larvae treated with *M. anisopliea* ranged from 4.3 to 10.3 days while those treated with *I. fumosorosea* ranged from 7.6 to 16.6 days. These values indicated that there was significant difference in the virulence of the two fungi. *Metathizium anisopliae* was consistently more virulent than *I. fumosorosea.* The ET_{50} value for larvae that were treated with the highest concentration of *I. fumosorosea* was about double that of larvae treated with the same concentration of *M. anisopliae* conidia. At the lowest concentration of 1×10^2 conidia mL^{-1}, *M. anisopliae* and *I. fumosorosea* gave ET_{50} values of 10.3 and 16.6 days respectively.

There were also large differences between fungi in the estimates of EC_{50}. The EC_{50} values on day 7 for larvae treated with *M. anisopliae* and *I. fumosorosea* were 4.2×10^3 conidia mL^{-1} with 95% confidence limits of 1.9×10^3–8.9×10^3 and 8.2×10^4 mL^{-1} with 95% confidence limits of 1.8×10^4– 2.3×10^6 mL^{-1} respectively (results not shown).

Field trial with *Metarhizium anisopliae*

Conidial suspension of *M. anisopliae* sprayed at concentration of 2×10^7 conidia mL^{-1} effectively controlled field populations of *A. sciodoxa* attacking 2-year-old saplings. In trial 1, the total numbers of larvae on saplings before treatment were 73 and 36 on treated and control saplings respectively. On day 7 after treatment, 1 larva was recorded from treated saplings, while 21 larvae still remained on untreated saplings (Table 2). In trial 2, before treatment, a total of 86 and 124 larvae were recorded on treated and control saplings respectively (Table 3). The number dropped to 1 and 105 on treated and control saplings respectively on day 7 after treatment. In trial 3, high *A. sciodoxa* infestation occurred on saplings (Table 4). Thus, highly infested saplings were selected. The total numbers of larvae on 10 selected saplings before treatment were 146 and 181 on treated and control saplings respectively. On day 7 after treatment, the number dropped to 13 on treated saplings, while 144 larvae still remained on control saplings. After considering the natural population reduction in controls, the corrected reduction values for treated populations in trials 1, 2 and 3 were

Table 1 Mortality of *Atteva sciodoxa* larvae caused by different concentrations of *Metarhizium anisopliae* and *Isaria fumosorosea* on day 10 and their median effective times in the laboratory

Isolate	Concentration (conidia mL^{-1})	Mortality (%)	ET_{50} (days)	95% limit (days)	
				Lower	Upper
Metarhizium anisopliae	1×10^2	48.0 ± 13.2 c	10.3	9.8	10.9
	1×10^3	48.0 ± 6.6 c	9.6	8.8	10.6
	1×10^4	84.0 ± 5.1 a	6.5	5.3	7.7
	1×10^5	88.0 ± 5.8 a	4.3	3.0	5.4
Isaria fumosorosea	1×10^2	26.0 ± 6.0 d	16.6	13.1	26.6
	1×10^3	40.0 ± 3.1 c	12.4	10.6	16.1
	1×10^4	44.0 ± 9.3 c	10.9	9.6	13.2
	1×10^5	62.0 ± 12.4 b	7.6	6.8	8.5

Means with the same letter in the same column are not significantly different at $p < 0.05$, Tukey's test; values for mortality = means ± standard errors; ET = effective time

Table 2 Number and per cent reduction of *Atteva sciodoxa* larvae on *Eurycoma longifolia* saplings before and after spraying with *Metarhizium anisopliae* at 2×10^7 conidia mL^{-1} in the first trial

Sapling	Treatment		Control	
	Before	After (% reduction)	Before	After (% reduction)
1	24	0 (100)	7	3 (57.14)
2	26	0 (100)	10	8 (20.00)
3	7	0 (100)	3	3 (0)
4	6	1 (83.33)	7	5 (28.57)
5	10	0 (100)	9	2 (77.77)
Total	73	1	36	21
% Mean ± SE	96.67 ± 3.33		42.69 ± 14.19	

$t = 3.70$, $df = 8$, $\alpha = 0.05$; SE = standard error

Table 3 Number and per cent reduction of *Atteva sciodoxa* larvae on *Eurycoma longifolia* saplings before and after spraying with *Metarhizium anisopliae* at 2×10^7 conidia mL^{-1} in the second trial

Sapling	Treatment		Control	
	Before	After (% reduction)	Before	After (% reduction)
1	30	0 (100)	26	11 (57.69)
2	10	0 (100)	5	3 (40)
3	2	0 (100)	1	1 (0)
4	26	1 (96.15)	15	13 (33.33)
5	3	0 (100)	6	6 (0)
6	6	0 (100)	5	5 (0)
7	4	0 (100)	16	13 (18.75)
8	5	0 (100)	50	45 (10)
Total	86	1	124	105
% Mean ± SE	99.52 ± 0.48		17.51 ± 7.48	

$t = 10.93$, $df = 14$, $\alpha = 0.05$; SE = standard error

Table 4 Number and per cent reduction of *Atteva sciodoxa* larvae on *Eurycoma longifolia* saplings before and after spraying with *Metarhizium anisopliae* at 2×10^7 conidia mL^{-1} in the third trial

Sapling	Treatment		Control	
	Before	After (% reduction)	Before	After (% reduction)
1	21	2 (90.48)	26	23 (11.53)
2	10	2 (80.00)	14	11 (21.43)
3	11	1 (90.90)	13	10 (23.07)
4	20	0 (100)	18	13 (27.78)
5	25	2 (92.00)	14	10 (28.58)
6	6	0 (100)	50	43 (14.00)
7	8	1 (87.50)	11	9 (18.18)
8	20	2 (90.00)	21	15 (28.57)
9	17	3 (82.35)	8	5 (37.50)
10	16	0 (100)	6	5 (16.66)
Total	146	13	181	144
% Mean ± SE	92.12 ± 2.41		22.73 ± 2.52	

t = 19.91, df = 18, α = 0.05; SE = standard error

94.19, 99.42 and 89.80%respectively (results not shown). All larvae that were sampled from treated saplings succumbed to fungal infection within 7 days after treatment. Those sampled from control saplings survived to adulthood. Figure 3 shows the progress of *M. anisopliae* infection on a treated larva in the field. The larva died on day 3 and subsequently mummified into a green cadaver 7 days after treatment.

DISCUSSION

Even though both fungi were pathogenic, they differed in virulence to *A. sciodoxa* larvae. *Metarhizium anisopliae* was more virulent than *I. fumosorosea*. The virulence of *M. anisopliae* was double that of *I. fumosorosea*. At 1×10^5 conidia mL^{-1}, *M. anisopliae* caused more than 80% mortality as opposed to less than 65% mortality caused by *I. fumosorosea* 10 days after treatment. The variation in virulence may be due to inherent characteristics of the fungal species (Hassanloui et al. 2006). The superiority of *M. anisopliae* to *I. fumosorosea* has been shown in *Melanoplus sanguinipes*, *Locusta migratoria migratorioides*, *Schistocerca gregaria* (Nowierski et al. 1996) and *Lygus lineolaris* (Liu et al. 2002). Studies have shown that the virulence of a microbial agent may vary between isolates and differ against species of the host insects even within a single fungal species (Shah & Pell 2003). Variability in virulence among entomopathogenic fungi is a natural phenomenon and may be attributed to genomic variability of the fungi (Bidochka et al. 1994). Apart from *M. anisopliae* and *I. fumosorosea*, *A. sciodoxa* larvae were also susceptible to *B. bassiana* (Abood et al. 2010). However, *B. bassiana* isolates were less virulent than either *M. anisopliae* or *I. fumosorosea*. Even their most virulent isolate Bba-Pp was less infective than *M. anisopliae* and *I. fumosorosea* when applied at the same concentration. The result from three consecutive field sprayings of *M. anisopliae* at 2×10^7 conidia mL^{-1} was very promising. The significant reduction of larval population recorded from treated saplings could be attributed to the behaviour of the pest. Unlike many lepidopteran larvae that move within the plant or from one plant to another, *A. sciodoxa* larvae as webworms do not move freely from one plant to another but invariably remain active within the open webs they construct on the aerial part of saplings. The silken webs that evidently capture strings of fine dewy conidia containing droplets from the mist created by the sprays could have served as a reservoir for the inocula. The webs also increase the spray coverage while maintaining relatively high humidity within the larval niche. These attributes increase the chances of actively-foraging larvae contracting inocula from the dews. Their agile and gregarious

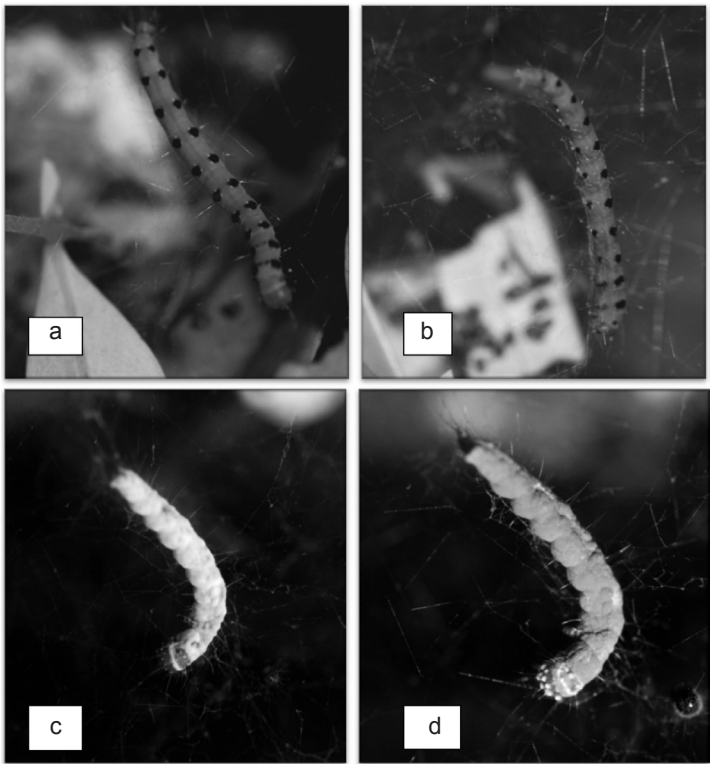

Figure 3 Progress of *Metarhizium anisopliae* infection on a treated larva in the field: (a) newly-treated larva—alive, (b) moribund larva 3 days after treatment, (c) fully white mycelia-covered larva 5 days after treatment and (d) mummified larva covered with green conidia 7 days after treatment

behaviour could have also facilitated horizontal transmission of conidia within larval population and consequently make them vulnerable to fungal infection. Even though the larvae can be easily infected from a single spray, the conidia may not survive the harsh environmental stress over a longer period of time. Germination rate can be affected by low humidity, high temperature and solar radiation (Zimmermann 1982, Braga et al. 2001). Thus, repeated sprays preferably in the morning are recommended to ensure the success of controlling *A. sciodoxa* larvae using entomopathogenic fungi in an open field of *E. longifolia*.

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REFERENCES

Abood F, Bajwa GA & Ibrahim YB. 2009. Developmental biology of the tiger moth, *Atteva sciodoxa* Meyrick (Lepidoptera: Yponomeutidae) under laboratory conditions. *Journal of Biological Sciences* 9: 458–463.

Abood F, Bajwa GA, Ibrahim YB & Sajap AS. 2010. Pathogenicity of *Beauveria bassiana* against the tiger moth, *Atteva sciodoxa* (Lepidoptera: Yponomeutidae). *Journal of Entomology* 7: 19–32.

Ang HH & Lee KL. 2006. Contamination of mercury in tongkat ali hitam herbal preparations. *Food and Chemical Toxicology* 44: 1245–1250.

Bidochka MJ, McDonald MA, St Leger RJ & Roberts DW. 1994. Differentiation of species and strains of entomopathogenic fungi by random amplification of polymorphic DNA (RAPD). *Current Genetics* 25: 107–113.

Braga GUL, Flint SD, Miller CD, Anderson AJ & Roberts DW. 2001. Both solar UVA and UVB radiation impair conidial culturability and delay germination in the entomopathogenic fungus *Metarhizium anisopliae*. *Photochemistry and Photobiology* 74: 734–739.

Cheong YL, Sajap AS, Hafidzi MN, Omar D & Abood F. 2010. Outbreaks of bagworms and their natural enemies in an oil palm, *Elaeis guineensis*, plantation at Hutan Melintang, Perak, Malaysia. *Journal of Entomology* 7: 141–151.

Daniel O, Meier MS, Schlatter J & Frischknecht P. 1999. Selected phenolic compounds in cultivated plants: ecological functions, health implications, and modulation by pesticides. *Environmental Health Perspective* 107: 109–114.

Godonou I, James B, Atcha-Ahowé C, Vodouhè S, Kooyman C, Ahanchédé A & Korie S. 2009. Potential of *Beauveria*

bassiana and *Metarhizium anisopliae* isolates from Benin to control *Plutella xylostella* L. (Lepidoptera: Plutellidae). *Crop Protection* 28: 220–224.

Hassanloui RT, Kharazi-Pakdel A, Goettel M & Mozaffari J. 2006. Variation in virulence of *Beauveria bassiana* isolates and its relatedness to some morphological characteristics. *Biocontrol Science and Technology* 16: 525–534.

Liu H, Skinner M, Parker BL & Brownbridge M. 2002. Pathogenicity of *Beauveria bassiana*, *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes), and other entomopathogenic fungi against *Lygus lineolaris* (Hemiptera: Miridae). *Journal of Economic Entomology* 95: 675–681.

Maketon M, Orosz-Coghlan P & Aengarun J. 2008. Field evaluation of *Isaria fumosorosea* in controlling the diamondback moth (*Plutella xylostella*) in Chinese kale. *Phytoparasitica* 36: 260–263.

Milner RJ, Huppatz RJ & Swaris SC. 1991. A new method for assessment of germination of *Metarhizium* conidia. *Journal of Invertebrate Pathology* 57: 121–123.

Nowierski RM, Zheng Z, Jaronski S, Delgado F & Swearingen W. 1996 Analysis and modeling of time–dose mortality of *Melanoplus sanguinipes*, *Locusta migratoria migratorioides*, and *Schistocerca gregaria* (Orthoptera: Acrididae) from *Beauveria, Metarhizium*, and *Paecilomyces* isolates from Madagascar. *Journal of Invertebrate Pathology* 67: 236–252.

Shah PA & Pell JK. 2003. Entomopathogenic fungi as biological control agents. *Applied Microbiology and Biotechnology* 61: 413–423.

Soper RA & Ward MG. 1981. Production, formulation and application of fungi for insect control. Pp 161–180 in Papavizas GC (ed) *Biological Control in Crop Production*. BARC Symposium No. 5. Allenheld, Osmun and Co, Montclair.

Zimmermann G. 1982. Effect of high temperatures and artificial sunlight on the viability of conidia of *Metarhizium anisopliae*. *Journal of Invertebrate Pathology* 40: 36–40.