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Pathogenicity of *Beauveria bassiana* against the Tiger Moth, *Atteva sciodoxa* (Lepidoptera: Yponomeutidae)

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Abstract: Seven isolates of *Beauveria bassiana* were screened for pathogenicity and infectivity at a concentration of 5×10^7 conidia mL^{-1} against *Atteva sciodoxa* at 27±2°C and 75±5% relative humidity with 12 h photoperiod. Based on screening results, isolates Bba-Pp and FS-11 were further bioassayed at 1×10^6, 5×10^6 and 1×10^7 conidia mL^{-1}. All the isolates were found to be pathogenic. However, the infectivity varied significantly among the isolates. The earliest mortality was recorded three days after inoculation. The most virulent isolate, Bba-Pp, caused 100% mortality with a median infective time (ET_{50}) of 3.6 days on day seven following inoculation while FS-11 caused 83.3% mortality with an ET_{50} value of 4.1 days. Bba-Sl3 was the least infective isolate with 24.9% mortality and 15.3 days of median effective time. Mycelia appeared on 24 to 48 h old cadavers. The highest level of sporulation on two-week old cadavers was 150.6×10^5 Bba-Pp conidia mg^{-1} cadaver while the lowest was 12.23×10^5 Bba-Sl3 conidia. The median effective concentration (EC_{50}) of Bba-Pp was 9.89×10^5 conidia mL^{-1} while that of FS-11 was 3.85×10^6 conidia mL^{-1}. The ET_{50} values for 1×10^6 and 1×10^7 conidia mL^{-1} of Bba-Pp ranged between 7.0 and 4.4 days, respectively, while that of FS-11 were 10.3 and 5.8 days. A strong negative correlation was found between inoculum concentrations and food consumption ($R^2 = -0.99$). The infection by Bba-Pp and FS-11 resulted in 55.8 to 72.5% reduction in food consumption by *A. sciodoxa* compared to the controls.

Key words: *Beauveria bassiana*, pathogenicity, *Atteva sciodoxa*, tiger moth, *Eurycoma longifolia*, biological control

INTRODUCTION

There are many deleterious effects of chemical pesticides. The social and eco-economical problems such as health hazards, contamination of food and water, resurgence of primary pest, replacement of primary pest with secondary one, not only by the destruction of natural enemies but also by changing pest behavior, dispersal, development and fecundity are well recognized. Realizing these problems, recent sustainable forest management policy and the certification schemes of forests and forest products have given paramount importance to the environmental aspects of pest management and emphasis for the non-chemical pest control methods (Kneeshaw *et al*., 2000; Mihajlovich, 2001).

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Entomopathogenic fungi could provide environmentally benign alternatives to chemical pesticides due to their environmentally friendly characteristics. Successful application of *Beauveria bassiana* against several economically important pest insects and its wide distribution has rekindled interest to use it in non-chemical pest control programmes (Arcas *et al*., 1999). *Beauveria bassiana* has more than 707 host insect species (Li, 1988) belonging to 521 genera and 149 families of 15 orders. It is found globally in alpine soil, heathland, peat bogs, soils with savannah type vegetation, forest and cultivated soils, sand blows and dunes, desert soils, running water, rhizoplane of peat bog plants, the rhizosphere of clover, dead bark, nests, feathers and droppings of free-living birds (Zimmermann, 2007) and phylloplanes of various plants species (Meyling and Eilenberg, 2006).

Despite having a wide spectrum of insect host species and cosmopolitan distribution, studies have shown that virulence of *B. bassiana* isolates tends to be host specific (Goettel *et al*., 1990; Vestergaard *et al*., 2004). Isolates from different sources have variable degrees of pathogenicity against an insect species and vice versa (Cottrell and Shapiro-Ilan, 2003; Shah and Pell, 2003; Castrillo *et al*., 2004). It is therefore necessary to screen isolates for pathogenicity vis-à-vis virulence against a target insect species to find out the most infective one for further applications.

The tiger moth, *Atteva sciodoxa* is a serious perennial pest in *Eurycoma longifolia* plantations, a commercial medicinal plant species in Malaysia and the South East Asian region. The plant is commonly known as tongkat Ali in Malaysia and pasak bumi in Indonesia. *Atteva sciodoxa* attacks young apical shoots, resulting in stunted plant growth and also mortality of plant in case of severe infestation (Abood *et al*., 2008). *Eurycoma longifolia* roots and leaves are widely consumed for medicinal purposes and it is thus essential that pest control methods do not involve undesirable chemicals.

This study was conducted to (1) screen the most infective isolate of *B. bassiana* against *A. sciodoxa*; (2) compare *B. bassiana* vegetative growth, germination and sporulation on the host cadavers; (3) estimate the median effective concentrations and times of the most infective isolate(s) and assess effects of fungal infection on the food consumption of *A. sciodoxa*.

MATERIALS AND METHODS

This study was conducted at Faculty of Forestry, Universiti Putra Malaysia, Malaysia during 2007-08.

Fungal Isolates and Conditions

Seven *B. bassiana* isolates from various insect species and geographic origins (Table 1) were bioassayed against third instar *A. sciodoxa* larvae at 27±2°C and 75±5% relative humidity with 12 h photoperiod.

Table 1: Isolates of *B. bassiana* screened against *A. sciodoxa* at 27±2ºC and 75±5% relative humidity with 12 h photoperiod

Isolate	Host species	Taxonomic order of host	Geographic origin
FS-11	*Metisa plana*	Lepidoptera	Malaysia
F-1	Unknown	-	Japan
F-8	Unknown	-	Japan
Bba-Pp	*Pteroma pendula*	Lepidoptera	Malaysia
Bba-Sl1	*Spodoptera litura*	Lepidoptera	Malaysia
Bba-Sl2	*S. litura*	Lepidoptera	Malaysia
Bba-Sl3	*S. litura*	Lepidoptera	Malaysia

Cultures and Inocula

Isolates FS-11, F-1 and F-8 were obtained from Malaysian Palm Oil Board (MPOB) while Bba-Sl1, Bba-Sl2 and Bba-Sl3 from the Entomology Laboratory, Faculty of Forestry, Universiti Putra Malaysia. The isolates were maintained at 4°C on Potato Dextrose Agar (PDA). Isolate Bba-Pp was isolated from *Pteroma pendula* collected from a teak plantation in Sungai Buluh, Selangor, Malaysia.

Prior to bioassay, the isolates were passaged through *A. sciodoxa*. Fifteen 3rd instar larvae were exposed to two-week old cultures of each isolate for 10 min. The inoculated larvae were reared on *E. longifolia* for seven days. Dead larvae were separated daily and kept in a humidity chamber in 9.0 cm Petri dish with moistened Whatman® filter paper and sealed with parafilm for sporulation. Upon sporulation the cadavers were kept as nuclei cultures. A selective medium comprising PDA (Difco Laboratories, Augsburg, Germany) enriched with 0.5% Yeast Extract (YE) to which were added 0.05% streptomycin sulphate and 0.03% chloramphenicol, (Sigma Aldrich Chemie GmbH, Steinheim, Germany) was used to isolate inocula from cadavers. Five evenly spaced spots were marked in each Petri dish and were inoculated using sterilized inoculating needle. The inoculated Petri dishes were incubated at 27°C in darkness. After 24 h of incubation, the germinating spots were transferred singly on PDA+0.5%YE and incubated for two weeks at 27°C. Conidia were harvested from these cultures and used for further sub-cultures. These sub-cultures were maintained on PDA+YE at 27°C in darkness.

Bioassays

Conidia were harvested by scraping with a sterile glass rod two-week old cultures mixed with 20 mL of 0.02% aqueous Tween 80 in PDA plates. To prepare a homogenous suspension the mixture was transferred into test tubes and vortexed at a speed of 3000 rpm for 5 min using IKA®MS 3-Digital Vortex (IKA Werke GmbH and Co KG, Staufen, Germany). The aliquot was filtered twice using cheesecloth. A dilution of 1:100 was prepared and the appropriate serial concentrations of conidia were determined using Hirschman® Neubauer improved haemocytometer (Hirschmann Laborgeräte, Germany).

Stock culture of *A. sciodoxa* was bred on *E. longifolia* in the laboratory at 27±2ºC with 75±5% relative humidity and 12 h photoperiod. A total of 50 third instar larvae selected at random were inoculated at a concentration of 5×10^7 conidia mL^{-1} per isolate along with *E. longifolia* leaves using Preval® TLC sprayer (Precision Valve Corporation, NY, USA). Eight mililitre of conidial suspension was used per treatment spray while only aqueous 0.02% Tween 80 was used for the control set. The inoculated larvae were confined to cylindrical containers (11 cm diameter×8 cm) lined with moist filter paper for 24 h and then transferred to new containers provided with fresh untreated leaves. Survival of the larvae was monitored daily and moribund larvae were placed in humidity chambers for mycelial growth and sporulation.

Based on the screening results, two superior isolates, Bba-Pp and FS-11, were used to estimate the median Effective Concentrations (EC_{50}) and median Effective Times (ET_{50}). The inocula of Bba-Pp and FS-11 were obtained from fresh cultures as previously described and concentrations of 1×10^6, 5×10^6, 1×10^7 and 5×10^7 conidia mL^{-1} were prepared. A total of 50 third instar larvae per concentration were randomly selected from the stock culture and inoculated with different concentration levels as described earlier.

Sporulation and Viability

Fungal growth on two-week old cadavers was used to determine the number of conidia mg^{-1} cadaver body weight and their percentage germination. Ten milligrams of

cadaver along with fungal growth was mixed in 10 mL of 0.02% aqueous Tween 80. The mixture was vortexed for 5 min to provide a homogenous suspension. The aliquot was filtered twice using cheesecloth. Concentration of the conidia was determined in 1:10 time diluted suspension using Neubauer improved haemocytometer and the number of conidia was calculated for each mg cadaver body weight.

A 0.1 mL suspension from a 1:10 dilution was pipetted on PDA+YE plate and spread evenly using a cell spreader. The inoculated plates were incubated at 27°C in darkness for 24 h. The germinating and non-germinating conidia were stained with lactophenol cotton blue. A rectangular piece of each PDA isolate (1.5×2) cm^2 was mounted on microscope slide and examined for conidial germination using a compound microscope (400x).

Effect of Fungal Infection on Food Consumption

Effect of infection by Bba-Pp and FS-11 on food consumption of *A. sciodoxa* was assessed using a gravimetric method as described by Waldbauer (1968) for concentrations of 1×10^6, 5×10^6 and 1×10^7 conidia mL^{-1}. For each treatment, two sets of *E. longifolia* leaves of equal weight were provided: one to feed the larvae while the other was a control to obtain the equivalent oven-dry weight of the exposed leaf. After 24 h, the remaining leaves in each set were removed and oven-dried. Daily mean food intake per larva was calculated by subtracting the oven-dry weight of the remaining leaf from oven-dry weight of equal weight obtained from blank set, divided by the number of larvae in the respective treatment.

Experimental Designs and Statistical Analysis

The trials were set up in completely randomized design with five replications. The percent corrected mortality over time was calculated as described by Abbott (1925). Overall degree of pathogenicity was analyzed by 1-Way ANOVA, while differences among isolates and concentrations were analyzed by Tukey's (HSD) test.

Median effective concentrations (EC_{50} and EC_{99}) and median effective time (ET_{50}) of Bba-Pp and FS-11 were calculated on the basis of seven days of post-inoculation using Probit Programme Version 1.5 (US Environmental Protection Agency). The intercepts and slopes were calculated by Linear Regression analysis and further subjected to χ^2 test. Pearson's Linear Correlation was used to determine the correlation between $\log_{10}$ transformed inocula and total food consumed over seven days. The effect of inoculum concentrations on food consumption was further quantified by calculating R^2 values from linear regression analysis.

RESULTS

Screening of the Isolates

All isolates tested were found to be pathogenic against *A. sciodoxa*. However, the infectivity among isolates was highly variable. The earliest mortality was recorded on day three after inoculation (DAI). On day seven after inoculation, an overall highly significant ($F_{7, 32}$: 377.88; $p<0.01$) larval mortality was observed. Bba-Pp caused the highest mortality of 100% while Bba-Sl3 caused the lowest of 24.9±2.1 (Table 2). The differences between Bba-Pp and FS-11; FS-11 and F-8 were significant (Tukey's HSD; $p = 0.05$; CV: 7.6), while the differences among F-1, Bba-Sl1 and Bba-SL2 were not significant. The mortality caused by Bba-Pp was 1.2 to 4.0 times greater as compared to other six tested isolates.

The mean survival time after exposure also varied among the isolates. The shortest survival time of 50% larval population was 3.6 days in Bba-Pp treatment and this was

Table 2: Mortality and median effective times of *B. bassiana* isolates at 5×10^7 conidia mL^{-1} against 3rd instar *A. sciodoxa* at 27±2°C and 75±5% relative humidity with 12 h photoperiod

			95% fiducial limits (days)		
Isolates	Mortality±SE (%)	ET_{50}(days)	Lower	Upper	χ^2-value
Bba-Pp	100.0±0.0a	3.6	3.3	3.9	2.75
FS-11	83.3±2.5b	4.1	3.6	4.6	0.67
F-8	60.5±1.8c	5.7	5.1	6.4	3.67
F-1	41.8±1.1d	7.8	6.6	11.9	0.86
Bba-Sl1	37.3±1.6d	7.8	6.7	10.8	3.60
Bba-Sl2	35.3±2.0d	8.1	7.0	12.1	1.31
Bba-Sl3	24.9±2.1e	15.3	-	-	0.11

Means within columns with the same letter are not significantly different (p = 0.05, Tukey's HSD)

0.24 times that observed with Bba-Sl3 (15.3 days). The time taken by Bba-Pp to kill 50% population of *A. sciodoxa* was 0.24 to 0.88 times greater than the other six tested isolates. The intercept and slope values for median effective time of Bba-Pp were -9.89±1.97 and 7.70±0.98, respectively whereas these were ranged from -2.05±5.70 to -5.87±2.89 and between 2.75±2.63 and 4.75±1.35 for other tested isolates. The intercept and greater slope value of Bba-Pp indicates good potential for rapid high mortality (knockdown) effect as compared to the other isolates.

The results of screening bioassay further indicated that with the progression of post-inoculation time, inter- and intra-isolate mortality rates also varied. In all isolates, the mortality rate increased to a certain time after inoculation. The highest mortality rate was within 24 to 48 h after first occurrence of mortality. This indicates maximum mortality occurred between day 4 and 5 after inoculation. The highest mortality rate for Bba-Sl2, FS-11, Bba-Sl3, Bba-Pp, F-8, F-1 and Bba-Sl1 between any two successive days was 55.5, 68.8, 74.0, 91.2, 286.1, 289.6 and 311.0%. It was observed that there was a relatively higher rate of increment in mortality, i.e., 77.5 and 106.7% between days four and five after inoculation in Bba-Sl2 and Bba-Sl3. This was likely due to low initial mortality of 6.0% in these isolates at 4 DAI as compared to Bba-Pp which resulted in 62.4% mortality. The mean mortality at 4 DAI for Bba-Pp was 7.8 and 10.4 times of Bba-Sl2 and Bba-Sl3, respectively. This suggests that the temporal mortality increment rates with each isolate is high for those isolates which produced low early mortality, such as Bba-Sl2 and Bba-Sl3 (between 4 to 5 DAI). The overall mortality level remained high in isolates with high early mortality.

The trend and level of isolate infectivity remained the same even after 7 DAI. At 12 DAI, highly significant ($F_{7, 32}$: 176.53; $p<0.01$) difference in survival of *A. sciodoxa* was recorded. The lowest larval survival was zero percent in Bba-Pp while the highest among test isolates was 68% in Bba-Sl3 (Fig. 1). In the control, survival was 96% and the mortality recorded was non-infectious because no fungal growth appeared on the cadavers. The differences in mortality observed between Bba-Sl1 and Bba-Sl2, Bba-Sl2 and Bba-Sl3 as well as F-1 and Bba-Sl1 were not significant ((Tukey HSD, p = 0.05; CV: 10.50).

From the overall results of infectivity, the isolates can be classified into three groups:

- Isolates of high infectivity with a mean survival of inoculated *A. sciodoxa* between 0% to 20% (Bba-Pp, FS-11)
- Isolates of intermediate infectivity with mean survival between 30 and 50% (F-1, F-8)
- Isolates with low infectivity with greater than 50% mean survival (Bba-Sl1, Bba-Sl2, Bba-Sl3)

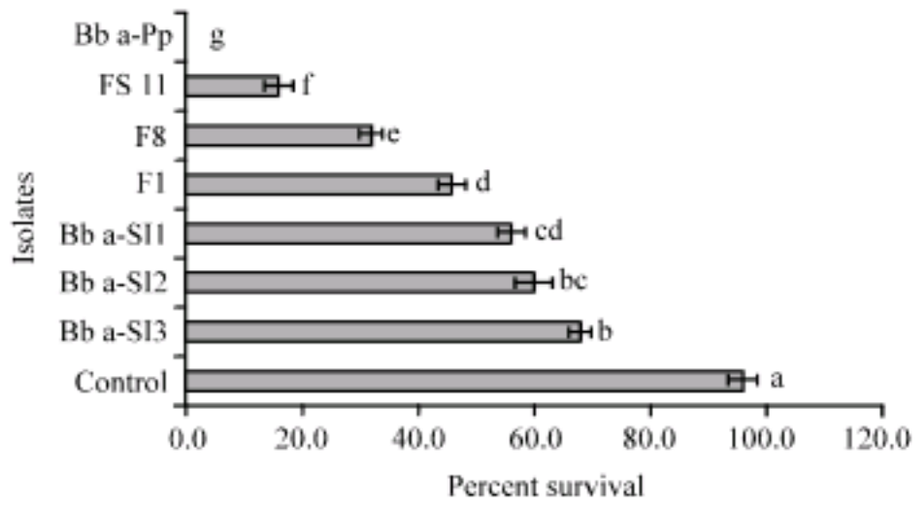

Fig. 1: Mean percent survival (±SE) of *A. sciodoxa* at 12 DAI with *B. bassiana* isolates at 27±2°C and 75±5% relative humidity with 12 h photoperiod

Table 3: Median effective concentration values (10^5 conidia mL^{-1}) of Bba-Pp and FS-11 against *A. sciodoxa* at 27±2°C and 75±5% relative humidity with 12 h photoperiod

		95% fiducial limits			95% fiducial limits		
Isolate	EC_{50}	Lower	Upper	EC_{99}	Lower	Upper	χ^2-value
Bba-Pp	9.89	4.9	15.2	287.0	150.0	1050.0	0.90
FS-11	38.45	19.2	64.2	13097.0	2980.0	300140.0	0.52

Median Effective Concentrations (EC_{50}) and Times (ET_{50})

The results showed considerable differences in both EC_{50} and EC_{99} values between Bba-Pp and FS-11. The difference between the two isolates was more profound at EC_{99} (Table 3). The EC_{50} of Bba-Pp showed that it required only 0.26 times inocula for the same level of effect as compared to FS-11 (9.89×10^5 conidia mL^{-1} and 38.45×10^5 conidia mL^{-1}) and for 99% mortality Bba-Pp required 2.87×10^7 conidia mL^{-1} which was 0.02 times that of FS-11 (130.97×10^7 conidia mL^{-1}). The estimated values of intercept of Bba-Pp and FS-11 were -4.53±1.81 and -1.05±1.18, while the estimated slope values were 1.59±0.28 and 0.92±0.17. The values of intercept and slope of Bba-Pp signifies a higher level of initial mortality as well as a steep mortality curve as compared to that of FS-11.

On day 7 after inoculation, a highly significant ($F_{8, 36}$: 309.86; $p<0.01$) inter-concentration effect, both in Bba-Pp and FS-11 was found. Isolate Bba-Pp at the concentration of 1×10^7 conidia mL^{-1} caused significantly (Tukey's HSD; $p = 0.05$; CV: 8.7) higher mortality than 5×10^7 conidia mL^{-1} of FS-11. The difference in mortality between 1×10^6 conidia mL^{-1} of Bba-Pp and that of 5×10^6 conidia ml^{-1} of FS-11 was not significant. Similarly, the difference in mortality between Bba-Pp at the concentration of 5×10^6 conidia mL^{-1} and FS-11 at the concentration of 5×10^7 conidia mL^{-1} was not significant. Isolate Bba-Pp at the concentrations of 1×10^6, 5×10^6, 1×10^7 and 5×10^7 conidia mL^{-1} caused 1.9, 1.4, 1.6 and 1.2 times greater mortality than that of the corresponding concentrations of FS-11 (Table 4). Within the isolates, the difference among concentrations was also highly significant (Bba-Pp $F_{4, 20}$: 729.96; $p<0.01$; FS-11 $F_{4, 20}$: 270.01; $p<0.01$) at 7 DAI. There was no significant difference in mean survival of *A. sciodoxa* when inoculated with 1×10^7 and 5×10^7 conidia mL^{-1} of Bba-Pp (Tukey's HSD; $p = 0.05$; CV: 6.5), while the difference between concentrations of 5×10^6 and 1×10^7 conidia mL^{-1} of FS-11 was not significant (Tukey's HSD; $p = 0.05$; CV: 8.4).

The median effective time (ET_{50}), of inoculated larvae varied with the concentration of inocula for both isolates. The ET_{50} values of Bba-Pp ranged between 3.6 and 7.0 days for concentrations of 5×10^7 to 1×10^6 conidia mL^{-1} while that of FS-11 were between 4.1 to 10.3 days. Bba-Pp at a concentration of 5×10^7 conidia mL^{-1} took 0.88 times the required time

Table 4: Median effective time for different concentrations (Conidia mL^{-1}) of Bba-Pp and FS-11 against *A. sciodoxa* at 27±2°C and 75±5% relative humidity with 12 h photoperiod

				95% fiducial limit (days)		
Isolate	Conc.	Mortality± SE	ET_{50} (days)	Lower	Upper	χ^2-value
Bba-Pp	1×10^6	50.0±1.8d	7.0	6.6	8.4	0.83
	5×10^6	81.7±1.9b	5.2	4.7	5.6	3.69
	1×10^7	95.0±2.2a	4.4	4.0	4.8	4.31
	5×10^7	100.0±0.0a	3.6	3.3	3.8	2.75
FS-11	1×10^6	NA				
	5×10^6	57.8±1.0cd	6.5	5.9	7.6	0.31
	1×10^7	60.9±3.0c	5.8	5.3	6.5	0.89
	5×10^7	83.3±2.5b	4.1	3.6	4.5	0.67

Means within columns with the same letter are not significantly different (p = 0.05, Tukey's HSD); NA: Larval mortality was less than 50% at 7 DAI

Table 5: Time to mycelial appearance (h) (±SE) of *B. bassiana* isolates on *A. sciodoxa* cadavers at 27±2°C and 90±5% relative humidity with 12 h photoperiod

	Time of death after inoculation (days)				
Isolates	3	4	5	6	7
FS-11	30.4±0.40c	27.6±0.98c	26.0±0.89b	24.0±0.00bc	24.0±0.00b
F-8	39.2±1.35b	34.8±1.20b	27.2±0.80b	24.0±0.00bc	24.0±0.00b
F-1	43.2±0.73a	36.0±0.00b	28.4±1.16b	24.0±0.00bc	24.0±0.00b
Bba-Pp	24.0±0.00d	22.4±0.98d	21.6±0.98c	20.0±1.09c	19.6±0.75c
Bba-Sl1	46.2±0.73a	37.6±0.98b	28.8±0.49b	26.0±1.26b	24.8±0.49b
Bba-Sl2	-	48.0±0.00a	43.2±0.73a	34.8±1.20a	29.2±0.49a
Bba-Sl2	-	48.0±0.00a	45.6±0.60a	36.0±1.26a	30.8±0.49a

Means within columns with the same letter are not significantly different (p = 0.05, Tukey's HSD)

to kill 50% of the inoculated larvae compared to that of FS-11. The difference in ET_{50} values was more evident at low concentrations than the higher concentrations. Bba-Pp at a concentration of 1×10^7 conidia mL^{-1} required almost the same duration for 50% mortality of inoculated larvae as that of FS-11 at a concentration of 5×10^7 conidia mL^{-1} (4.4 days and 4.1 days). An inoculum of 1×10^6 conidia mL^{-1} of Bba-Pp needed 0.68 times duration to kill 50% inoculated larvae that of FS-11 at the same inoculum level (Table 4).

Vegetative Fungal Growth and Sporulation

Mycelial appearance and time to mycelial appearance on cadavers are important parameters to assess larval death by fungal infection (Moorhouse *et al*., 1993; Vandenberg, 1996) and to determine the growth rate of the fungus. The results indicated that the time to mycelial appearance on cadavers is correlated to infectivity potential of the isolate as well as the time taken to death following inoculation. Mycelia of Bba-Pp appeared on 24 h old cadavers when larvae died at 3 DAI. This mycelial appearance of Bba-Pp was 0.79, 0.61, 56 and 0.52 times faster than that of FS-11, F-8, F-1 and Bba-Sl1, respectively. At 4 DAI, the time to mycelial appearance of isolates Bba-Sl2 and Bba-Sl3 was 2.1 times that of Bba-Pp. In all the isolates, the time to mycelial appearance on cadavers decreased as the time between inoculation and death increased (Table 5).

The time to mycelial appearance on cadavers ranged between 19.6 to 31.8 h when larvae died at 7 DAI. Based on the time to mycelial appearance on cadavers at 7 DAI, the isolates can be classified into three groups:

- Bba-Pp < 24 h
- FS-11, F1 and F-8 = 24 h
- Bba-Sl1, Bba-Sl2 and Bba-Sl3 > 24 h

Table 6: Mean number and viability of conidia on two-week old cadavers of *A. sciodoxa* at 27±2°C in darkness

Isolate	Conidial concentration (1×10^5 conidia mg^{-1})	Germination (%)
Bba-Pp	150.6±3.30a	95.6±0.51^{ns}
FS-11	109.1±2.45b	95.6±0.25
F-8	74.6±2.47c	95.2±0.66
F-1	58.4±0.64d	95.8±0.58
Bba-Sl1	60.1±1.58d	95.4±0.60
Bba-Sl2	37.5±1.20e	95.8±0.58
Bba-Sl3	12.2±0.84f	94.6±0.58
CV	14.2	-

Means within columns with the same letter are not significantly different; (p = 0.05, Tukey's HSD); ns: Not significant

The mean daily reduction in time to mycelial appearance on the cadavers from 3 DAI to 7 DAI for Bba-Pp, FS-11, F-8, F-1 and Bba-Sl1 were 3.7, 4.2, 7.8, 8.9 and 9.3%, respectively while the daily mean reduction in time to mycelial appearance on the cadavers from 4 DAI to 7 DAI for Bba-Sl2 and Bba-Sl3 was 9.8 and 9.0%. There was an apparent positive correlation between mortality caused by the isolate and time to mycelial appearance on cadavers.

The isolates which caused high early mortality, such as Bba-Pp and FS-11, appeared earlier on the cadavers as compared to isolates with low and delayed mortality, such as Bba-Sl1, Bba-Sl2 and Bba-Sl3. The rate of mean daily reduction in time to mycelial appearance on cadavers in low virulent isolates was relatively greater as compared to high virulent isolates. This observation may be attributed to the longer initial time taken for first mycelial appearance (4 DAI, 48 h) by low virulent isolates. The high virulent isolates appeared in less time (3 DAI, 24 h) therefore mean daily rate of reduction in mycelial appearance time was low.

Production of conidia by different isolates of *B. bassiana* may also affect the degree of pathogenicity because certain amount of inoculum is required to kill the target insect species. As such, conidial production, apart from abiotic and biotic factors, depended on the *B. bassiana* isolate (Luz *et al*., 1999); this was also exhibited in the present study, whereby, a highly significant ($F_{6, 28}$: 235.6; $p<0.01$) difference in conidial production was observed in the seven tested *B. bassiana* isolates. Isolate Bba-Pp was the most prolific isolate producing 150.6×10^5 conidia mg^{-1} on two-week old cadavers (Table 6). The differences between F-8 and Bba-Sl2; Bba-Sl1 and Bba-Sl2; F-1 and Bba-Sl3 were not significant (Tukey's HSD, p=0.05; CV: 14.20). Isolate Bba-Pp produced 1.4 to 12.3 times greater number of conidia mg^{-1} of cadaver as compared to other tested isolates. The production of conidia followed the trend of mortality and vegetative growth of the isolates (Table 2 and 5). The results indicated that pathogenicity and virulence are apparently positively correlated to vegetative growth and sporulation of the isolate.

The results of conidial germination ranged between 94.4±0.58 and 95.8±0.58%, thus indicating high viability of the isolates (Table 6). The overall difference in conidial germination of the seven isolates was not significant ($F_{6, 28}$: 0.53; $p>0.05$).

Effect of Fungal Infection on Food Consumption

Isolates Bba-Pp and FS-11 showed significant negative effect on chronological food consumption of *A. sciodoxa* (Fig. 2). However, there was no significant difference (p = 0.05) in food consumption within the first two days after inoculation. Initially there was a normal increase in food consumption in all treatments relative to the controls attributed to increase in larval age. At 3 DAI onwards, a significant ($F_{6, 28}$:17.3; $p<0.01$; CV: 1.1) reduction in food consumption was recorded. At 7 DAI, there was a highly significant ($F_{6, 28}$: 1496.6; $p<0.01$) difference among different concentrations of Bba-Pp, FS-11 and the control. In the control, larvae consumed significantly (Tukey's HSD; p = 0.05; CV: 0.95) more leaves (22.8 mg dry leaf $larva^{-1}$). The highest food consumption at 7 DAI among different

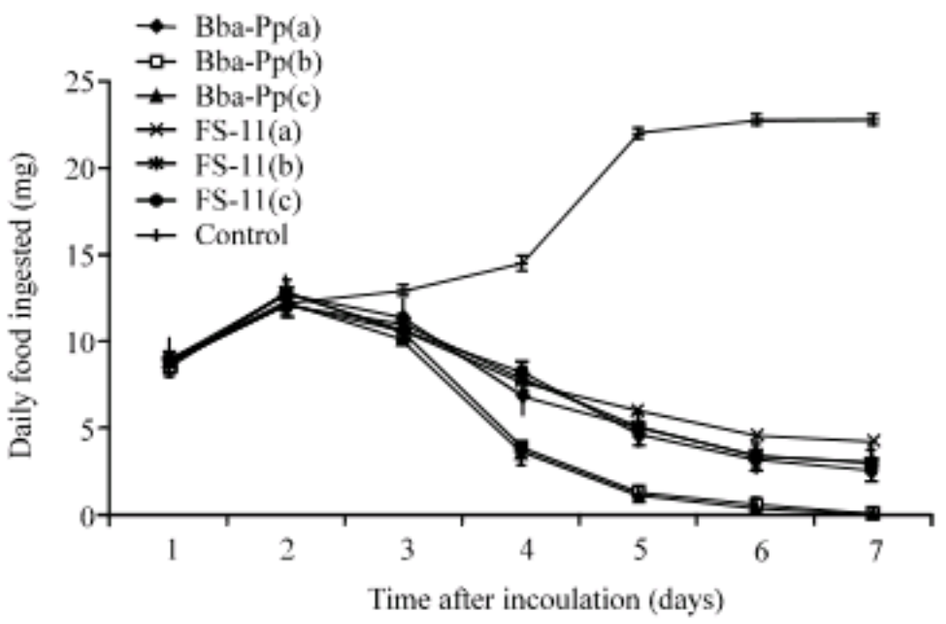

Fig. 2: Chronological food consumption by *A. sciodoxa* inoculated with Bba-Pp and FS-11 at 27±2°C and 75±5% relative humidity with 12 h photoperiod (a = 1×10^6 conidia mL^{-1}; b = 5×10^6 conidia mL^{-1}; c = 1×10^7 conidia mL^{-1})

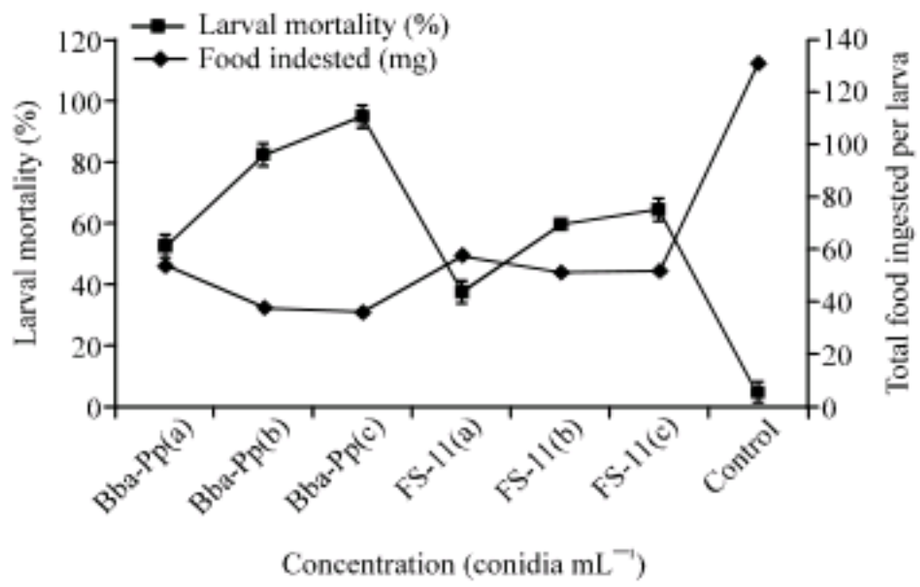

Fig. 3: Cumulative mortality (±SE) and amount of food ingested (mg dry leaf per larva) (±SE) by *A. sciodoxa* inoculated with different concentrations of Bba-Pp and FS-11 at 27±2°C and 75±5% relative humidity with 12 h photoperiod (a = 1×10^6; b = 5×10^6; c = 1×10^7)

concentrations of two isolates was observed in FS-11 at a concentration of 1×10^6 conidia mL^{-1} with a consumption of 4.2 mg dry leaf per larva. The difference in food consumption between larvae inoculated with 5×10^6 and 1×10^7 conidia mL^{-1} of Bba-Pp was not significant. Similarly, the difference in food consumption between larvae inoculated with Bba-Pp at a concentration of 1×10^6 conidia mL^{-1} and FS-11 at concentrations of 5×10^6, 1×10^7 conidia mL^{-1} were not significant.

The overall cumulative food consumed in different treatments over 7 DAI varied highly significantly ($F_{6, 28}$: 1570.7; $p<0.01$). Significantly, more food (131.3 mg dry leaf $larva^{-1}$) was consumed in the control (Fig. 3). The greatest (72.5%) reduction in food consumption was in Bba-Pp at 1×10^7 conidia mL^{-1} while the lowest was in FS-11 (55.8%) at 1×10^6 conidia mL^{-1} as compared to the control. There was significant difference in food consumption between Bba-Pp at concentration of 1×10^6 conidia mL^{-1} and FS-11 at 1×10^6 and 5×10^6 conidia mL^{-1}. Similarly there was no significant (Tukey's HSD, p = 0.05; CV 3.8) difference between

concentrations of 5×10^6 and 1×10^7 conidia mL^{-1} of Bba-Pp. A strong linear negative correlation was present between fungal inoculation and food consumption for both Bba-Pp and FS-11, with a correlation coefficient value of -0.99 for both.

The regression equations for Bba-Pp and FS-11 were y = 115.52-1.25x and y=114.75-9.58x, respectively. The coefficient values of correlation and regression analyses of both Bba-Pp and FS-11 showed that isolates were equally effective in reducing food consumption by *A. sciodoxa*.

DISCUSSION

Pathogenicity is a complex phenomenon that starts with the germination and generally percutaneous penetration of fungal germ tube and culminates in the death of the host. Apart from conidial germination and penetration, *in vivo* fungal growth, production of metabolites and toxins are important for pathogenicity. The germination of conidia may be affected by ambient climatic conditions especially cuticular temperature and moisture content (Keller and Zimmermann, 1989; Fuxa, 1995), availability of food on cuticle surface (free amino acids and a carbon source) (Smith and Grula, 1981), age of the insect host and presence of inhibitory compounds on cuticular surface (Hajek and St. Leger, 1994). The successful accomplishment of penetration of the germ tube is pre-requisite for pathogenicity. Besides activities during the course of penetration of the host cuticle, the pathogenicity and virulence of *B. bassiana* also depends on physiological characteristics of a fungal isolate and its potential of production of biochemical compounds within the insect host body (Bidochka and Khachatourians, 1990; Gupta *et al*., 1994).

The present pathogenicity results show that exogenous and endogenous conditions are conducive for *Atteva sciodoxa-Beauveria bassiana* pathogenic interactions. The occurrence of mortality on day three following inoculation reflects the pathogenic potential of the isolates. Previously, Adane *et al*. (1996) reported pathogenicity of different *B. bassiana* isolates to *Sitophilus zeamais* with considerable variation in the virulence among the isolates. The best isolates commenced mycosis as early as on day three after inoculation with mycelia appearing on 24 to 48 h old cadavers. Our findings also indicated that isolates that caused early mortality following inoculation (on day three after inoculation) resulted in greater mortality than those isolates with delayed mortality. These findings on time to mycelial appearance on cadavers are in corroboration with that of Adane *et al*. (1996). This signifies that commencement of mortality is an important criterion to assess the degree of pathogenicity. The early mortality also likely to help prevent crop losses by virtue of reduced food consumption.

The present variation in virulence of *B. bassiana* isolates may be due to different physiological characteristics and enzyme production potential of the isolates as reported previously by Leland *et al*. (2005). This observed variation in virulence of the isolates may also be attributed to geographic origins and insect host species. Our isolates are principally from two geographic origins and four insect host species. The virulence of two isolates of Japanese origin (F-1, F-8) was moderate while the isolates of Malaysian origin gave different results. The significant variation in virulence of the Malaysian isolates may be assigned to their distinct niche and host species. Bba-Pp and FS-11 were isolated from the bagworms, *Pteroma pendula* and *Metisa plana* infesting the teak, *Tectona grandis* and the oil palm, *Elaeis guineensis* grown in forest plantations, while Bba-Sls were isolated from the tobacco caterpillar, *Spodoptera litura* infesting the tobacco plants, *Nicotiana tobacum* grown in an agro-forestry system. The trend in virulence of Malaysian isolates seems to

follow the habitat of the host insect and plant species. *Atteva sciodoxa* has similar habitats to *P. pendula* and *M. plana* as *E. longifolia* is currently grown in plantations with *T. grandis* and *E. guineensis*. Previously influence of geographic origins on the pathogenicity of *B. bassiana* has been reported by Soper and Ward (1981) and Vandenberg (1996).

The present trend of median effective concentration (9.89×10^5 conidia mL^{-1}) and median effective time (3.6 days at inoculating concentration of 5×10^7 conidia mL^{-1}) of Bba-Pp is comparable with previous findings with different fungal isolates and insect species. For example, Feng and Johnson (1990), Ekesi (1999) and Sabbahi *et al*. (2008a, b) obtained LC_{50} values of 0.57×10^5, 1.8×10^5, 7.8×10^5 and 5.3×10^5 conidia mL^{-1} by using other isolates of *B. bassiana* against the Russian wheat aphid, *Diuraphis noxia*, the pod sucking bugs, *Clavigralla tomentosicollis*, *Lygus hesperus* and *L. lineolaris*, respectively. They found median effective times of 4.2, 4.1, 4.5 and 4.4 days at concentrations of 10^7, 10^8, 10^8 and 10^8 conidia mL^{-1}, respectively. The variation in calculated LC_{50} and LT_{50} values of different isolates of *B. bassiana* may be attributed to vegetative growth, sporulation characteristics, geographic origin and insect hosts of the isolates as previously reported by Feng and Johnson (1990). The differences in present EC_{50} and ET_{50} values and previously reported further be explained in terms of variations in virulence of different isolates to an insect species or variations in virulence of single isolate to related species of the host insect. Such variations have earlier been recorded by Khachatourians (1992) and Poprawski *et al*. (2000).

In addition to mortality, *B. bassiana* infection may also cause a spectrum of changes in behavior of host insect particularly in feeding. Our results showed that *B. bassiana* infection decreased significantly food consumption by *A. sciodoxa*. These findings are in corroboration with Tefera and Pringle (2003) where they found reduced mean daily food consumption associated with *B. bassiana* infection in the spotted borer, *Chilo partellus*. The reduction in food consumption became evident three to four days after inoculation and consequently food consumption decreased by 70 to 85%. Our study also showed significant reduction in mean daily food consumption starting on day three after inoculation with 55.8 to 72.5% less food consumption at different concentrations of FS-11 and Bba-Pp. The difference in the present results of overall reduced food consumption and those of Tefera and Pringle (2003) is likely to be due to the larval stage and inoculating concentrations used; the food consumption decreased with increasing inoculating concentration. They used 1×10^8 conidia mL^{-1} concentration against second instar larvae while we used a maximum 1×10^7 conidia mL^{-1} concentration against third instar larvae.

The effect of fungal infection on food consumption has also been reported in other insect pests, such as *B. bassiana* on *L. decemlineata* (Fargues *et al*., 1994) and *C. tomentosicollis* (Ekesi, 1999), *Entomophaga maimaiga* on the hairy caterpillar, *Lymantria dispar* (Hajek, 1989), *Metarhizium flavoviride* on the desert locust, *Schistocerca gregaria* (Moore *et al*., 1992) and *Nomuraea riley* on *Plathypena scabra* (Thorvilson *et al*., 1985). Reduction in feeding rate and ultimately cessation is likely to be due to progressive toxic effects of enzymes, metabolites and physical injury to the host tissues by mycelial growth. Samuels *et al*. (1988) and Vey and Quiot (1989) found that metabolites of fungi act on insect tissues, including the midgut, which adversely affects the feeding rate. These reduced feeding rates may help to prevent crop damage even insects are not killed by infection (Noma and Strickler, 2000).

CONCLUSION

Selection of a viable and virulent isolate is crucial for a successful microbial control of any insect pest. The seven isolates of *B. bassiana* used in this study exhibited pathogenicity

against *A. sciodoxa.* Bba-Pp was the most virulent with 100% mortality followed by FS-11 (83.3% mortality) on day seven after inoculation. The results further showed that isolates which have fast vegetative growth and high sporulation potential such as Bba-Pp and FS-11 were more virulent. The EC_{50} and ET_{50} values of Bba-Pp were superior to FS-11 and comparable to other virulent *B. bassiana* isolates tested against other insect pests. There was a strong negative correlation between fungal infection and food consumption. A 72.5% reduction in food consumption due to Bba-Pp infection suggests that the isolate has the potential to reduce plant damage. Based on these results of mortality, time to mortality and food consumption, it is concluded that isolate Bba-Pp has good potential for control of *A. sciodoxa* on *E. longifolia*. Finally, as isolate Bba-Pp is native to the tropical region thus has less environmental risks associated with field applications as compared to non-indigenous isolates.

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