

Potential of Selected Isolates of Entomopathogenic Fungi in Causing Lethal Infection against Various Developmental Stages of the Cocoa Pod Borer, *Conopomorpha cramerella* (Snellen) (Lepidoptera: Gracillariidae)

Yusof Bin Ibrahim

Department of Agricultural Science,
Faculty of Technical and Vocational Education,
Sultan Idris Education University,
35900 Tanjong Malim, Perak, MALAYSIA.
Email: yusofib@yahoo.com.my

Shari Fuddin Bin Sha'ari

Cocoa Research and Development Centre (MCB),
KM 27, Simpang Jalan Tingkayu/Kunak,
W.D.T. 175, 91207 Kunak, Sabah, MALAYSIA

Abstract – The pathogenicity of three species comprising 15 isolates of entomopathogenic fungi was evaluated in the laboratory against the eggs, last instar larvae (prepupae), pupae and adults of the cocoa pod borer (CPB), *Conopomorpha cramerella* (Snellen). The fungal isolates used were from *Metarhizium anisopliae* [Ma(SBN), Ma(Chi), Ma(SIB), Ma(SB8), Mman, MaPs, Ma1, Ma2, MMaj, MaGm], *Beauveria bassiana* [BBgc, Bb1, BbPs, BbPc] and *Iseria fumosoroseus* (Pf). Effective isolates were also tested for their field efficacy against the adults. Larvae (prepupa) inoculated with 0.5 mL conidial suspension of 1×10^6 conidia mL^{-1} in 0.05% aqueous Tween 80 began to manifest external disease symptoms three days after inoculation (DAI). All the isolates could cause infection with cumulative larval mortalities ranging from 55-100% at a concentration of 1×10^6 conidia mL^{-1} . Five superior isolates with the mean percentage mortalities in excess of 95% were BBgc, Ma(SBN), Ma(Chi), Ma(SB8) and Ma(SIB) with 100, 100, 97.8, 97.5 and 95.0% mortality, respectively. Adults inoculated with 0.5 mL of these five superior isolates at concentrations ranging from 1×10^4 to 1×10^8 conidia mL^{-1} began to show disease symptoms at 3 DAI. Six days after fungal application BBgc was the most efficacious recording 100% mortality followed by Ma(SBN) with 97.14%, Ma(SIB) with 94.29%, Ma(Chi) with 91.43% and Ma(SB8) with 82.86%. Eggs inoculated with 0.5 mL of 1×10^6 conidia mL^{-1} of the preceding two most effective isolates, BBgc and Ma(SBN), in 0.05% aqueous Tween 80 did not prevent hatching and subsequent boring of the pods, hatchability being 100%. Pupae inoculated with 0.5 mL conidial suspension of 1×10^7 conidia mL^{-1} using these isolates revealed that none could cause more than 50% infection at 7 DAI; however, isolate BBgc gave significantly higher mean percentage mortality of 43.12% compared to 26.3% for isolate Ma(SBN). Tests against the adults showed infected moths became moribund within 3-4 days and fully infected by the fifth day of treatment (DAT). Only isolate BbGc recorded complete mortality followed by Ma(SBN) with 97.14%, Ma(SIB) with 94.29%, Ma(Chi) with 91.43% and Ma(SB8) with 82.86% at 6 DAT. A field spray consisting of a suspension of 10 mL of 1×10^8 conidia mL^{-1} of isolates BbGc, Ma(SBN) and their 50:50 combination in 0.05% aqueous Tween 80 applied against caged adults significantly gave a complete kill compared to the control by the 6 DAT, and their efficacy was equal to that of the insecticide Decis®.

Keywords – *Conopomorpha cramerella*, Efficacy, Entomopathogenic Fungi, *Theobroma Cacao*.

I. INTRODUCTION

Cocoa, *Theobroma cacao* L., an indigenous crop of South America, originated from the rain forest of inter-tropical Americas. The crop was introduced into Malaysia through the Philippines in the 17th century. However, commercial planting only started in 1953 in Jerangau, Terengganu and followed later in 1955 by Borneo Abaca Limited (BAL) Plantation in Tawau, Sabah, Malaysia. By 1976 the price of dry cocoa beans in the world market had doubled that triggered a dramatic expansion in the cocoa planting area. Due to ease in land availability, the hectareage of cocoa rapidly increased in Sabah, while in Peninsular Malaysia planting as an intercrop with coconuts was much encouraged and had contributed to the rapid expansion in cultivation and production of cocoa. The hectareage reached its peak in 1989 with area planted in excess of 414,200 hectares producing about 247,000 tons of dried cocoa beans.

As an industrial crop, cocoa is the third important agricultural export commodity in Malaysia after palm oil and rubber, and ranked third in Asia and Oceania, after Indonesia and Papua New Guinea [1]. The area cultivated has progressively declined and currently recorded at about 20,543 hectares of which about 3,000 hectares are in productive phase. This declining trend is due, among others, to low price of cocoa beans, the cocoa pod borer *Conopomorpha cramerella* (Snellen) (CPB), black pod disease, labour constraint and competition from alternative crops like oil palm and rubber. Cocoa plantation area in Malaysia would be expanded and rehabilitated to 40,000 hectares in eight years' time at the rate of 2,000 hectares per year and producing an expected 60,000 tons of beans by the year 2020 [6]. According to the Director General of the Malaysian Cocoa Board, in 2012 Malaysia processed about 295,00 tonnes of imported cocoa beans from Indonesia, Ivory Coast and Ghana and have stabilized for the last three years, but cocoa grinding in Malaysia is seen to go up by 20% by the year 2020 [16].

The CPB, formerly known as *Acrocercops cramerella* Snellen, outbreak was first confirmed in Tawau in late 1980's and spread throughout Sabah within a period of 2.5 years [11], and it was subsequently detected in Peninsular

Malaysia in late 1986 [13]. Crop loss was due mainly from aborted pod development, small beans, non-extractable beans, premature ripening resulting in reduced bean weight and reduced cocoa butter content. This pest is also found on at least seven fruit plants [23]-[12]-[13], but they tend to be more seasonal and might not provide the right conditions for permanent establishment [21]. Currently, with good husbandary practices, limited control has been obtained using 'pod sleeving' with plastic sleeves and 'biocoater' sprays with tuber extract from the elephant yam *Amorphophallus muelleri* [22]. CPB have been eradicated from Queensland, Australia in under three years following an eradication programme ran under Emergency Plant Pest Response Deed (EPPRD) [14]. Monitoring was done for more than two years after the initial detection and prompt action was taken by spraying and stripping pods from trees thus breaking the life cycle of the pest.

A great deal of information has been generated on the efficacy of entomopathogenic fungi against insect pests. However, relatively little information exists for the cocoa pod borer (CPB), *Conopomorpha cramerella* Snellen. In the light of the ecological basis for the management of the cocoa pod borer, it is thus the objective of this paper to evaluate the infectivity of selected isolates of entomopathogenic fungi against the various developmental stages of *Conopomorpha cramerella* (Snellen) followed by comparative field sprays against the adults.

II. MATERIALS AND METHODS

A. Experimental Environment

Laboratory experiments were conducted in the laboratory of Plant Protection Department, Universiti Putra Malaysia (UPM) and Plant Pathology Laboratory of Malaysian Cocoa Board, Tawau, Sabah. All tests were conducted under an ambient laboratory environment of 28±2°C, 12:12 h (L:D) and 60-90% R.H.. A field experiment was carried out at a farmer's smallholding in Merotai, Tawau, Sabah, Malaysia. No rain was recorded during the duration of the experiment.

B. Insect Culture

The CPB took almost a month to complete its life cycle from egg to adult emergence. The culture was maintained systematically such that each life stage could be made available continuously when needed.

Infested cocoa pods collected from the field were placed on the floor in a heap of 150-200 pods covered with dried cocoa leaves in the laboratory maintained at 28±2°C, 12:12 h (L:D) and 60-90% R.H.. The resident last instar larvae would tunnel out of the pod but enter into a short prepupal stage and spin the cocoons within the dried leaves before metamorphose into obtect pupae. These pupae were separated daily from the leaves and placed in plastic containers measuring 23x12x16 cm each covered with a piece of muslin cloth. The moths that emerged about 6-8 days later were transferred to oviposition cages provided with 5% honey solution as food.

C. Preparation of Clean Pods

Cherelles measuring 4-7 cm long were sleeved by inserting each through a transparent polyethylene bag in

order to protect them from CPB or the mosquito bug *Helopeltis clavifer* (Walker) (Hemiptera: Miridae). The sleeves were removed two weeks later and the clean pods were then brought to the laboratory.

D. Source of Fungal Isolates and Culture

A selection of entomopathogenic fungi, namely *Metarhizium*, *Beauveria* and *Isaria* comprising of a total of 15 isolates (Table 1) mostly from a personal collection maintained at UPM was used in this study. One *B. bassiana* isolate was obtained from a scarabaeid beetle. Spores from this infected beetle, which was covered with a white cottony fungal growth, were scraped off using a sterile scalpel and were allowed to grow on potato dextrose agar (PDA). Fungal purification followed the standard microbiological technique. Two *M. anisopliae* isolates were obtained from cocoa rhizosphere by life bating technique using laboratory cultured mealworms, *Tenebrio molitor* (Tenebrionidae). These mealworms were placed on the surface of the soil samples in plastic containers (15mm diameter x 30mm) taken from the upper 30cm layer under the cocoa canopy of a farmer's small holding. These preparations were tightly sealed and placed in the dark at 28±2°C for two weeks. The fungus that grew over the larval cadavers was isolated following the standard laboratory protocol.

E. Preparation of Stock Culture

Malt Extract Agar (MEA) (Oxoid®) was used as the artificial growth media. Autoclaved at 121°C for 20 minutes at a pressure of 1.05kg cm⁻³, the sterilized media was left to cool to about 40°C before an amount of 15ml was transferred into a Petri plate and left to solidify. The fungal inoculum was transferred onto the plate by streaking it over the surface of the media using a sterilized inoculating loop needle. The plate was sealed with a masking film (Parafilm M®) and left to incubate in the dark. Sporulation occurred after 14 days of incubation. All test isolates were maintained on this media.

The stock cultures were prepared for longer storage using 9ml PDA media prepared in a universal bottle kept slanted at a 45° angle. Only a single spore of the fungus was isolated into a slant PDA using a single spore isolation technique in order to secure purity and virulence of the isolate. The lid was kept tightened and the media was left to incubate in the dark for about two weeks. These bottles were kept refrigerated at 4°C for subsequent studies.

F. Preparation of Conidial Suspension and Production of Conidia

Spores from a fully grown plate were scraped from the surface of the media using a sterile scalpel and suspended in a 0.05% aqueous Tween 80 (polyoxyethylenesorbitan monooleate). The suspension was sieved through four layers of muslin cloth to separate fragmented mycelium and other dirt. The conidial concentration was estimated using a Neubauer haemocytometer and subsequent appropriate dilutions were made thereof.

Isolates were mass-produced using a solid state fermentation technique following the method of Daoust *et al.* [4]. A 15% broken rice constituted the substrate for *Metarhizium* while broken grain corn was found suitable

for *Beauveria*. These grains were washed three times through tap water and rinsed for five minutes before filling with 250g of them into an autoclaveable polypropylene bag which was rounded with a 3cm PVC pipe ring at the top. These bags were sterilized at 121°C for 30 minutes at a pressure of 1.05kg cm⁻³. After 24h of cooling period, each bag was inoculated with 10ml of the respective conidial suspension, plugged with a cotton clump and then incubated in the dark at 28±2°C for two weeks. The bag was shaken once a day for the first three days to distribute the inoculum evenly. After two weeks the established spores with the substrate were spread evenly on clean paper towels and air dried for 3-5 days in the laboratory. Dried spores were harvested by sieving through a 125µm particle size sieve using an orbital shaker. The conidia were kept tightly in a universal bottle and refrigerated at 4°C.

G. Pathogenicity test on larvae

From the heap of cocoa leaves of the CPB culture in the laboratory, the light green last instar larvae (prepupae) were removed from the cocoon before they turned into obtect pupae three days later. Tests on the larvae were conducted in Petri dishes layered with moistened filter paper. Ten larvae placed in each treatment dish were inoculated with freshly prepared suspension of 0.5mL of 1x10⁶ conidia mL⁻¹ in 0.05% aqueous Tween 80 of the respective isolates (Table 1) using a Sigma[®] hand atomiser. The atomizer could deliver an even volume diameter droplet spray of 75µm (VMD). The treatments were completely randomised with four replications. Observation for fungal infection was recorded six days after inoculation. Five most effective isolates that gave larval mortality in excess of 95% were selected for subsequent test on adults. A single spore was re-isolated from these infected larvae to ensure that only mutant virulent conidia were isolated for subculture on artificial media. This was done to avoid decreasing capacity to sporulate and weakened virulence [5]. The virulence, viability and field efficacy of the pathogens are also influenced by storage conditions and their formulations [2], hence fresh cultures were used in these studies.

H. Pathogenicity Test on Adults

An experimental cage was assembled consisting of a 9cm glass Petri dish as the base, a cylindrical hard transparent polyethylene (9cm diameter x 20cm) fitted into the glass base covered with a piece of muslin cloth. The glass base was layered with a moist cotton pad to provide a constant relative humidity and was separated by a fine-sized mosquito net in order to prevent the insect from being stuck to the moist cotton. A serial concentration of fresh conidial suspensions ranging from 1x10⁴ to 1x10⁸ conidia mL⁻¹ in 0.05% aqueous Tween 80 were prepared. Ten 3-day old CPB moths placed in each treatment cage were inoculated with 0.5mL of conidial concentration of the respective five superior isolates [Ma(SBN), BBgc, Ma(Chi), Ma(SB8) and Ma(SIB)] with the aid of a Sigma[®] hand atomizer. The cages were completely randomised and each treatment was replicated three times with an untreated control. Mortality was recorded daily for seven days post-inoculation. The dead and moribund adult with

fungal growth was recorded as a successful infection. Pathogenicity was assessed by estimating the median effective concentration (EC₅₀) and regression relationship line with 95% fiducial limit (FL) using an EPA Probit Analysis Programme (Version 1.5, Ohio, USA). The two most pathogenic isolates against the adult [Ma(SBN), BBgc] were selected for subsequent studies.

I. Pathogenicity Test on Eggs

The eggs were prepared by placing a pair of adults in an experimental cage provisioned with 5% honey solution and allowed to mate for three nights. A medium size freshly harvested clean pod was hung and left overnight for oviposition. The pods with eggs were collected the following day. A fresh and fertile egg is orange in colour, ovoid and decorated with rectangular indentations. Pods with 24h eggs were cut into portions of small blocks and arranged in a 2x2 cm area containing a total of 20 eggs. The blocks were then placed in a plate layered with two-ply of moistened filter paper. Inoculation was done by evenly spraying over the blocks 0.5mL of freshly prepared suspension of 1x10⁶ conidia mL⁻¹ of isolates Ma(SBN) and BBgc in 0.05% aqueous Tween 80 with the aid of a Sigma[®] hand atomizer. The plates were completely randomised with three replications and a control without the conidia treatment. Egg hatching was observed daily for three consecutive days with the aid of a magnifying glass.

J. Pathogenicity Test on Pupae

To initiate tests on pupae, infected pods were hung 60 cm above the laboratory floor layered with dried leaves as pupal substrate. The respective conidial suspension of freshly prepared 0.5mL of 1x10⁷ conidia mL⁻¹ of isolates Ma(SBN) and BBgc in 0.05% aqueous Tween 80 was sprayed evenly over the upper surface of the dried leaves with the aid of a Sigma[®] hand atomizer. Treatments were completely randomised with three replications with the addition of an untreated control. Pupae formed on the dried leaves were transferred individually into Petri dishes layered with moistened filter paper. The pupae were observed for seven consecutive days for fungal infection.

K. Field Experiment

A smallholder's plot at Merotai, Tawau, Sabah was chosen where 20-year old cocoa trees were rehabilitated with new clones five years earlier and grown without shade trees. Pests and diseases were managed following the usual estate practices; insecticides were sprayed 18-20 times a year to control the cocoa pod borers. Trees with properly oriented horizontal branches and good canopy to provide good shades were selected; only branches protected from direct sunlight were chosen. These branches measuring approximately 100cm long were cleared from any ant colonies while small twigs and leaves were removed. Only trees with homogenous canopy were included in a completely randomised design.

A cylindrical wire sleeve (30cm diameter x 100cm long) made of muslin cloth was assembled such that it enveloped the selected pod bearing branch with both ends of the sleeve secured tightly and smeared with grease to prevent intrusion of ants and other crawling predators. In each of the sleeve supplied with 5% honey solution as food, ten 3-day old CPB moths (undetermined sex) were

released. Once all the moths had rested under the branch, a freshly prepared suspension of 10mL of 1×10^8 conidia mL⁻¹ of isolates Ma(SBN), BBgc and their 50:50 mixture in 0.05% aqueous Tween 80 was sprayed into the sleeve using a Sigma® hand atomizer. As comparisons, a treated control consisting of deltamethrin (Decis®) and a control with 0.05% Tween 80 only were used. All treatments were replicated three times. Mortality was recorded daily for seven consecutive days and the results were analysed using 1-way ANOVA and LSD [17].

III. RESULTS

A. Larval Mortality

Under the ambient laboratory environment, the treated larvae began to manifest external disease symptoms three days of post-inoculation; all the isolates caused infection resulting in mortalities ranging from 55-100% at a concentration of 1×10^6 conidia mL⁻¹ recorded six days after inoculation. The percentage cumulative larval mortalities upon exposure to the isolates are shown in Fig. 1. All the larvae in the controls survived to adulthood. The five most superior isolates with the mean percentage mortalities in excess of 95% were Ma(SBN), BBgc, Ma(Chi), Ma(SB8) and Ma(SIB) with 100, 100, 97.8, 97.5 and 95.0% mortality, respectively. These five most pathogenic isolates were selected for subsequent pathogenicity tests against the adults, and two most superior isolates thereof were used against the eggs and the pupae.

B. Adult Mortality

Pathogenicity tests indicated that all the four isolates of *M. anisopliae* and one isolate of *B. bassiana* were able to infect the CPB adults (Table 2). The adults showed disease symptoms three days after inoculation. Six days after fungal application BBgc was the most efficacious recording 100% mortality followed by Ma(SBN) with 97.14%, Ma(SIB) with 94.29%, Ma(Chi) with 91.43% and Ma(SB8) with 82.86%. Isolates BBgc and Ma(SBN) were subsequently used for pathogenicity tests against the eggs and pupae.

The infected moths became moribund within 3-4 days and fully infected at five days post-treatment at a concentration of 1×10^6 conidia mL⁻¹. Both *M. anisopliae* and *B. bassiana* mycelial growth over the cadaver had sporulated with dark green colour and powdery white on the 12th day, respectively. Estimates of three best EC₅₀ with 95% FL computed beginning with the lowest value were 1.123×10^4 conidia mL⁻¹ (BBgc) > 1.133×10^5 conidia mL⁻¹ (Ma(SBN)) > 3.65×10^6 conidia mL⁻¹ (Ma(SIB)) (Table 3). The BBgc isolate was the most pathogenic, being approximately 10 times more pathogenic than Ma(SBN) and 325 times more than Ma(SIB).

C. Egg and Pupal Mortality

A fresh and fertile egg is orange, flat, oval and decorated with rectangular indentations. None of the two selected superior isolates was pathogenic on the eggs, hatchability being 100%. A close examination revealed that the first instar (neonate) larva affixed itself on the floor of the egg corion and immediately tunneled into the pod.

In the case of the pupa, not more than 50% infection was observed seven days of post-inoculation; however, isolate BBgc significantly gave higher mean percentage mortality (43.12%) compared to isolate Ma(SBN) (Table 4).

D. Field Experiment

Results of the field experiments were consistent with that of the laboratory studies whereby both *B. bassiana*, *M. anisopliae* and their combination significantly recorded the highest mortality compared to the control, and their efficacy was equal to that of the insecticide Decis® (Table 5).

At 2-DAT, deltamethrin (Decis®) significantly inflicted the highest mortality, understandably due to the nature of the immediate effect of the insecticide compared to the slow acting infection of the entomopathogenic agents. From 3-DAT onwards, the fatal effect of the fungi began to take effect as the hydrophobic conidia began to germinate within 8-16 h of contact followed by germ tube penetration of the cuticle. Both fungal isolates and their combination gave 100% kill by the 6th day.

IV. DISCUSSION

From all the experiments conducted, the isolates BBgc and Ma(SBN) had demonstrated to be the most successful entomopathogenic fungal agents against the CPB and consistently inflicted high mortality on the adults, but to a lesser degree on the pupa. Results of laboratory tests against the larvae varied among the isolates due to the factor of virulence among strains besides the degree of susceptibility of the insect species [18]. Nevertheless, the chances of fungal infection occurring on the larva in the field are practically nil since upon hatching the larva would penetrate immediately into the cocoa pod and become very well protected once inside it. Therefore, it is not prudent for the entomopathogenic fungal application to be directed on the larval stage.

The chances of infection to occur on the eggs are also practically nil. The neonate larva positioned itself on the floor of the egg corion and survived by immediately tunneling into the pod. Hendrawan [7] reported similar result by the rice moth *Corcyra cephalonica* whereby no fungal infection on the eggs was observed. In contrast, Priyatno [15] indicated that *M. anisopliae* could infect the eggs of the flea beetle *Phyllotreta striolata*, while egg hatching was also inhibited in the red spider mite *Tetranychus urticae*, however, *B. bassiana* could not infect the spider mite eggs [8]. The eggs of the latter two arthropod pests are much smaller and hence took a shorter time and easier for the fungal germ tube to penetrate through the corion. On the contrary, in the case of the CPB, the egg is larger such that the germ tube failed to reach the embryo in time; the embryo remained at the floor of the corion ready for hatching. Since the duration of the egg stage is short, the germ tube penetration could not completely occur before egg hatching.

A low infection on the pupae was probably due to the last instar larvae formed into an obtect pupa, similar to a chrysalis, within a cocoon and perhaps had become more

resistant to fungal invasion. A closer observation revealed that when a wider space existed between the exuviae and the new cuticle formed during pupation there was inhibition of the germ tube penetration. Cloyd [3] reported that some insects have developed physiological mechanism to reduce fungal infection by avoiding infection through rapid moulting or developing a new integument before the mycelial hyphae could penetrate the new cuticle. Also, the surface condition of the insect's cuticle could either enhance or inhibit the infection [24]-[19]-[20]. Pupal infection in the field is expected to be low; however, it could still indirectly reduce the population of the following generations.

Temperature and humidity are part and principal to the factors that influence efficacy of entomopathogenic fungi. Temperatures above 30°C would inhibit vegetative growth of most fungi, while high moisture is essential for germination of propagules. The cocoa canopy provides a cooler temperature and maintains a suitable moisture where little light reaches the lower branches. As such during the day time these lower branches become conducive for CPBs to rest which incidentally also provides a conducive environment for the germination of conidia. As such both fungal isolates and their combination gave 100% kill by the end of the week; the death could be delayed if and when the field environment becomes unfavourable [9]. The dual-culture treatment showed combined infection thus no antagonistic effect was evident between both pathogens. As suggested by Tanada and Kaya [18], the combined effect could be greater than a single pathogen. When applied at the recommended dosage Decis® resulted in a maximum kill of only 83.33%, i.e. a situation that could be attributed to the development of some degree of resistance as stated by Lee [10] who reported that a higher dosage of deltamethrin was required of up to 4.45 times to completely kill CPB moths.

In a large scale field spray of entomopathogenic fungi, the spray residue would have accumulated on the dried leaf litter layer and would inevitably inflict an additional mortality on the pupae in the leaf litter, hence providing an indirect contribution to the total mortality.

Notwithstanding that the laboratory and field results revealed effective control on the adults, a large scale field study is being planned to validate the real efficacy. The frequency of field application needs to be determined in order to establish an optimum level of fungal sprays as well as to manage a low CPB infestation. The timing of sprays is important since sunlight inactivates fungal vegetative growth. It would be wise to presume that spraying in late afternoon to evening would benefit the pathogen since the CPB adults are active and become easy targets. The spray droplets that eventually fall onto the leaf litters underneath the canopy would incidentally infect the last instar larvae that drop out from the infested pods to pupate.

V. CONCLUSION

Results from these studies showed that infection by both isolates *B. bassiana* (BBgc) and *M. anisopliae* (Ma(SBN))

was more successful on the adult stage and consistently inflicted high mortality than the pupae or the eggs. The likelihood of infection on the egg or early larval stages in the field is practically nil. Therefore, the strategy of using entomopathogenic fungi would be best targeted against the adults, either replace or complement chemical insecticides in an integrated pest management programme for a better control approach against the cocoa pod borer *C. cramerella*.

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Table 1: Entomopathogenic fungal isolates, their original hosts and countries of origin

Fungus	Code	Host	Country of Origin
<i>Beauveria bassiana</i> (Balsamo) Vuillemin	Bb1	Scarabaeid beetle	Malaysia (Sarawak)
<i>Metarhizium anisopliae</i> (Metch) Sorokin	Ma1	Cocoa rhizosphere	Malaysia (Sarawak)
Personal Collection:	Ma2	Cocoa rhizosphere	Malaysia (Sarawak)
<i>Beauveria bassiana</i> (Balsamo) Vuillemin	BBgc	<i>Glenia celia</i> (Cerambycidae)	Malaysia (Sabah)
	BbPs	<i>Phyllotreta striolata</i> (Chrysomelidae)	Malaysia (Selangor)
	BbPc	<i>Phyllotreta cruciferae</i> (Chrysomelidae)	Malaysia (Selangor)
<i>Metarhizium anisopliae</i> (Metch) Sorokin	MMaj	<i>Oryctes rhinoceros</i> (Scarabaeidae)	Malaysia (Selangor)
	Mman	<i>Oryctes rhinoceros</i> (Scarabaeidae)	Malaysia (Selangor)
	Ma(SBN)	<i>Coptotermes curvignathus</i> (Rhinotermitidae)	Malaysia (Selangor)
	MaPs	<i>Phyllotreta striolata</i> (Chrysomelidae)	Malaysia (Selangor)
	MaGm	<i>Galleria melonella</i> (Pyralidae)	Malaysia (Selangor)
	Ma(SIB)	Termite nest	Malaysia (Selangor)
	Ma(Chi)	Termite nest	Malaysia (Selangor)
	Ma(SB8)	Termite nest	Malaysia (Selangor)
<i>Iseria fumosoroseus</i> (Wise) Brown & Smith	Pf	<i>Pteroma pendula</i> (Psychidae)	Malaysia (Selangor)

Table 2: Mean percentage mortality of CPB adults upon exposure to selected entomopathogenic fungal isolates

Isolates	Days after treatment				
	2	3	4	5	6
BBgc	0	22.85a	71.43a	91.43a	100.00a
Ma(SBN)	0	25.72a	85.71a	91.43a	97.14ab
Ma(SIB)	0	22.86a	74.29a	88.57a	94.29ab
Ma(Chi)	0	34.29a	34.29a	80.00a	91.43ab
Ma(SB8)	0	31.43a	68.57a	82.86a	82.86 b
Control	0	0	0	0	5.70 c

Mean percentage mortality of 5 adults per replicate, 7 replicates at a dose of 1×10^6 conidia mL⁻¹. N=210 moths
Means within columns followed by the same letter are not significantly different at p=0.05 according to LSD.

Table 3: Probit analysis for BBgc, Ma(SBN) and Ma(SIB) isolates against CPB adults

Isolate	a Intercept	b ± SE Slope	χ ²	EC ₅₀ (conidia mL ⁻¹)	95% FL (conidia mL ⁻¹)
BBgc	3.830	0.289 ± 0.36	12.30 (7 df)	1.123 E + 04	2.383 E + 03 - 3.887 E + 04
Ma(SBN)	3.783	0.241 ± 0.34	2.496 (7 df)	1.133 E + 05	2.45 E + 04 - 4.58 E + 05
Ma(SIB)	2.744	0.343 ± 0.077	24.19 (7 df)	3.654 E + 06	3.654 E + 05 - 1.92 E + 08

Table 4: Mean percentage mortality of CPB pupae upon exposure to selected entomopathogenic fungal isolates

Isolates	Mean percentage mortality
<i>B. bassiana</i> (BBgc)	43.12 a
<i>M. anisopliae</i> (Ma(SBN))	26.33 b
Control	0 c

Means followed by the same letter are not significantly different at $p=0.05$ according to LSD.

Table 5: Mean percentage cumulative mortality of CPB adults upon exposure to selected entomopathogenic fungal isolates

Treatments	Days after treatment (DAT)					
	1	2	3	4	5	6
<i>B. bassiana</i> (BBgc)	0.00b	6.66ab	36.66a	86.66a	100.0a	100.0a
<i>M. anisopliae</i> (Ma(SBN))	3.33a	20.00ab	43.33a	80.00a	86.66a	100.0a
Mixture (50:50)	0.00b	3.33b	30.00a	70.00a	86.66a	100.0a
Decis®	50.0a	73.33a	80.00a	83.33a	83.33a	83.33a
Control	0.00b	0.00b	0.00b	3.33b	3.33b	6.66b

Mean percentage mortality of 10 adults per replicate, 3 replicates at a dose of 1×10^8 conidia mL^{-1} . N=150 moths
Means within columns followed by the same letter are not significantly different at $p=0.05$ according to LSD.

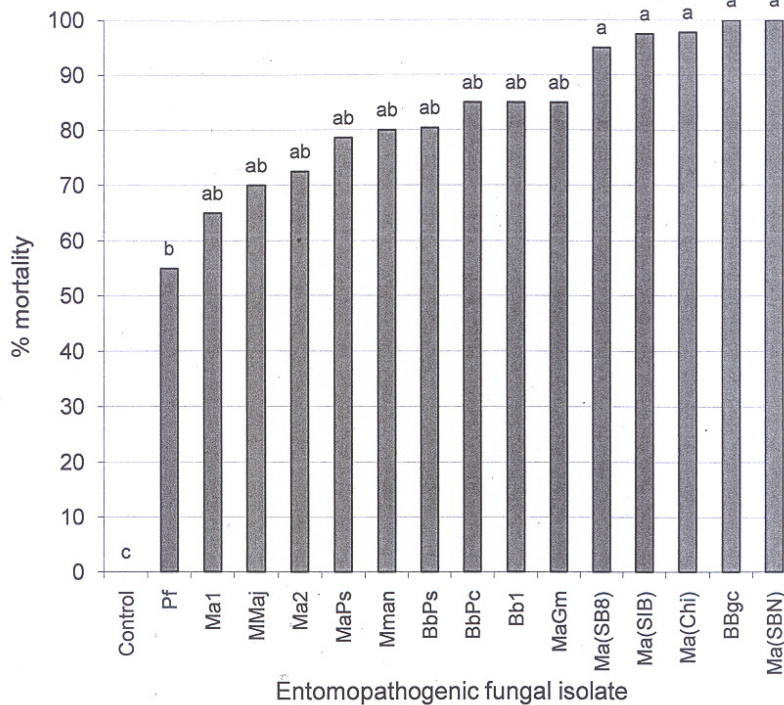


Fig.1. Mean percentage cumulative mortality of last instar larvae upon exposure to entomopathogenic fungal isolates at six days of post-treatment.