Chiang Mai J. Sci. 2012; 39(3) : 464-477
http://it.science.cmu.ac.th/ejournal/
Contributed Paper

Evaluation of Effective Entomopathogenic Fungi to Fruit Fly Pupa, *Bactrocera* spp. and Their Antimicrobial Activity

Thet Thet Mar and Saisamorn Lumyong*

Microbiology Division, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.

*Author for correspondence; e-mail: scboi009@gmail.com , saisamorn.l@cmu.ac.th

Received: 24 August 2011
Accepted: 13 January 2012

ABSTRACT

The activities of six entomopathogenic fungal isolates, collected from naturally infected insects, were tested against fruit fly *Bactrocera* spp. pupa (Diptera: Tephritidae) and evaluated *in vitro* with different conidial concentrations. All tested isolates were pathogenic to fruit fly pupa. According to the 50% lethal concentration, *Metarhizium flavoviride* CMUCDCT01, *Paecilomyces lilacinus* CMUCDMT02 and *Beauveria bassiana* CMUCDMF03 had the highest pathogenicity at the conidial concentration of (1×10^8 spore ml^{-1}). Mortality of pupa varied from 25.89% to 100% in *M. flavoviride*, 22.22 to 100% in *P. lilacinus* and 29.67% to 100% in *B. bassiana*. In soil bioassays, pupal mortality was not significantly different between drenching and premixing the conidial suspension with soil. However, the mycelia structure of fungal isolate CMUCDMF03 was recorded in premixing of the conidial solution with soil. According to our investigation of antimicrobial activity of fungal isolates, certain isolates produce antibacterial and antifungal activity in different cultured media without the addition of insect-derived materials.

Keywords: pathogenicity, fruit fly, drenching, premixing, mortality, antimicrobial activity

1. INTRODUCTION

Fruit flies belonging to the family Tephritidae (Order: Diptera) are considered a very destructive group of insects that cause enormous economic losses in agriculture, especially in a wide variety of fruits, vegetables and flowers [1]. All countries in Southeast Asia and the Pacific Islands suffer from major economic losses from infestations of tropical fruit flies [2]. The total number of species within this family exceeds 4,000. Approximately 10% of them are serious pests distributed around the world in temperate, subtropical and tropical areas [3-5].

In particular, two species belonging to this family are of great importance in Thailand, namely the Melon fly (*Bactrocera cucurbitae* (Coquillet)) and the Oriental fruit fly (*Bactrocera dorsalis*). The production of fruits and vegetables in Thailand is an important source of income. These crops represent an integral part of the gastronomic culture for

Thai people [6].

The use of insecticides as the only way to control pests in fruits and vegetables causes environmental pollution and hygienic problems that represent a risk for people and animals [7]. In the last four decades the use of synthetic pesticides such as organophosphate and carbamates in an intensive way has led to the development of insecticide resistant pest species [8-10] and in Thailand residues of organophosphate and organochloride and other compounds have been detected in soil, water and crops [11]. Insecticide resistance is a serious problem in agriculture, ending up with the tolerance and/or adaptation of insects to insecticides [12]. The indiscriminate use of pesticides to control fruit flies causes a serious ecological imbalance and triggers the emergence of populations of other pests by eliminating natural enemies, and leads to human infections and the environmental degradation [13]. Other approaches to fruit fly management, such as the use of protein baits has been more or less ineffective possibly due to the ecology of the fruit flies [6]. The microbial control of fruit flies can be a process that can partially replace other methods of control in an integrated management program for these insects, especially the use of agrochemicals, presenting economic and environmental advantages for the tropics [14].

Entomopathogenic fungi (EPFs) are classified as fungi that infect, invade, and eventually kill their host insects [15]. These fungi are also a rich source of natural bioactive compounds. The presence of antibioactive compounds in entomopathogenic fungi has been investigated by several studies. Moreover, various secondary metabolites produced by EPFs are beneficial for humans, particularly when they infect the corresponding host insects, these organisms have been considered as promising sources of novel bioactive compounds. Secondary fungal metabolites represent a diverse group of bioactive compounds characterized by their origin and biosynthetic pathways. In fungi they serve as regulators, chemical messengers in developmental processes, or as a defense system for the survival of the organism against their environment [16].

However, because of low availability and cultivation difficulties, there have been only a few studies on the characterization of the products of entomopathogenic fungi, such as destruxins from *Metarhizium anisopliae* [17-19], beauvericins from *Beauveria bassiana* [20], and cordycepin (a nucleoside analogue 3′-deoxyadenosine) from *Cordyceps sinensis* [21, 22]. Nowadays, there is a drive to find new bioactive agents [23]. But the pathogenicity of locally isolated entomopathogenic fungi to fruit fly has not yet been studied. In nature, fruit fly pupation takes place in the soil, and the control strategies of the pupal stage is one of the appropriate method in maintaining the population of fruit fly to some extent. Recently we have been isolated entomophagous fungi from natural habitats. To apply these fungi for biocontrol of insect pest we need virulence strain together with the high productivity of spore.

Therefore, the purposes of this study were:(1) to evaluate the virulence strains of EPFs against fruit fly pupa, (2) to screen the productivity of antimicrobial activity in collected EPFs.

2. MATERIALS AND METHODS

2.1 Fruit Fly Collection and Handling

The infested fruits with ovipositional scars or marks of larval infestation of tephritid fruit fly were collected from rose apple and star fruit trees. Last instar larvae of average size 7-11 mm in length were collected from the infested fruits and preserved in the plastic bottle containing a layer of dry sand

at the bottom [24] in order to pupate in the soil. When pupae turn brown, at least 2 days after pupation, were sterilized with 0.5% (v/v) sterilized sodium hypochlorite solution prior to bioassay.

2.2 Fungal Isolates and Preparation of Conidial Suspensions

Six entomopathogenic fungal isolates: *Metarhizium flavoviride* CMUCDCT01, *Beauveria bassiana* CMUCDMF03, *M. anisopliae* CMUCDMF04, *Paecilomyces lilacinus* CMUCDMT02, *Isaria tenuipes* CMUCDMF02 and *B. bassiana* CMUCDMG03 collected from natural habitats were used to test the pathogenic activity against fruit fly pupa. Fungal strains were cultured on PDA medium and incubated at 25±2°C.

Conidia were harvested by scraping the surface of 14-day-old culture. Spores were suspended in 0.1% Tween-80 solution in glass vials. Conidial suspensions were vortexed into a homogeneous suspension. Conidia were then quantified with a haemocytometer following serial dilutions (10^1, 2, 3, etc.) in sterile distilled water containing Tween-80. The viability of conidia was determined by plate count method on PDA (slight modification of [25]). Percent germination was determined by counting 100 spores for each plate.

2.3 *In vitro* Screening of Virulence Isolates Against Fruit Fly Pupa

A laboratory bioassay of collected EPFs was conducted in order to screen out the effective strains against the fruit fly, *Bactrocera* spp. The *in vitro* screening was carried out according to a method outlined by [26] with some modification. Two-three day-old pupae were sterilized with 0.5% (v/v) sodium hypochlorite and dipped in four serial dilutions (1×10^5 conidia ml^{-1} to 1×10^8 conidia ml^{-1}) of conidial suspension for 2 min. For each dilution, 10 pupa ml^{-1} were used. The treated pupae were transferred to 15ml sterilized glass vials containing wetted cotton wool and incubated in a BOD chamber (25 ± 2°C and 70 ± 2% RH). Control pupae were treated with sterile distilled water containing 0.1% Tween-80. Treated pupae were examined for their emergence and mycosis daily after inoculation. Mycosis was confirmed by microscopic examination. Dead pupae with fungal growth were transferred to PDA plates for confirmation of infecting species. Each experiment conducted in triplicate was replicated three times.

2.4 Soil Bioassays

A test of pathogenicity of selected virulent strains in controlling *Bactrocera* pupa was done during February, 2011 to April, 2011 at the Faculty of Science, Chiang Mai University. Three selected strains: *M. flavoviride*, *P. lilacinus* and *B. bassiana* CMUCDMF03 were used in the soil bioassay experiments. Soil bioassay was done using pots (7×5×5½ cm) and conidial suspensions were applied in two ways, through drenching and pre-mixing [26]. Autoclaved soil was used and sterilized pupae were placed below the soil surface. Conidial suspension was prepared to the final volume of 10^8 conidia g^{-1} in both experiments. Pots were kept under a shade net and the temperature ranged between 26.1 ± 2°C to 29.1 ± 2°C. The RH of soil was maintained at about 80% throughout the bioassay. Dead pupae were examined under the microscope after washing with sterilized distilled water. The experiments were carried out in three replicates.

2.5 Detecting Antimicrobial Activity

2.5.1 Organisms

Seven bacteria and six fungi were used to screen the antimicrobial properties of

six EPFs. Of the bacteria isolates, two were Gram-positive; *Staphylococcus aureus* and *Bacillus cereus*, and the rests were Gram-negative; *Proteus mirabilis*, *Ralstonia solanacearum*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*. Of the fungi examined, five were filamentous fungi; *Alternaria solani*, *A. brasicola*, *Collectotrichum* sp., *Rhizoctonia solani*, *Sclerotium solani* and one was a yeast, *Candida albicans*.

2.5.2 Cultivation media and methods

Fungal strains were grown in five submerged-culture media. Three media, SMY liquid medium; Gelatin semi-solid medium; 802C liquid medium [27] without Sanagiko (powdered silk worm pupa, MARUQ company, Japan); PDB, and F1 (2% glucose; 0.5% soybean meal; 0.01% $CaCl_2$; 0.1% KH_2PO_4; 0.05% $MgSO_4.7H_2O$; pH 6.0) media were used for the detection of antimicrobial activities. Fungi were cultured in 250-ml Erlenmeyer flasks containing 50 ml of liquid media. Mycelia disc of fungal isolates were inoculated in broth and grown at room temperature (25 ± 2°C) for 7 days on a reciprocal shaker (125 rpm) [28]. The mycelium was then harvested by filtration. The filtrates were used for extracellular antimicrobial activity against the tested organisms.

2.5.3 Antifungal and antibacterial assay

The antimicrobial assay was performed using an agar well diffusion method [28]. A well was prepared in the plates previously seeded with the test microorganisms using a sterile cork borer (*ca* 0.85 cm). A culture filtrate of EPFs (50 µl) was filled in each well. Plates were inoculated at 37°C for tested bacteria and 25±2°C for tested fungi and yeast. Inhibition zones developed due to active antimicrobial metabolites were measured after 24 hours of incubation for bacteria and 48 hours of incubation for fungi. Streptomycin (25mg ml^{-1}) and ampicillin (25mg ml^{-1}) were used as positive control for tested bacteria, and Benomyl (25mg ml^{-1}) and nystatin (25mg ml^{-1}) were used as positive control for fungi. For negative controls, sterilized culture broth media were used. Each experiment was conducted in triplicate and the results are expressed in average values.

2.6 Statistical Analysis

Pupal mortality was adjusted for natural mortality in the control using Abbott's formula [29]. Lethal concentrations and lethal time were calculated by using probit analysis. The treatment means were compared using Tukey's HSD Post-hoc test at 5% probability by using the SPSS program version 16.0 (SPSS Inc., Chicago, IL).

4. RESULTS AND DISCUSSION

4.1 Virulence of Fungal Isolates

In this experiment, all tested fungal isolates were pathogenic to fruit fly pupa. Mortality of pupae was assessed by analyzing dead pupae and emergence as well as mortality rates for emerged adult flies. This result confirms the previous findings of the entomopathogenic activity of fungal isolates on fruit fly species [30-38, 40]. However, pathogenic activity of isolates differed significantly between each other. Significant differences in pupal mortality were observed between different isolates with different concentrations. In screening bioassay, *M. flavoviride*, *P. lilacinus* and *B. bassiana* isolate CMUCDMF03 showed the highest efficacy against *Bactrocera* pupa. Mortality of these isolates caused 81.44% ($F = 25.49$; d.f. $= 9, 29$; $P < 0.0001$), 85.22% ($F = 25.93$; d.f. $= 9, 29$; $P < 0.0001$), and 85.22% ($F = 17.39$; d.f. $= 9, 29$; $P < 0.0001$) at 10^7 conidia ml^{-1} and 100% in each at 10^8 conidia ml^{-1}, respectively.

The control mortality was only 10%. No significant differences in infectivity were found among these three isolates (Table 1). These findings of pupal mortality verify a dose- dependent pattern. This concurs with the results Angel-Sahagun et al. [41], that the infection of *H. irritans* by three *M. anisopliae, I. fumusorosea* isolates and one *B. bassiana* isolate was found at the dose of 10^8 conidia ml^{-1}.

Nevertheless, fruit fly mortality was significantly affected by conidial ($P < 0.01$) concentrations. The higher the concentration level, the greater the number of dead fruit flies. These results are consistent with those of Bernardine et al. [42] who reported that the adult emergence of *M. domestica* larvae reduced as concentration increased. On the other hand, the pathogenic activity of seven isolates of *M. anisopliae,* five isolates of *B. bassiana* and two isolates of *P. fumosoroseus* against adult *Bactrocera zonata* and *B. cucurbitae* was demonstrated at a conidial concentration of 1×10^6 conidia ml^{-1}[40].

Table 1. Percent mortality of dead *Bactrocera* pupae after 10 days exposure of eight fungal isolates with various conidial concentrations.

Isolates	Percent mortality of fruit fly pupa 10 days after treatment			
	1×10^5	1×10^6	1×10^7	1×10^8
CMUCDCT01	25.89c	29.67c	81.44b	100a
CMUCDMT02	22.22b	33.33b	85.22a	100a
CMUCDMF02	18.56c	22.22c	40.78b	59.22a
CMUCDMF03	29.67d	48.11c	85.22b	100a
CMUCDMF04	18.56b	22.22b	44.44ab	66.67a
CMUCDMG03	14.78b	25.89b	40.78ab	63.00a

Note: The results are mean of three replicates. Data with different letters within row indicates a significant difference at $P < 0.001$ according to Tukey's HSD Post-hoc test within the same treatment.

From virulence assay, *I. tenuipe*, *M. anisopliae* and one strain of *B. bassiana* CMUCDMG03 showed less virulence towards pupae when compared to others, even at the highest dose of conidia.

For the concentration of 10^5 conidia ml^{-1}, the highest level of pupal mortality ranged from 14.78-29.67% between isolates. This mortality value was not significantly different from that of at the concentration of 10^6 (Table 1). Fruit fly pupae were significantly sensitive to all tested isolates after 3 days at a concentration of 10^8 conidia ml^{-1}. The LD_{50} value of isolate CMUCDMF03 was lower than the others but this was not significantly different from CMUCDMT02 and CMUCDCT01 (Table 2). According to the 50% lethal concentration, *B. bassiana* (CMUCDMF03) was found to be highly pathogenic to fruit fly pupa followed by *P. lilacinus* and *M. flavoviride*. This is in agreement with the findings Aemprapa [43] that *Beauveria* isolate 6241 killed 50% of *B. dorsalis* pupa.

The time taken for the isolates to kill 50% of fruit fly pupae is shown in Table 2. *Paecilomyces lilacinus* achieved the shortest time to attain 50 % mortality (LT_{50} = 3.54 days), whereas the time for isolate CMUCDMF03 was 3.71 days and 4.15 days *for M. flavoviride. B.bassiana* killed adult house flies within five days [44], while Watson et al. [45]

Table 2. Lethal concentrations, lethal times and respective confidence intervals (95% CI) of each tested isolates infected fruit fly, *Bactrocera* spp. for 10 days.

Isolates	LC_{50} (95%CI) log(conidia/ml)	LC_{90} (95%CI) log(conidia/ml)	LT_{50} (95%CI) (days)	LT_{90} (95%CI) (days)
CMUCDCT01	5.22(3.99-6.18)	8.81(7.55-11.66)	4.15(3.83-4.46)	5.87(5.46-6.44)
CMUCDMT02	5.17(3.86-6.16)	8.64(7.39-11.56)	3.54(3.22-3.84)	5.15(4.76-5.71)
CMUCDMF02	7.47(6.35-9.56)	13.95(11.17-21.55)	7.60(7.08-8.21)	11.41(10.44-12.85)
CMUCDMF03	4.79(3.99-5.43)	8.26(7.41-9.63)	3.71(3.37-4.03)	5.53(5.11-6.11)
CMUCDMF04	7.05(6.05-8.62)	12.91(10.59-18.65)	7.14(6.65-7.68)	10.76(9.91-11.99)
CMUCDMG03	7.28(6.24-9.09)	13.39(10.87-19.92)	7.04(6.46-7.72)	11.64(10.50-13.37)

mentioned the mortality of *Musca domestica* occurred within seven days. However, the adult house fly mortality was observed within six days for *B. bassiana* isolates [46]. In their studies, none of the isolates of *P. lilacinus* were pathogenic to these adult house flies, in contrast to our results with *Bactrocera* spp. in Thailand.

The entomopathogenic activity of tested isolates was confirmed by the presence of fungal hyphae on the bodies of dead flies (Figure 1). Percent mycosis in dead flies was significant among isolates. CMUCDMT02, CMUCDCT01 and

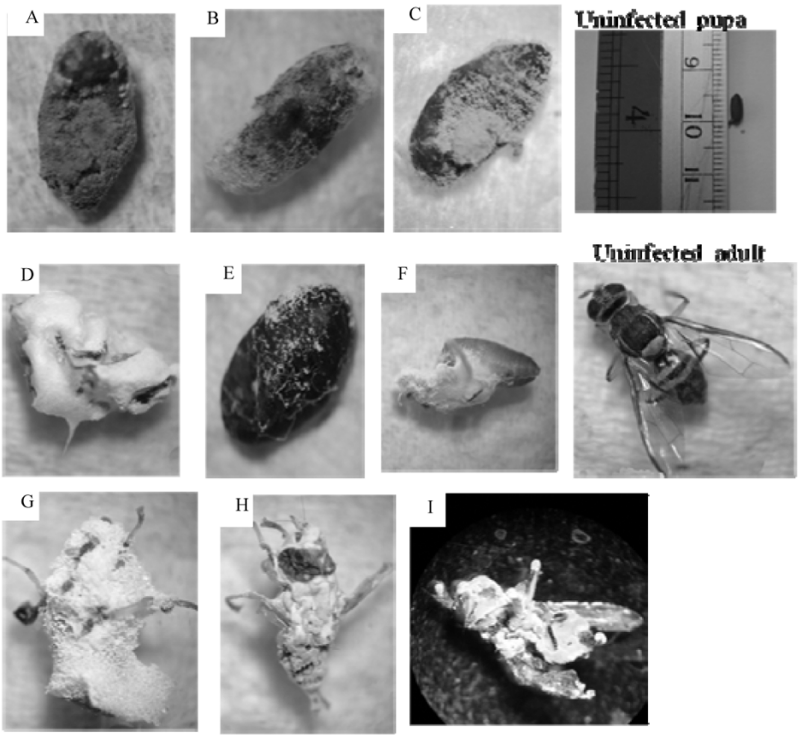

Figure 1. Fungal mycosis of tested entomopathogenic isolates on infected fruit fly (*Bactrocera* spp.) pupae and adult. A. *Metarhizium flavoviride*; B. *M. anisopliae*; C. *Paecililacinus lilacinus*; D. *Beauveria bassiana* CMUCDMF03; E. *B. bassiana* CMUCDMG03; F. *Isaria tenuipe*; G. *B. bassiana* CMUCDMF03 (adult); H. *M. flavoviride* (adult); I: *B. bassiana* CMUCDMG03 (adult).

CMUCDMF03 showed the highest mycosis in dead pupa after 5 days. The appearance of mycosis on dead pupae was shown first in isolate CMUCDMT02 followed by CMUCDCT01 and CMUCDMF03. Though isolate CMUCDMG03 showed less virulent infectivity to fruit fly pupa, percentage of fungal mycosis reached the highest in 5 days treated. The least amount of mycosis was observed in *M. anisopliae* even at 10^8 conidia ml^{-1}, and the appearance was delayed when compared with others. Emerged adults from pupae of treated isolates showed mycosis on the surface in all treatments. Virulent isolates were fast-growing with compact and dense mycelium, producing a high yield of conidia on the surface of the culture. These characters lead to the highest infective activity against hosts. Daily mortality rate is shown in Figure 2.

The Koch's postulate was used for confirmation of infected isolates. Each fungus was re-isolated from surface of infected pupa and transferred to PDA plates. The results indicated that all pure cultures were same morphological and cultural characteristics of each infected fungus.

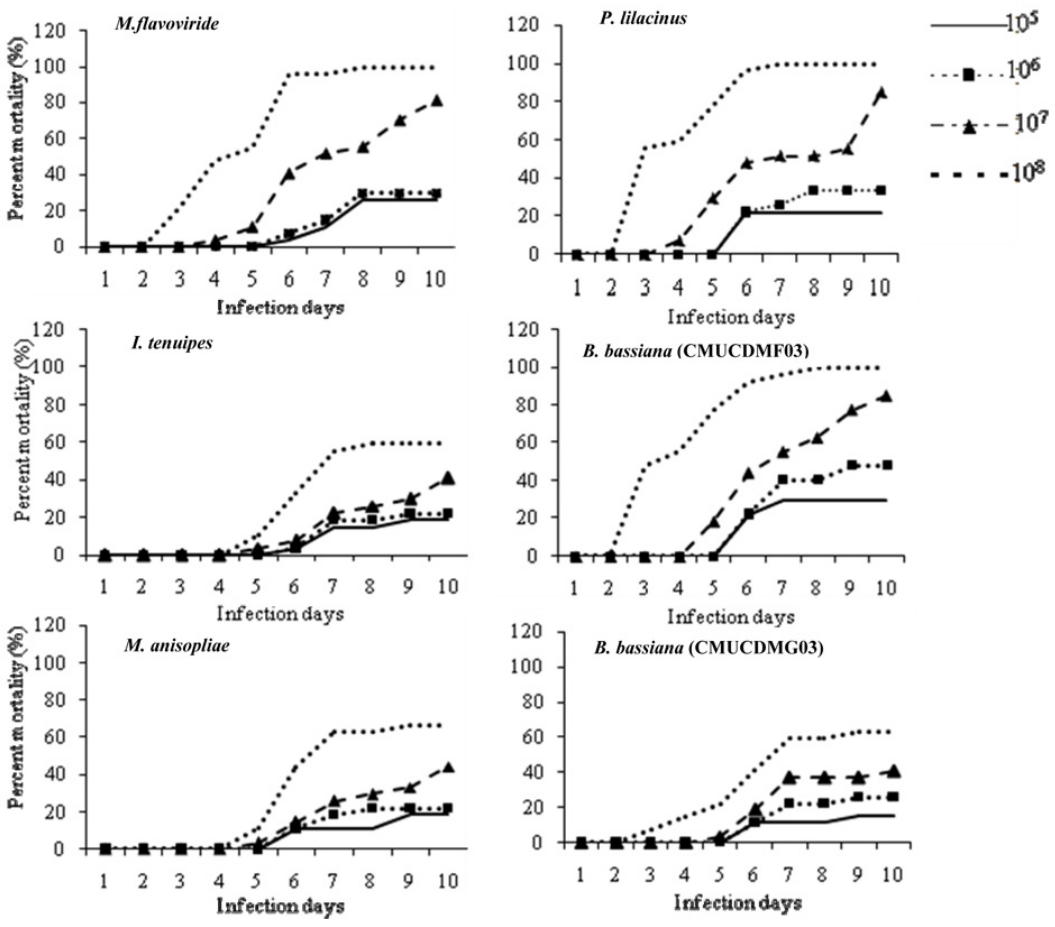

Figure 2. Percentage of daily mortality of fruit fly (*Bactrocera* spp.) infected by fungal isolates with different conidial concentrations.

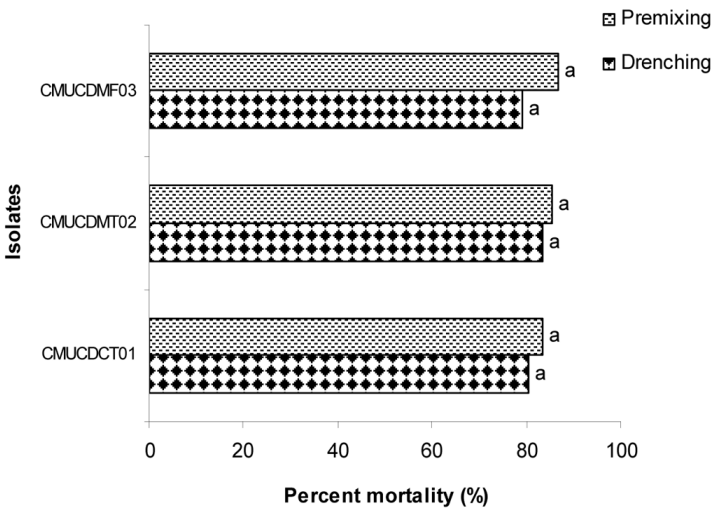

Figure 3. Mean percent mortality of fruit fly in soil bioassays at a concentration of 10^8 conidia ml^{-1} after 14 days treated.

4.2 Pathogenic Activity of Fungal Isolates in Soil Bioassay

The entomopathogenic activity of selected isolates showed no significant difference in both drenching and premixing of conidial suspension with soil. However, pupal mortality was observed first in the premixing with soil treatment (Figure3). In addition, external formation of mycelium was observed on *B. bassiana*, CMUCDMF03, when pupae were treated with the premixed conidial solution with soil.

4.3 Determination of Antimicrobial Activity

Entomopathogenic fungi produce various bioactive compounds, including toxins that kill the insect hosts upon infection and invasion. Successful production of antimicrobial activities was detected from EPFs in different growth media. Screening new organisms for antibacterial activity and searching for new antibacterial drugs is important due to the constant generation of new antibiotic-resistant strains of pathogenic bacteria [47]. In this experiment, the antibacterial and antifungal activities were examined from naturally infested insect pathogenic isolates collected from the field. The antibacterial activity of the tested EPFs was apparent in four bacteria, *Bacillus cereus*, *Staphylococcus aureus*, *Proteus mirabilis* and *Ralstonia solanacearum* out of seven except the CMUCDCT01 isolate, which did not demonstrate antimicrobial activity. On the other hand, antibacterial activity was detected in different cultivation media in different isolates (Table 3).

Takahashi et al. [49] reported that species of the genus *Beauveria* have been detected various types of pigments during cultivation of *B. bassiana*. Likewise, in this study red pigment was produced when *B. bassiana* (MF03) was cultured in liquid broth. The pigment producing MF03 strains displayed antibacterial activity against *Bacillus cereus* (Gram-positive) and *Proteus mirabilis* (Gram-negative) bacteria. This was the contrary results with the finding of Pegram et al. [50] who found that red pigment produced strain of *B. bassiana* showed antibiotic activity towards Gram-positive. Nevertheless, any clear zone was detected in the rest of the three media.

Table 3. Positive results of antibacterial activity of fungal extract from different culture media against tested bacteria.

Cultured Media	Tested microorganisms and diameter of clear zones (mm)			
	Staphylococcus aureus	*Bacillus cereus*	*Proteus mirabilis*	*Ralstonia solanacearum*
***P. lilacinus* (MT02)**				
PDB	0	8	0	0
SMY	0	0	17	0
***I. tenuipes* (MF02)**				
PDB	0	13	13	0
SMY	0	15	18	0
F1	0	13	0	0
802C	0	13	0	0
***B.bassiana* (MF03)**				
PDB	0	12	12	0
SMY	0	17	18	0
***M.anisopliae* (MF04)**				
PDB	16	12	18	0
SMY	16	12	17	0
F1	18	13	0	0
802C	13	0	0	0
GSSM	18	0	0	0
***B.bassiana* (MG03)**				
SMY	0	9	16	0
802C	8	0	0	0
Ampicillin	25	20	44	18
Streptomycin	30	24	39	19

In CMUCDMT02, the extract of fungal metabolite against *Bacillus cereus* and *Proteus mirabilis* found in PDB and SMY media, respectively. This was in opposition to the result of Mikami et al. [51] who stated that paecilotoxin isolated from the different strains of *P. lilacinus* showed antimicrobial activity against Gram-positive bacteria. Strain *Isaria tenuipe* produced anti-*Bacillus* activity in all liquid media except GSSM, and anti- *Proteus* activity was detected in PDB and SMY media.

Fortunately, results showed anti-*Staphylococcus* activity in all cultured media of *M. anisopliae* whereas anti- *Bacillus* activity was observed in PDB, SMY media and a clear zone of anti- *Proteus* activity was found in PDB, SMY and F1 media. Anti-*Bacillus* and anti- *Proteus* activities of CMUCDMG03 were apparent in SMY media, and anti- *Staphylococcus* activity was found in 802C medium. Anti-*Staphylococcus* activity was not detected in the tested entomopathogenic fungal isolates except *M. ansopliae* and *B. bassiana* CMUCDMG03. Amongst tested Gram-negative bacteria, only *Proteus mirabilis* was susceptible to extracts of EPFs, whereas both Gram- positive bacteria were found to be the most susceptible.

Table 4. Positive responses of antifungal activities from six entomopathogeic fungal isolates of different culture media.

Cultured Media	Tested microorganisms and diameter of clear zones (mm)					
	Alternaria solani	*Alternaria brasicola*	*Collectotrichum* sp.	*Rhizoctonia solani*	*Sclerotium solani*	*Candida albicans*
M.flavoviride CMUCDCT01						
PDB	0	0	0	0	0	14
I.tenuipes CMUCDMF02						
802C	10	0	0	0	0	0
B.bassiana CMUCDMF03						
SMY	11	8	0	0	0	15
M.anisopliae CMUCDMF04						
PDB	12	11	0	0	0	14
SMY	18	0	0	17	0	0
B.bassiana CMUCDMG03						
SMY	12	11	0	0	0	0
802C	0	0	0	17	0	17.7
GSSM	0	0	0	0	0	15.7
Benomyl	25	25	0	7	0	11
Nystatin	18	9	5	8	11	8

In all EPFs tested for antifungal activity of *Collectotrichum* and *Sclerotium solani*, no inhibition zones were detected in all cultured media. In addition, the water extract of *P. lilacinus* and *M. flavoviride* have no antifungal activities except anti-*Candida albicans* activity in *M. flavoviride*. *Isaria tenuipes* exhibited activity only (1/6; 17%) against tested fungi in 802C media (Table 4). Inhibition zones of *B. bassiana* isolate CMUCDMF03 against *Alternaria solani* and *A. brasicola* were 11 mm and 8 mm in SMY medium. *Metarhizium anisopliae* showed antifungal activity against *A. solani*, *A. brasicola* and *Rhizoctonia solani* in their specific media. The extract of isolate CMUCDMG03 from SMY culture was active against *A. solani* and *A. brasicola*, whereas the antifungal activity against *R. solani* was detected in 802C media. The inhibition zone of *M. flavoviride, B. bassiana* (CMUCDMF03), *M. anisopliae* and *B. bassiana* (CMUCDMG03) was 14 mm in PDB, 15mm in PDB, 14 mm in PDB, and 17.7 mm in 802C and 15.7 mm in GSSM, respectively, when the anti-yeast activity against *C. albicans* was measured.

The results demonstrate that EPF isolates have certain antimicrobial activity against some tested bacteria and fungi in different cultured media without insect-

derived materials. This is not in agreement with the finding of Lee et al. [27] who found that *Verticillium lecanii* HF238 produced a clear antibiotic activity against *Bacillus* and *Saccharomyces*, but only in the presence of insect-derived materials. Finally, it was clear that growth media plays an important role in the determination of antibacterial and antifungal activities.

5. CONCLUSION

The experiments clearly demonstrated that all evaluated entomopathogenic fungal strains were capable of infecting *Bactrocera* spp. pupae. Based on the LD_{50} and percent mortality, *P. lilacinus*, *B. bassiana* (CMU CDMF03) and *M. flavoviride* are the most virulent strains and can be used as effective BCAs in controlling fruit fly pupa (*Bactrocera* spp.). However, a further investigation of the infectivity of these strains to hosts will be necessary.

The results of present study clearly show that certain entomopathogenic fungal isolates evaluated for antimicrobial activity produced some antibacterial and antifungal compounds without the addition of any insect-derived materials. Finally, these findings represent the first analysis of the pathogenicity of fungal isolates of natural insect pathogens collected from northern Thailand and their antimicrobial activity. The specific antimicrobial compounds present in these collected insect pathogenic fungi from nature should be investigated further in the future by purification of fungal extracts.

6. ACKNOWLEDGEMENT

A special thank goes to the Third World Organization for Woman in Science (TWOWS), Italy and Sida (Swedish International Development Cooperation Agency); the Office of Higher Education Commission, Thailand under the National Research University program, and the Graduate School of Chiang Mai University,Chiang Mai, Thailand for granting a scholarship for this research.

7. REFERENCES

[1] Diamantidis A.D., Carey J.R. and Papa dopoulus N.T., *Life- History of an Invasive Tephritid*. Department of Agriculture, Crop Production and Rural Environmental, University of Thessaly, Phytokou St. N, Ionia 384 46, Magnisias, 2008.

[2] www.fao.org/docs/eims/upload/207694/7_1_2_cases.PDF.1994. Management of Fruit Fly Problems in the South Pacific Region.

[3] Christenson L.D. and Foote R.H., Biology of fruit flies, *Ann. Rev. Entomol.*, 1960; **5**: 171-192.

[4] Weems H.V Jr. and Heppner J.B., Oriental Fruit Fly, *Bactrocera* (= Dacus) *dorsalis* (Hendel) (Insecta: Diptera: Tephritidae), Florida Department of Agriculture and Consumer Services, Division of Plant Industry, and T.R. Fasulo, University of Florida Publication, 1999.

[5] Singh S., Effects of aqueous extract of neem seed kernel and azadirachtin on the fecundity, fertility and post-embryonic development of the melonfly, *Bactrocera cucurbitae* and the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae), *J. Appl. Entomol.*, 2003; **127**: 540-547.

[6] Sarango V.M.G., Monitoring and Pest Control of Fruit Flies in Thailand: New Knowledge for Integrated Pest Manage ment. SLU, Institutionen för ekologi, Uppsala, 2009.

[7] Gallo J., Integrated Plant Control: System and Management, *Encyclopedia of Pest Management*, 2007; **2:** 279-287.

[8] Casida J.E. and Quistad G.B., Golden age of insecticide research: Past, present, or future? *Annu. Rev. Entomol*., 1998; **43**: 1-16.

[9] Claudianos C., Russell R.J. and Oakeshott J.G., The same amino acid substitution in orthologous esterases confers organophosphate resistance on the house fly and a blowfly, *Insect Biochem. Mol. Biol*., 1999; **29:** 675-686.

[10] Hsu J.C. and Feng H.T., Development of resistance to spinosad in oriental fruit fly (Diptera: Tephritidae) in laboratory selection and cross-resistance, *J. Econ. Entomol.*, 2006; **99(3):** 931-936.

[11] Thapinta A. and Hudak P.F., Pesticide use and residual occurrence in Thailand, *Environ. Monit. Assess*., 1998; **60:** 103-114.

[12] Aldridge S., Insecticide Resistance: from Mechanisms to Management. Association of British Science Writers. London. [online] Available: http://www.absw.org.uk/Briefings/insecticide_resistance.htm, 2009.

[13] Mendes P.C.D., Ambrosano E.J., Guirado N., Rossi F., Arevalo R.A. and Groppo G.A., Avaliação populacional de moscas-das-frutas (Diptera: Tephritidae) e de seus Parasitóides Larvais (Hymenoptera: Braconidae), *Revista Brasileira de Agroecologia, Porto Alegre*, 2007; **2:** 690-693.

[14] de Oliveira F.Q., Biological control of fruit fly, *Revista Verde* (Mossoró - RN - Brasil), 2010; **5(1):** 23 - 30.

[15] Singkaravanit S., Kinoshita H., Ihara F. and Nihira T., Geranylgeranyl diphosphate synthase genes in entomo pathogenic fungi, *Appl. Microbiol. Biotechnol*., 2010; **85:** 1463-1472.

[16] Schneider P., Misiek M. and Hoffmeister D., *In vivo* and *in vitro* production options for fungal secondary metabolites, *Mol. Pharm*., 2008; 234-242.

[17] Loutelier C., Cherton J.C., Lange C., Traris M. and Vey A., Studies on the dynamics of the production of destruxins by *Metarhizium anisopliae*: Direct high-performance liquid chromatographic and fast atom bombardment mass spectrometric analysis correlated with biological activity tests, *J. Chromatogr. A*., 1996; **738:** 181-189.

[18] Vey A., Hoagland R. and Butt T.M., Toxic Metabolites of Fungal Biocontrol Agents. In Butt, T. M., Jackson, C. and Magan, N. ed., *Fungi as Biocontrol Agents: Progress, Problems and Potential*, CAB International, Oxon, 2001: 311-345.

[19] Pedras M.S.C., Irina Z. L. and Ward D. E., The destruxins: synthesis, biosynthesis, biotransformation, and biological activity, *Phytochem.*, 2002; **59:** 579-596.

[20] Grove J. and Pople M., The insecticidal effect of beauvericin and the enniatin complex, *Mycopathologia,* 1980; **70:** 103-105.

[21] Cunningham K., Manson W., Spring F. and Hutchinson S., Cordycepin, a metabolic product isolated from cultures of *Cordyceps militaris*, *Nature,* 1950; **166:** 949 pp.

[22] Kuo Y., Lin C., Tsai W., Wu C., Chen C. and Shiao M., Growth inhibitors against tumor cells in *Cordyceps sinensis* other than cordycepin and polysaccharides, *Cancer Invest.*, 1994; **12:** 611-615.

[23] Schachter B., Slimy business-the biotechnology of biofilms, *Nat. Biotechnol*, 2003; **2:** 361-365.

[24] Htar Htar Naing, Wint War Kyaw, Si Thu Zaw, Sai Than Zaw Tun, San Li Seng, Khin Htar Nge, Sandar Hlaing, Kyaw Min Han, Chit Darli, Win Min Thant, Aung Kyaw Oo and Ye Min Oo., Exploration of Species Composition of Fruit Fly and Monitoring its Population on Jujube (*Ziziphus jujube* Mill) in Yezin area, Final Year Special Problem, Yezin Agricultural University, Myanmar, 2008; 10 pp.

[25] Goettel M.S. and Inglis G.D., Fungi: Hyphomycetes. In: Lacey, L.A. ed., *Manual of Techniques in Insect Pathology*. Academic Press, San Diego, 1997; 211-249.

[26] Anand R., Prasad B. and Tiwary B.N., Relative susceptibility of *Spodoptera litura* pupae to selected entomopathogenic fungi, *Biol. Control*, 2009; **54:** 85-92.

[27] Lee S.Y., Nakajima I., Ihara F., Kinoshita H. and Nihira T., Cultivation of entomopathogenic fungi for the search of antibacterial compounds, *Mycopathologia*, 2005; **160:** 321-325.

[28] Thakur D., Yadav A., Gogoi B.K. and Bora T.C., Isolation and screening of *Streptomyces* in soil of protected forest areas from the states of Assam and Tripura, India, for antimicrobial metabolites, *Journal de Mycologie Médicale*, 2007; **17:** 242-249.

[29] Abbott W.S., A method of computing the effectiveness of an insecticide, *J. Econ. Entomol*, 1925; **18:** 265-267.

[30] Garcia A.S., Messias C.L., De Souza H.M.L. and Piedramuena A.E., Patogenicidade de *Metarhizium anisopliae* var. *anisopliae* a *Ceratitis capitata* (Wied) (Diptera, Tephritidae), *Rev. Bras. Entomol*, 1984; **28:** 421-424.

[31] Castillo M., Moya P., Hernandez E. and Primo-Yufera E., Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopathogenic fungi and their extracts, *Biol. Control*, 2000; **19:** 274-282.

[32] Lezama-Cutiérrez R., Trujillo-De-La Luz A., Molina-Ochoa J., Rebolledo-Dominguez O., Pescador A.R., Lopez- Edwards M. and Aluja M., Virulence of *Metarhizium anisopliae* (deuteromycotina: hyphomycetes) on *Anastrepha ludens* (Diptera: Tephritidae): Laboratory and field trials, *J. Econ. Entomol*, 2000; **93:** 1080-1084.

[33] De La Rosa W., Lopez F.L. and Liedo P., *Beauveria bassiana* as a pathogen of the Mexican fruit fly (Diptera: Tephritidae) under laboratory conditions, *J. Econ. Entomol*, 2002; **95:** 36-43.

[34] Ekesi S., Maniania N.K. and Lux S.A., Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*, *Biocontrol Sci. Technol.*, 2002; **12:** 7-17.

[35] Dimbi S., Maniania N.K., Lux S.A. and Mueke J.M., Host species, age and sex as factors affecting the susceptibility of the African tephritid fruit fly species, *Ceratitis capitata*, *C. cosyra* and *C. fasciventris* to infection by *Metarhizium anisopliae*, *J. Pest. Sci*., 2003a; **76:** 113-117.

[36] Dimbi S., Maniania N.K., Lux S.A., Ekesi S. and Mueke J.M., Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, to three adult fruit fly species: *Ceratitis capitata* (Wiedemann), *C. rosa* var. *fasciventris* Karsch and *C. cosyra* (Walker) (Diptera: Tephritidae), *Mycopathologia*, 2003b; **156:** 375-382.

[37] Uziel A., Levy K. and Yuval B., Infection of *Ceratitis capitata* by two species of the *Entomophthora muscae* species complex (Zygomycetes: Entomophthorales) in the field, *Phytoparasitica*, 2003; **31:** 1-3.

[38] Mochi D.A., Monteiro A.C., De Bortoli S.A., Doria H.O.S. and Barbosa J.C., Pathogenicity of *Metarhizium anisopliae* for *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) in soil with different pesticides, *Neotrop. Entomol*., 2006; **35:** 382-389.

[39] Quesada-Moraga E., Ruiz-Garcia A. and Santiago-Alvarez C., Laboratory evaluation of entomopathhogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against puparia and adults of *Ceratitis capitata* (Diptera: Tephritidae), *J. Econ. Entomol*., 2006; **99:** 1955-1966.

[40] Sookar P., Bhagwant S. and Awuor Ouna E., Isolation of entomopathogenic fungi from the soil and their pathogenicity to two fruit fly species (Diptera: Tephritidae), *J. Appl. Entomol*., 2008; **132:** 778-788.

[41] Angel-Sahagun C.A., Lezama-Gutierrez R., Molina-Ochoa J., Galindo- Velasco E., Lopez-Edwards M., Rebolledo-Dominguez O., Cruz-Vazquez C., Reyes-Velazquez W.P., Skoda S.R. and Foster J.E., Susceptibility of biological stages of the horn fly, *Haematobia irritans*, to entomopathogenic fungi (Hypho mycetes), *J. Insect. Sci*., 2005; **5:** 50.

[42] Bernardine D.M., Pinto J.S., do N., Rineiro B. and da Silva C.L., Efeito dos Fungos Entomopatogenicos *Metarhizium anisopliae e Beauveria bassiana* sobre o Desenvolvimento da *Musca domestica* L. (Diptera: Muscidae) em Laboratorio, *Arq. Inst. Biol*., 2006; **73:** 127-129.

[43] Aemprapa S., Entomopathogenic fungi screening against fruit fly in Thailand, *KMITL Sci. Tech. J.*, 2007; **7:** 122-126.

[44] Geden C.J., Rutz D.A. and Steinkraus D.C., Virulence of different isolates and formulations of *Beauveria bassiana* for house flies and the parasitoid *Muscidifurax raptor, Biol. Control*, 1995; **5:** 615-621.

[45] Watson D.W., Geden C.J., Long S.J. and Rutz D.A., Efficacy of *Beauveria bassiana* for controlling the house fly and stable fly (Diptera: Muscidae), *Biol. Control*, 1995; **5:** 405-411.

[46] Mwamburi L.A., Laing M.D. and Miller R.M., Laboratory screening of insecticidal activities of *Beauveria bassiana* and *Paecilomyces lilacinus* against larval and adult house fly (*Musca domestica* L.), *Afr. Entomol*., 2010; **18(1):**38-46.

[47] Janes D., Umek A. and Kreft S., Evaluation of antibacterial activity of extracts of five species of wood colonizing fungi, *J. Basic Microbiol*., 2006; **46(3):** 203-207.

[48] Parine N.R., Pathan A.A.K., Sarayu B, Nishanth V.S. and Bobbarala V., Antibacterial efficacy of secondary metabolites from entomopathogenic fungi *Beauveria bassiana*, *Int. J. Chem. Anal. Sci.*, 2010; **1(5):** 94-96.

[49] Takahashi S., Uchida K., Kakinuma N., Hashimoto R., Yanagisawa T. and Nakagawa A., The structures of pyridovericin and pyridomacrolidin, new metabolites from the entomopathogenic fungus, *Beauveria bassiana*, *J. Antibiot.,* 1998; **51:** 1051-1054.

[50] Pegram R.A., Wyatt R.D. and Smith T., Oosporein-toxicosis in the Turkey poultry, *Avian Dis*., 1982; **26:** 47-59.

[51] Mikami, Y., Yazawa, K., Fukushima, K., Arai, T., Udagawa, S. and Samon, R.A., Paecilotoxin production in clinical or terrestrial isolates of *Paecilomyces lilacinus* strains, *Mycopathologia*, 1989; **108:**195-199.