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Original Article

Occurrence and effectiveness of indigenous *Metarhizium anisopliae* against adults *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae) in Southern Thailand

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Abstract

The indigenous entomopathogenic fungus, *Metarhizium anisopliae*, was isolated from soil samples and infected insect cadavers in fruit orchards of peninsular Thailand. Sixty-four isolates of indigenous *M. anisopliae* were obtained from fruit orchards in Nakhon Si Thammarat, Patthalung, and Songkhla provinces by using selective media. The soil samples from fruit orchards of Songkhla province gave the most fungal isolates (39), while the soil samples from Patthalung and Nakhon Si Thammarat gave 14 and 5 isolates, respectively. Six more isolates were from the infected insect cadavers, collected from the orchards in Nakhon Si Thammarat (4) and Songkhla (2). The conidial morphologies of the isolates were divided into four groups based on length to width (L/W) ratio: 1.69-1.96, 2.01-2.47, 2.55-2.94, and 3.14-3.16. The efficiency of isolated *M. anisopliae* against adult stage *Zeugodacus cucurbitae* was evaluated in laboratory conditions. The fungal isolates PSUM02, PSUM05 and PSUM08 were the most effective with complete 100% mortality of *Z. cucurbitae* within seven days from inoculation. These fungi in the soils of different fruit orchards should be further assessed for their efficacies against different insect pests. In particular, future studies should look into the three efficient isolates identified, and determine their key differences from the other isolates representing the same habitat or geological origin.

Keywords: indigenous entomopathogenic fungi, *Metarhizium anisopliae,* biocontrol agents, *Zeugodacus cucurbitae*

1. Introduction

Entomopathogenic fungi are estimated to include 700 species in approximately 90 genera; the predominant ones are *Metarhizium, Beauveria, Paecilomyces, Verticillium, Isaria* and *Hirsutella* (Lacey *et al*., 2001). Some species are effective and relatively easy for mass production and are frequently used globally, especially *Metarhizium anisopliae* (Bidochka *et al*., 1998; Roberts & St. Leger, 2004), which is the most notable soil inhabiting entomopathogen with effective potential to control plant and animal pests (Ghanbary *et al*., 2009). This species retains its ability to destroy a number of insect species and it has been developed into commercial mycoinsecticide products (Faria & Wraight, 2007).

Few reports of the entomopathogenic fungus, *M. anisopliae*, isolated from soil and infected insect cadavers in

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agricultural areas and national parks have been published and show variations in pathogenicity and virulence in the tested insect pests (Mar & Lumyong, 2012; Tangthirasunun *et al*., 2010; Thungrabeab *et al*., 2006). However, for successful biological control with entomopathogenic fungi one needs to consider the availability and the acquisition cost of virulent strains from germplasm collections and the regulations and legal restrictions to transport and release of the exotic fungal strains. An approach that avoids these problems with exotic strains is to search for promising, virulent fungal strains *in situ* in the local environment.

The melon fruit fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae), is an economically important insect pest in the tropical areas (Dhillon *et al*., 2005; Hendrichs *et al.,* 2015; White & Elson-Harris, 1994). Its host range covers over 81 plant species of the family Cucurbitaceae (Dhillon *et al*., 2005). This fly species is a cosmopolitan and is also distributed in Asia and Southeast Asia (Allwood *et al*., 1999), particularly throughout Thailand (Clarke *et al*., 2001). The damage caused by the larvae of the flies negatively impacts quality and quantity of fruit crops (Dhillon *et al*., 2005; Singh *et al*., 2000).

The objectives of this study were twofold: firstly, to isolate the indigenous entomopathogenic fugus, *M. anisopliae,* from agricultural soil and infected insect cadavers in Peninsular Thailand; and secondly, to evaluate the virulence of some of the isolates against the adult melon fly, *Z. cucurbitae*, which is an important insect pest species in the study area.

2. Materials and Methods

2.1 Sampling sites

Soil samples were collected from June to September 2012 in mixed fruit orchards, at least five-year old, of longkong (*Lansium domesticum* Correa) (Meliaceae), durian (*Durio zibethinus* L.) (Bombacaceae), and mangosteen (*Garcinia mangostana* L.) (Guttiferae), in the three provinces of Southern Thailand, namely Nakhon Si Thammarat (N 08° 36' 10.89, E 099° 46' 20.12), Patthalung (N 07° 13' 07.20, E 100° 04' 42.32), and Songkhla province (N 06° 58' 34.04, E 100° 18' 54.39) (Figure 1A and 1B). The collection sites have not been exposed to commercial entomopathogenic microorganisms.

In each orchard, five sampling sites were randomly assigned while maintaining at minimum 10 meters distance between all sampling sites. Ten samples were taken at 50 centimeter distances from each sub-sampling site producing 50 soil samples per habitat, with a total of 150 soil samples. Samples were collected with sterilized soil core at a depth of 10-15 cm under the soil surface. Between soil core samples the sampler was sterilized with 1% NaOCl to avoid potential cross contamination. Two hundred grams of each soil sample were kept in a plastic bag, and put in an ice box at 4°C for transportation to the laboratory. Temperature, moisture, and pH of the soil samples were recorded (Table 1). Additionally, insect cadavers infected with the fungus were randomly observed and collected in sterile vials for isolation and identification of the entomopathogenic fungi.

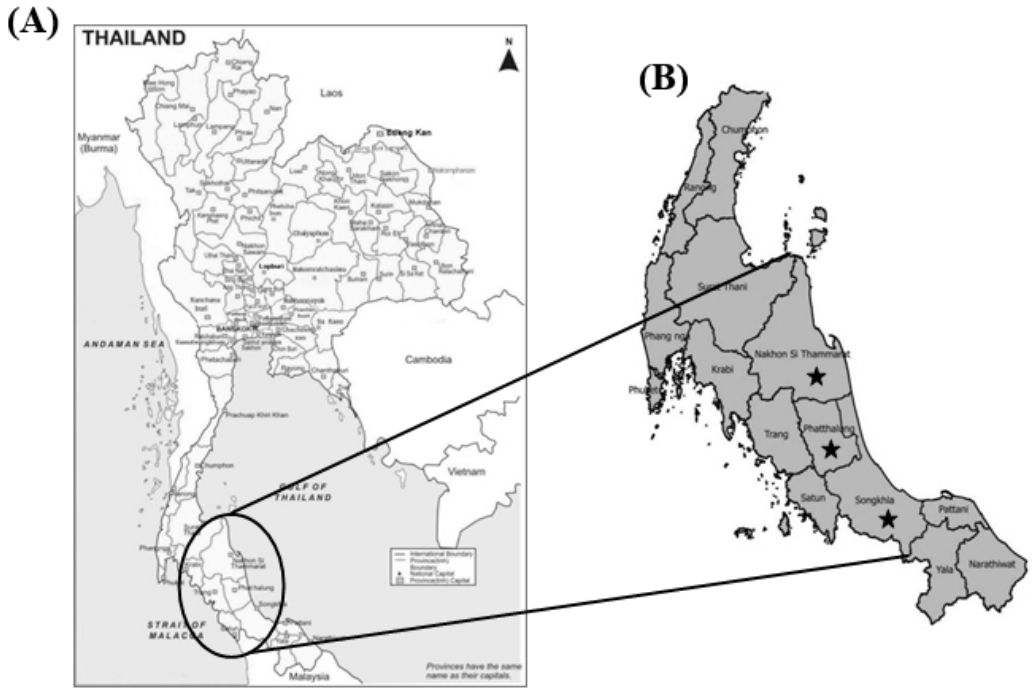

Figure 1. Sample collection sites (fruit orchards) for isolating the indigenous entomopathogenic fungus, *Metarhizium anisopliae,* in the three provinces of southern Thailand (A). Black stars indicate Nakhon Si Thammarat, Patthalung, and Songkhla provinces (B). Maps modified from http://eng.kiangwan.com/index.php?lay=show&ac=article&Id= 322141 and http://www.mapofthailand.org

Table 1. Soil properties and prevalences of indigenous *Metarhizium anisopliae,* in soil samples from different locations.

Location	Soil pH	Soil temperature	Soil humidity (%)	Total samples	Number of isolates[1/]	% prevalence
Nakhon Si Thammarat	6.68 ± 0.14	27.67 ± 0.06	6.35 ± 3.70	50	5**[2/]	8.62
Patthalung	6.20 ± 0.71	25.20 ± 0.42	4.58 ± 1.35	50	14[ns3/]	24.14
Songkhla	6.32 ± 0.34	27.14 ± 0.34	3.74 ± 0.72	50	39**	67.24
		Total		150	58	

[1/]Numbers of isolates of the indigenous entomopathogenic fungus were highly significantly different (P<0.01), according to c^2 test of goodness-of-fit. [2/]** = highly significantly different (P<0.01) when tested with Bonferroni correction. [3/]ns = not significantly different (P>0.05) from others when tested with Bonferroni correction.

2.2 Isolation of entomopathogenic fungi and morphological characterization

Ten grams of each soil sample were mixed by sterile spatula and placed in 100 ml of sterile distilled water in a 250-ml Erlenmeyer flask. The sample was agitated vigorously by vortex (1,000 rpm) for approximately one minute. The resulting suspension was serially diluted (10^{-1}, 10^{-2}, and 10^{-3}) in test tubes (1.5×15 cm). Aliquots of 250 µl were spread on Sabouraud Dextrose Agar (SDA) supplemented with 2.5 g/l yeast extract, 0.5 g/l Chloramphenicol, and 0.001 g/l Thiabendazole (modified from Fernandes *et al*., 2010), and incubated at 28±2°C in the dark for 7-14 days. Then, conidial morphology of a single colony of the fungus was observed under a compound microscope to confirm its taxonomy (Luangsa-ard *et al.,* 2007, 2009) and transferred to SDAY (SDA with yeast, but without antibiotic or fungicide) for purification. Other than isolation of the fungi from soil samples, fungal isolates from the infected insect cadavers were also taken. Each infected insect cadaver was individually surface sterilized by dipping in 0.1% NaOCl for one minute, washed twice with sterile distilled water and placed in a moist chamber at 28±2°C in the dark until fungal sporulation. Later, a single spore of the fungus was transferred onto new SDAY and incubated similarly as described above. Initially the identification was done by observation of colony, conidial, and mycelial morphology. Length and width of the conidia were measured and length to width ratio (L/W ratio) was calculated (Tangthirasunun *et al*., 2010). All the pure isolates were transferred to SDAY slants (1.5×10 cm) and incubated at the above conditions until sporulation, and then the slants were kept at 4°C for stock culture collection and experimental use.

2.3 Melon fly cultures and bioassays

Adult melon flies, *Zeugodacus cucurbitae* (Couqillett) (Diptera: Tephritidae), were collected from naturally infested cucumber (*Cucumis sativus* L.), and laboratory colonies of the fly were cultivated for one to two generations on artificial media (modified from Swaine *et al*., 1978). Pupae were kept in a clear plastic box (10×10×10 cm) with a ventilation hole on the lid covered with gauze. After eclosion, flies were transferred to a cage (30 cm × 30 cm × 30 cm) and supplied with enzymatic yeast hydrolysate, sugar cubes, and water. Test flies were maintained in the laboratory at 25-28°C, 75-90% relative humidity (RH) and 12:12 hrs of light and dark. Test flies were reared until 10 days of age for sexual maturation before bioassay, following Dimbi *et al*. (2003b) and Thaochan and Chinajariyawong (2008).

Selected isolates with flat colonies, i.e. smooth matlike mass of mycelia and conidia, were cultured on SDAY at 28±2°C for 15 days in darkness. Isolates showing clearly abundant sporulation with high number of conidia and fast growth on agar plates were selected for bioassays. The highly spore producing and fast growing isolates might be suitable for large scale applications. Conidia of each isolate obtained from solid medium were transferred with a sterile spatula and suspended in sterile distilled water with 0.01% Tween 80. The suspension was filtered through three layers of sterile cotton cloth and adjusted to a concentration of 1×10^8 spores/ml by using haemocytometer counts.

Bioassays were conducted to evaluate fungal virulence in a completely randomized design (CRD) with five replications. The experimental units were the isolates of entomopathogenic fungi. Five adults of each sex, males and females, 10 days old, of *Z. cucurbitae,* were transferred to a glass bottle 5 cm in width and 10 cm in height, containing one milliliter fungal spore suspension (1×10^8 spores/ml) by dipping method, and held for one minute.

After inoculation, the flies were transferred to plastic boxes (10×15×20 cm) with a gauze covered ventilation hole on each lid. Each box held 10 flies (five each of males and females) and was supplied with enzymatic yeast hydrolysate, sugar cubes, and water soaked in wettex. Insect mortality was assessed daily for 20 days after inoculation. The dead insects were surface sterilized by dipping in 0.1% NaOCl for one min, and washed twice with sterile distilled water, then placed in a sterile 90 mm diameter petri dish with moist filter paper (Whatman® #1) and incubated at 28±2°C in the dark until fungal sporulation. Sporulation was considered a confirma-

tion that the cause of death was attributable to the entomopathogenic fungi. Test insects sprayed with sterile distilled water were used as the control group.

2.4 Data analyses

The number of the entomopathogenic fungi isolated from soil was compared between locations by the extact binomial goodness-of-fit test with an equal proportion of cases expected in each group of the categorical variable, and with Bonferroni correction for test of each location (McDonald, 2014). To assess the differences in insect mortality between fungal isolates, analysis of variance (ANOVA), and post-hoc Tukey's mean comparisons were used for the bioassay data. The mortality of female fruit flies was arcsine transformed before analysis, to reach normality and homogeneity of variance required by the parametric statistics (Gomez & Gomez, 1984). The lethal time at 50 and 90 (LT_{50} and LT_{90}) percent mortalities of the infected adult fruit flies was subjected to probit analysis. All the statistical analyses were carried out with the SPSS 11.0 program for Windows (Statistical Package for the Social Science for Windows [SPSS], 2001).

3. Results and Discussion

3.1 Occurrence of fungi

There are a few reports on the indigenous entomopathogenic fungus *M. anisopliae* in fruit orchards of southern Thailand. However, prior studies have investigated isolates exclusively from central northern and eastern parts of the country (Mar & Lumyoung, 2012; Tangthirasunun *et al*., 2010; Thungrabeab *et al*., 2006). In our study, 64 isolates of *M. anisopliae* were extracted from soil (Table 1) and infected insect cadavers (Table 2) sampled from fruit orchards in southern Thailand. The entomopathogenic fungus, *M. anisopliae*, was common in the sampled areas, but showed marked differences in abundance by locality. In our study, the fungus was retrieved from 38.67% of the soil samples (n=150), which is a much higher prevalence than in previous studies (3-22%) of *Metarhizium* sp. in soil samples (Sánchez-Peña *et al*., 2011; Rocha *et al*., 2013). The entomopathogenic fungal species of *M. anisopliae* detected in southern Thailand were similar to those previously reported, in producing green cylindrical conidia with rounded ends from closely packed hyphae (Bidochka *et al*., 1998; Bing & Xing, 2008; Meyling & Eilenberg, 2007; Sánchez-Peña *et al*., 2011; Sun & Lui, 2008).

For the entomopathogenic fungi recovered from the soil samples, which consisted of 58 isolates, the highest prevalence was in Songkhla at 67.24% (39 isolates), followed by Patthalung 24.14% (14 isolates), and Nakhon Si Thammarat 8.62% (5 isolates). The number of indigenous entomopathogenic *M. anisopliae* from Songkhla was highly significantly different from the other locations ($\chi^2 = 56.182$, df = 2, $P = 0.000$) (Table 1). The prevalence of *M. anisopliae* in the present study (Table 1) was higher than those reported by Mar and Lumyong, (2012), Tangthirasunun *et al*. (2010), and Thungrabeab *et al*. (2006) which might be due to different isolation techniques applied.

St. Leger *et al*. (1992) reported that this fungus was screened from a wide range of environments, i.e., forest, urban, and agricultural soils with diverse ecologies. The frequency of occurrence of the indigenous entomopathogenic fungus *M. anisopliae* in our investigation was similar to other reports (Freed *et al*., 2012; Sánchez-Peña *et al*., 2011). Previous studies suggested that *M. anisopliae* is common in agricultural areas and relatively tolerant to contamination by some pesticides (Bruck, 2004; Mochi *et al*., 2005, 2006; Quesada-Moraga *et al*., 2007). In our study, information on pesticides used and soil types in the orchards in three different provinces were not recorded. However, studies are under way to analyze the relationship between the occurrence of these fungi and the environmental factors. Low density of entomopathogenic fungi in arable land seems to be related to the scarcity of hosts, due to soil cultivation, input of fertilizers, and pesticide use (Mochi *et al*., 2005; Rodrigues *et al*., 2005).

Additionally, six isolates of *M. anisopliae* were randomly found and isolated from infected insect cadavers (Table 2); and they were labeled as isolates PSUM01, PSUM02, PSUM03, PSUM04, PSUM06, and PSUM10. *M. anisopliae* PSUM01 and PSUM06 were isolated from dead *Proreus simulans* Stallen found on the soil, and this insect is considered a good natural enemy for use in insect pest control (Jamjanya *et al*., 2008). The *M. anisopliae* PSUM03 and 04 were isolated from dead *Brontispa longissima* Gestro inhabiting young coconut leaves. The remaining isolates *M. anisopliae* PSUM02 and PSUM10 were isolated from dead *P. simulans* and *Pycnoscelus surinamensis* (L.) that inhabited longkong trees.

Various soil characteristics may affect the occurrence of entomopathogenic fungi, especially pH and moisture. The observed pH values ranged from 6.20 to 6.68. The relatively low pH (6.20±0.71) was observed for soil samples from Patthalung. Soil temperature at all sampling sites was similar in the range 25.20-27.67°C (Table 1). The moisture content in soil was low in samples from Songkhla (3.74±0.72%), and high in Nakhon Si Thammarat (6.35±3.70%) (Table 1).

Soil samples from Songkhla were relatively acidic with low moisture content, and these gave the highest abundance of *M. anisopliae*. The fungus accommodates to slightly acidic soils better than other entomopathogenic fungi (Issaly *et al*., 2005; Padmavathi *et al*., 2003), which could explain why in our study *M. anisopliae* predominated in soils with pH lower than 7. Moreover, Rath *et al*. (1992) found that one specific isolate of *M. anisopliae* was able to grow across a wide pH range (4 to 7.8). However, soil samples from Nakhon Si Thammarat showed the lowest number of *M. anisopliae* with pH nearly 7.0 and high soil humidity (Table 1). The isolates representing Nakhon Si Thammarat during the wet season were less active (high activity = high mortality and low % of

recovery) than others (Table 1).

High infection rate was found in cadavers from Nakhon Si Thammarat, although the level of humidity influences the disease transmission in insects. High environmental moisture has been found to be important for fungal entomopathogens to sporulate, and to produce infective asexual conidia on the host cadavers during the necrophytic phase of the fungus (Benz, 1987; Carruthers & Soper, 1987; Ekesi *et al*., 2003; Hajek & St. Leger, 1994; Hall & Papierok, 1982).

3.2 Colonial and conidial morphology

Colonial morphology of the indigenous entomopathogenic fungus, *M. anisopliae*, on SDAY medium is illustrated in Figure 2. Most of the isolates obtained, both from soil samples and insect cadavers, had similar general appearance of colonies flat in form, with smooth mycelia and mat-like mass of conidia. The variation of length to width conidial ratio in the isolated fungi was large, so the isolates were divided into four groups by L/W ratio: 1.69-1.96 (10 isolates), 2.01-2.47 (43 isolates), 2.55-2.94 (9 isolates), and 3.14-3.16 (2 isolates) (Table 3). Representatives of each L/W ratio group, of the indigenous entomopathogenic fungus *M. anisopliae,* are shown in Figure 3.

The conidia of *M. anisopliae* in our study showed variations in their morphology and naturally fell into four groups by the L/W ratio. Other investigators have reported that these fungi could be partially characterized by using morphological traits such as features of colony morphology, and size and shape of conidia (Luangsa-ard *et al.,* 2007, 2009; Tangthirasunun *et al*., 2010). In our study, the colonial length of the fungi was less than 10 µm conforming to the reports by Boucias and Pendland (1998) and Bischoff *et al*. (2009)

3.4 Bioassay test on the adult melon fruit fly, *Z. cucurbitae*

In bioassays the isolated fungi were tested for virulence against adult melon flies, *Z. cucurbitae*, and caused a wide range of mortalities (4-100%) (Table 4 and 5). *M. anisopliae* isolates PSUM02 and PSUM05 induced complete 100% mortality within seven days, in both sexes of *Z. cucurbitae* (Table 4 and 5). The eight isolates PSUM09, PSUM33, PSUM35, PSUM36, PSUM38, PSUM39, PSUM42, and PSUM44, caused below 50% mortality within seven days of

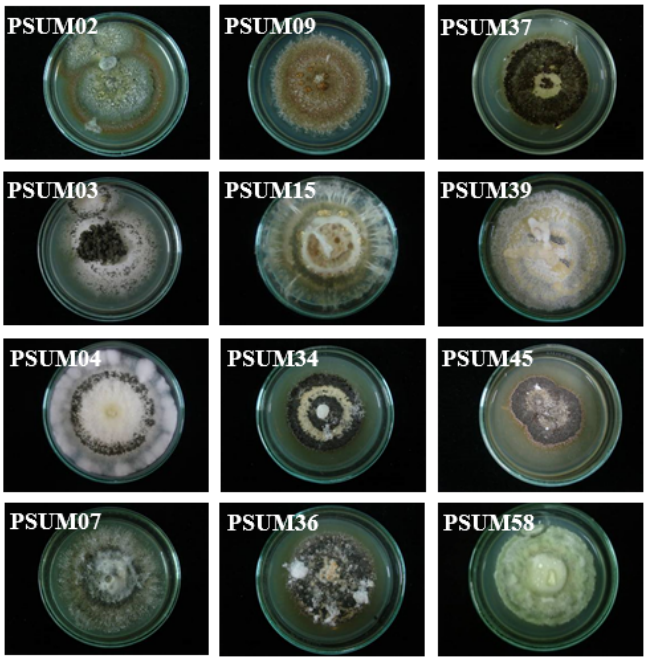

Figure 2. Colonial morphology of some *Metarhizium anisopliae* isolates on culture media.

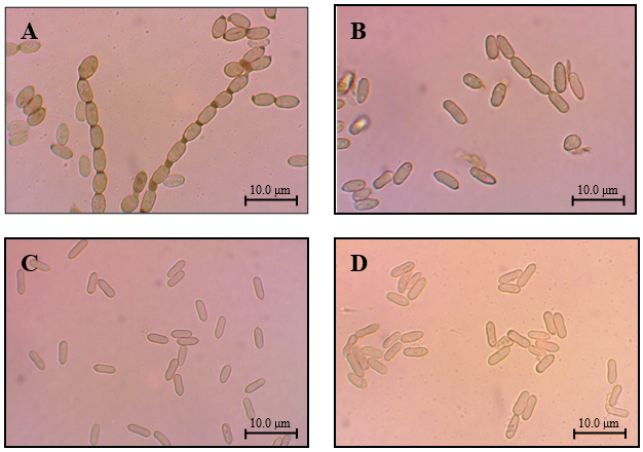

Figure 3. Conidial morphology of the group representatives of indigenous entomopathogenic fungus *Metarhizium anisopliae,* from groups with different length/width ratios (L/W ratio) of conidia: (A) group 1 PSUM38 (1.87), (B) group 2 PSUM07 (2.31), (C) group 3 PSUM15 (2.94), and (D) group 4 PSUM25 (3.16).

Table 2. Isolates of *Metarhizium anisopliae* collected from infected insect cadavers.

Isolate	Insect species	Province
PSUM01	*Proreus simulans* Stallen (Dermaptera: Chelisochidae)	Songkhla
PSUM02	*Tibicen* sp. (Hemiptera: Cicadidae)	Nakhon Si Thammarat
PSUM03	*Brontispa longissima* Gestro (Coleoptera: Chrysomelidae)	Nakhon Si Thammarat
PSUM04	*B. longissima*	Nakhon Si Thammarat
PSUM06	*P. simulans*	Songkhla
PSUM10	*Pycnoscelus surinamensis* (Linnaeus) (Blaberoidea: Blaberidae)	Nakhon Si Thammarat

Table 3. Categories of isolates, clustered by conidial length to width ratio, representing indigenous entomopathogenic *Metarhizium anisopliae*.

Isolate	Number of isolates	Length (µm)	Width(µm)	L/W ratio
PSUM37, PSUM38, PSUM39, PSUM40, PSUM42, PSUM44, PSUM46, PSUM47, PSUM52, PSUM55	10	5.04-5.95	2.62-3.16	1.69-1.96
PSUM01, PSUM02, PSUM03, PSUM04, PSUM05, PSUM07, PSUM08, PSUM09, PSUM10, PSUM11, PSUM12, PSUM13, PSUM16, PSUM17, PSUM18, PSUM19, PSUM21, PSUM22, PSUM23, PSUM24, PSUM27, PSUM28, PSUM29, PSUM31, PSUM32, PSUM33, PSUM34, PSUM35, PSUM36, PSUM41, PSUM43, PSUM45, PSUM48, PSUM49, PSUM50, PSUM51, PSUM53, PSUM54, PSUM55, PSUM57, PSUM59, PSUM62, PSUM63	43	5.09-7.20	2.26-3.04	2.01-2.47
PSUM06, PSUM14, PSUM15, PSUM20, PSUM26, PSUM30, PSUM58, PSUM61, PSUM64	9	5.38-6.82	2.07-2.41	2.55-2.94
PSUM25, PSUM60	2	6.33-6.63	2.00-2.11	3.14-3.16

inoculation, in both sexes of adult *Z. cucurbitae*. These findings are in line with the susceptibility of fruit flies to some isolates of *M. anisopliae* reported previously (Castillo *et al*., 2000; Cossentine *et al*., 2010; Cutiérrez *et al*., 2000; De La Rosa *et al*., 2002; Ekesi *et al*., 2002; Garcia *et al*., 1984; Lezama- Dimbi *et al*., 2003a, b; Quesada-Moraga *et al*., 2006; Sookar *et al*., 2008; Thaochan & Chinajariyawong, 2008).

Lethal times for male flies varied from 2.9 to 12.0 days (LT_{50}, time for 50% mortality), and from 4.8 to 16.0 days (LT_{90}) (Table 4). For female flies the corresponding time ranges were 4.4-11.0 days and 6.0-18.0 days, respectively (Table 5). In our investigation, *M. anisopliae* isolates PSUM02 and PSUM05 showed the highest virulence against adult *Z. cucurbitae*. These isolates represented different sources: PSUM02 and PSUM05 were isolated from insect cadavers and agricultural soil, respectively. Concerning the virulence, it appears unrelated to the original host or the geographic origin (Moorhouse *et al*., 1993; Prior, 1990). The original host of entomopathogenic fungi does not significantly affect their virulence to insect pests (Feng & Johnson, 1990). In this study the indigenous entomopathogenic fungi isolates from insect cadavers (PSUM02) or soils (PSUM05 and PSUM08) had high virulence against melon flies, and this feature appears not attributable to other known circumstances.

4. Conclusions

The indigenous entomopathogenic fungus *M. anisopliae* was sampled from soils and infected insect cadavers in fruit orchards in the southern part of Thailand: Nakhon Si Thammarat, Patthalung and Songkhla provinces. Among the total of 64 isolates, the occurrence of the fungus by locality was affected by some soil properties, *i.e.,* soil pH and soil humidity. Soil with low acidity and low humidity gave the highest occurrence of fungi. The isolates varied in their colonial morphologies and conidia sizes. Most of them had virulence against adult melon fruit fly, *Z. cucurbitae*. The isolates PSUM02, PSUM05 and PSUM08 gave 100% mortality within seven days post inoculation, and appear promising for such pest control. Origins of isolates were not related to their virulence. Some isolates of this entomopathogenic fungus are considered good candidates for mycoinsecticide development for insect pest control.

Acknowledgements

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Table 4. Mortality of adult male melon flies, *Zeugodacus cucurbitae* (Coquillett), after exposure to indigenous *Metarhizium anisopliae* isolates.

Isolate	% Mortality (% ± S.E.)*	LT_{50} (95% CI)	LT_{90} (95% CI)	Slope (±S.E.)
PSUM02	100.0 ± 0.0^{a}	3.8 (3.3-4.3)	4.9 (4.4-6.1)	1.09 ± 0.08
PSUM05	100.0 ± 0.0^{a}	2.9 (2.5-3.4)	4.8 (4.2-5.7)	0.69 ± 0.04
PSUM07	88.0 ± 4.9^{ab}	5.0 (4.0-5.9)	8.3 (7.2-9.9)	0.39 ± 0.02
PSUM08	96.0 ± 4.0^{a}	4.7 (3.1-6.4)	7.7 (6.2-11.5)	0.45 ± 0.02
PSUM09	16.0 ± 7.5^{efg}	10.2 (9.6-10.8)	15.4 (14.6-16.6)	0.25 ± 0.01
PSUM24	84.0 ± 7.5^{abc}	4.9 (4.2-5.6)	7.8 (6.9-9.3)	0.45 ± 0.02
PSUM33	28.0 ± 8.0^{defg}	10.1 (9.3-11.1)	15.2 (13.7-14.5)	0.26 ± 0.01
PSUM34	64.0 ± 17.2^{abcd}	6.4 (5.6-7.4)	11.2 (9.6-13.8)	0.27 ± 0.02
PSUM35	36.0 ± 11.7^{defg}	8.8 (8.3-9.3)	12.8 (11.9-13.8)	0.32 ± 0.02
PSUM36	48.0 ± 8.0^{cdef}	8.2 (7.6-8.8)	11.9 (11.1-13.2)	0.34 ± 0.02
PSUM37	52.0 ± 4.9^{bcde}	7.0 (6.9-7.2)	8.6 (8.3-8.9)	0.84 ± 0.07
PSUM38	12.0 ± 4.9^{fg}	10.9 (10.4-11.5)	15.0 (14.0-16.5)	0.31 ± 0.02
PSUM39	12.0 ± 4.9^{fg}	10.9 (10.2-12.4)	14.2 (12.7-17.3)	0.39 ± 0.05
PSUM41	88.0 ± 8.0^{ab}	5.5 (5.2-5.8)	7.3 (6.9-7.7)	0.72 ± 0.04
PSUM42	48.0 ± 10.2^{cdef}	8.7 (8.1-9.5)	14.1 (12.9-15.8)	0.24 ± 0.01
PSUM43	88.0 ± 4.9^{ab}	5.1 (4.8-5.4)	6.7 (6.3-7.3)	0.80 ± 0.05
PSUM44	36.0 ± 13.3^{defg}	7.9 (7.5-8.4)	11.6 (10.9-12.5)	0.35 ± 0.02
PSUM45	80.0 ± 6.3^{abc}	5.6 (5.3-5.8)	7.4 (7.1-7.9)	0.69 ± 0.04
PSUM47	96.0 ± 4.0^{a}	4.7 (4.3-5.1)	6.7 (6.2-7.5)	0.63 ± 0.04
PSUM49	52.0 ± 8.0^{bcde}	6.8 (6.5-7.2)	8.9 (8.5-9.7)	0.60 ± 0.04
Control	0.0 ± 0.0^{g}	-	-	-

*The mortality of male melon fly at 7 days post-treatment. Each point is the mean of five replicates. Values in the same column followed by different superscripts are highly significantly different ($P<0.01$) according to Tukey's HSD test. The LT_{50} and LT_{90} (in days) with 95% confidence intervals (CI) and the slopes are also indicated.

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Table 5. Mortality of adult female melon flies, *Zeugodacus cucurbitae* (Coquillett), after exposure to isolates of indigenous *Metarhizium anisopliae*

Isolate	% Mortality (%±S.E.)*	LT_{50} (95% CI)	LT_{90} (95% CI)	Slope (±S.E.)
PSUM02	100.0 ± 0.0^{a}	4.3 (4.2-4.4)	4.9 (4.8-5.2)	1.96±0.19
PSUM05	100.0 ± 0.0^{a}	5.4 (4.7-6.2)	6.9 (6.2-8.8)	0.79±0.05
PSUM07	96.0 ± 4.0^{ab}	4.4 (3.4-5.3)	6.4 (5.4-8.3)	0.63±0.03
PSUM08	100.0 ± 0.0^{a}	5.1 (4.9-5.4)	6.3 (5.9-6.9)	1.05±0.08
PSUM09	4.0 ± 4.0^{f}	12.2 (11.4-12.9)	16.9 (15.9-18.6)	0.26±0.01
PSUM24	$68.0 \pm 12.0^{a-e}$	6.0 (5.4-6.7)	10.3 (9.3-11.9)	0.29±0.02
PSUM33	52.0 ± 13.6^{bcde}	8.6 (7.8-9.6)	12.5 (11.2-14.9)	0.32±0.02
PSUM34	$68.0 \pm 12.0^{a-e}$	5.6 (5.3-6.5)	9.8 (8.8-11.3)	0.33±0.02
PSUM35	40.0 ± 11.0^{def}	7.4 (7.2-7.6)	9.5 (9.1-9.9)	0.62±0.04
PSUM36	44.0 ± 11.7^{cdef}	7.8 (7.4-8.3)	11.1 (10.4-12.3)	0.38±0.02
PSUM37	40.0 ± 11.0^{cdef}	7.0 (6.2-8.1)	9.3 (8.2-11.9)	0.56±0.03
PSUM38	20.0 ± 11.0^{ef}	8.4 (8.1-8.8)	10.8 (10.2-11.5)	0.55±0.04
PSUM39	4.0 ± 4.0^{f}	10.0 (9.5-10.8)	12.5 (11.6-14.3)	0.51±0.05
PSUM41	88.0 ± 8.0^{abc}	5.6 (5.4-5.8)	7.3 (7.1-7.6)	0.75±0.05
PSUM42	36.0 ± 16.0^{def}	7.9 (7.6-8.3)	11.7 (11.1-12.4)	0.34±0.02
PSUM43	76.0 ± 9.8^{abcd}	5.7 (5.5-5.9)	7.5 (7.3-7.8)	0.71±0.04
PSUM44	36.0 ± 9.8^{def}	8.5 (8.2-8.9)	12.7 (12.1-13.5)	0.30±0.02
PSUM45	72.0 ± 8.0^{abcd}	5.9 (5.5-6.3)	8.3 (7.8-9.1)	0.53±0.03
PSUM47	88.0 ± 8.0^{abc}	5.1 (4.9-5.3)	7.3 (7.0-7.7)	0.57±0.03
PSUM49	$72.0 \pm 8.0^{a-e}$	6.2 (5.9-6.3)	7.8 (7.6-8.2)	0.76±0.05
Control	0.0 ± 0.0^{f}	-	-	-

*The mortality of female fruit flies at 7 days post-treatment. Each point represents the mean of five replicates. Values in the same column followed by different superscripts are highly significantly different ($P<0.01$) according to Tukey's HSD test. The LT_{50} and LT_{90} with 95% confidence intervals (CI) and the slopes are also indicated.

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