

The Occurrence of Entomopathogenic Fungi on Mineral and Peat Soils in Peninsular Malaysia

¹Pong Kuan Kin, ¹Wahizatul Afzan Azmi,
²Norman Kamarudin, ²Siti Ramlah Ahmad Ali and ²Ramle Moslim

¹School of Marine and Environmental Sciences,
Universiti Malaysia Terengganu (UMT), Kuala Terengganu, Terengganu, Malaysia
²Applied Entomology and Microbiology Unit,
Malaysia Palm Oil Board (MPOB), Bandar Baru Bangi, Selangor, Malaysia

Article history

Received: 12-01-2016

Revised: 06-01-2017

Accepted: 07-01-2017

Corresponding Author:

Ramle Moslim

Applied Entomology and
Microbiology Unit, Malaysia
Palm Oil Board (MPOB),
Bandar Baru Bangi, Selangor,
Malaysia

Email: ramle@mpob.gov.my

Abstract: The aims of this study are to isolate and identify Entomopathogenic Fungi (EPF) from mineral and peat soils in relation with the soil physico-chemical parameters. The mineral soil was sampled from the MPOB Research Station Hulu Paka in Terengganu, whereas peat soil was sampled from the MPOB Research Station Teluk Intan in Perak. Isolation of these fungi was carried out using a selective medium. Morphological characteristics of fungi were studied by observing the mycelium and conidia grown on agar plates using a light microscope. Soil physico-chemical parameters such as pH, water content, carbon and nitrogen content were also determined. Two species of EPF isolated from both types of soils were identified as *Isaria amoenerosea* and *Metarhizium anisopliae*. On potato dextrose agar, the colony of *I. amoenerosea* was pink in colour and slow growing with floccose mycelium which producing conidiophores with 3 to 4 phialides. The conidia were subglobose or irregular shapes between 2.0-3.0 µm long × 1.7-2.0 µm wide. The colony of *M. anisopliae* was whitish yellow and turned to dark green when matured; slow growing with floccose mycelium. The conidia were cylindrical with the dimension ranging from 6.0-7.0 µm long × 2.0-2.8 µm wide. The result shows that the occurrence of *I. amoenerosea* was more dominant than *M. anisopliae*. In mineral soil, out of 30 samples, *I. amoenerosea* was isolated from 25 soil samples (83%), while the *M. anisopliae* was only found in 15 samples (50%). In peat soil, out of 36 samples collected, 26 samples (72%) were found with *I. amoenerosea*, while the fungus *M. anisopliae* was isolated from eight samples (22%). In this study, the occurrence of EPF on mineral soil was higher than from peat soil, which was possibly due to low water content, high soil temperature and low C/N ratio.

Keywords: Entomopathogenic Fungi, *Isaria amoenerosea*, *Metarhizium anisopliae*, Soil Physico-Chemical Parameters

Introduction

Entomopathogenic Fungi (EPF) are effective biological control agents of various insect pests. Among them, four well known genus are *Metarhizium*, *Beauveria*, *Isaria* and *Lecanicillium* (Charnley and Collin, 2007; Wraight *et al.*, 2007; Vega *et al.*, 2012). The EPF have unique mechanisms in which they can infect insects through direct penetration of cuticle by fungal mycelia and later causing death to insects (Carlile *et al.*, 2001; Charnley and Collins, 2007;

Wraight *et al.*, 2007). The EPF are widely distributed in terrestrial ecosystem (Vega *et al.*, 2012). Most of the EPF can be found naturally in the soil, as soil provides a good environmental shelter, which protected them from solar radiation and against high temperature (Vega *et al.*, 2009). High distribution of soil-borne microorganisms in the agriculture soil indicates that the soil is fertile and healthy, thus give EPF higher chances to grow (Meyling and Eilenberg, 2006). The occurrence of EPF in soil is influenced by soil physico-chemical properties such as pH, organic matter, carbon/nitrogen ratio and cropping

systems (Asensio *et al.*, 2003; Quesada-Moraga *et al.*, 2007; Oddsdóttir *et al.*, 2010). Organic carbon and nitrogen are two important components to measure soil fertility and microbial activity (Kauffman *et al.*, 1998).

Malaysia is the world's second largest oil palm producer and exporter after Indonesia, covering total planted areas of more than 5.6 million hectares in 2015 (MPOB, 2015a; 2016). In Malaysia, the oil palm is mostly planted in mineral soil, but in some areas, the palm also growing well in peat soil. Many studies on the occurrence and distribution of EPF in agricultural soils have been carried out by various researchers especially in temperate countries (Chandler *et al.*, 1997; Jabbour and Barbercheck, 2009; Meyling *et al.*, 2011). However, studies on the occurrence and distribution of EPF in tropical soil, such as soil from oil palm plantation are still lacking. In addition, the indigenous isolates of EPF found from soils in this study could be potentially used as biological agents to control major insect pests of oil palm such as rhinoceros beetles, nettle caterpillars, termites and bunch moths (Kamarudin and Wahid, 2007).

In previous studies, the use of EPF to control oil palm insect pests was focused on EPF isolated from infected insects (Ramlah *et al.*, 1994; Ramle *et al.*, 1999; Shalina *et al.*, 2010). However, the EPF from infected insects is hardly to find, therefore, most of the work has been focusing on isolating them from the soils. Various isolation methods of EPF from soil have been developed, but the methods are mostly suitable for isolation of EPF from soil in temperate countries (Klingen *et al.*, 2002; Keller *et al.*, 2003; Jarmul-Pietraszczyk *et al.*, 2012). Therefore, the objectives of this study are to isolate and identify EPF from mineral and peat soils in relation with the soil physico-chemical parameters.

Materials and Methods

Soil Sampling

Sampling of mineral soil was conducted in oil palm plantation at Malaysian Palm Oil Board (MPOB) Research Station Hulu Paka in Terengganu (Fig. 1). The area is 832 hectares which falls within latitudes N4° 36.478 and longitudes E103° 16.187. Mineral soil is low in organic matter and low in C/N ratio (Heritage *et al.*, 1999). Soil classification indicates that there are five soil series in this area. For each series, two samples were collected from different field blocks. The distances between sampling points varied ranging from 26 to 755 m, depending on the locality of the block. Overall, there were 30 samples were collected from this area.

Sampling of peat soil was conducted in oil palm plantation at MPOB Research Station Teluk Intan in Perak (Fig. 1). The area is 425 hectares which falls within latitudes N3° 49.166 and longitudes E101° 05.568. Peat

soil has high organic carbon and organic matter, low total nitrogen and high C/N ratio (Paramanathan, 2012). The peat soil in this area is classified as sapric, as the organic materials in the soil are completely decomposed (Andriese, 1988). The field has 36 blocks. For each block, two samples were collected either from the center of the block or between fourth and fifth oil palm tree from the roadside. The distances between sampling points were ranging from 90 to 260 m. There were 36 soil samples collected from this area.

In both sampling sites, the soil samples were collected using a soil auger at a depth of 15 cm from the soil surface. There was no fertilizer or herbicides applied in the plantations for at least a month before the soil sampling was conducted. To minimize the effect of abiotic factors such as sunlight and moisture on survival of fungi, the soil samples were placed into a zip-lock plastic bag and stored in polystyrene box at 15°C between 2 to 4 h before they were transported to the laboratory (Inglis *et al.*, 2001; Quesada-Moraga *et al.*, 2007).

Preparation of Soil Sample

In the laboratory, the soil samples were thoroughly mixed manually inside the plastic bag until homogeneous mixture was formed. The mixture was then divided into two parts. The first part was used for isolation of fungi and the second part was used for physico-chemical analysis.

Soil Physico-Chemical Properties

Before the physico-chemical analyses were conducted, 100 to 200 g of the soil was air-dried at room temperature for two to three days. The soil was ground using a mortar and pestle, then sieved using a 2 mm mesh sieve. Gravimetric soil water content was determined by drying 5 g of wet soil in an oven at 105°C for 24 h as described by Van Reeuwijk (1986). Soil pH was analyzed using a pH meter from soil: Water ratio of 1:2 solutions (Patiram *et al.*, 2007). Total carbon (C) and nitrogen (N) contents of the soil were determined using a CN analyzer (Wright and Bailey, 2001).

Preparation of Selective Medium

A selective medium for isolation of EPF from soil was developed based on Ramle *et al.* (1999), but with slight modification. The medium was made from 1 g peptone, 0.50 g yeast extract, 0.20 g chloramphenicol, 0.07g rose Bengal, 25 g Bacto agar and 1000 mL distilled water. These ingredients were mixed and dissolved by boiling in a microwave oven and then the medium was autoclaved at 120°C for 15 min. The medium was allowed to cool down between 60-65°C and then 0.367 g Cetyl Tri-methyl Ammonium Bromide (CTAB) was added into the medium before poured into petri dishes.

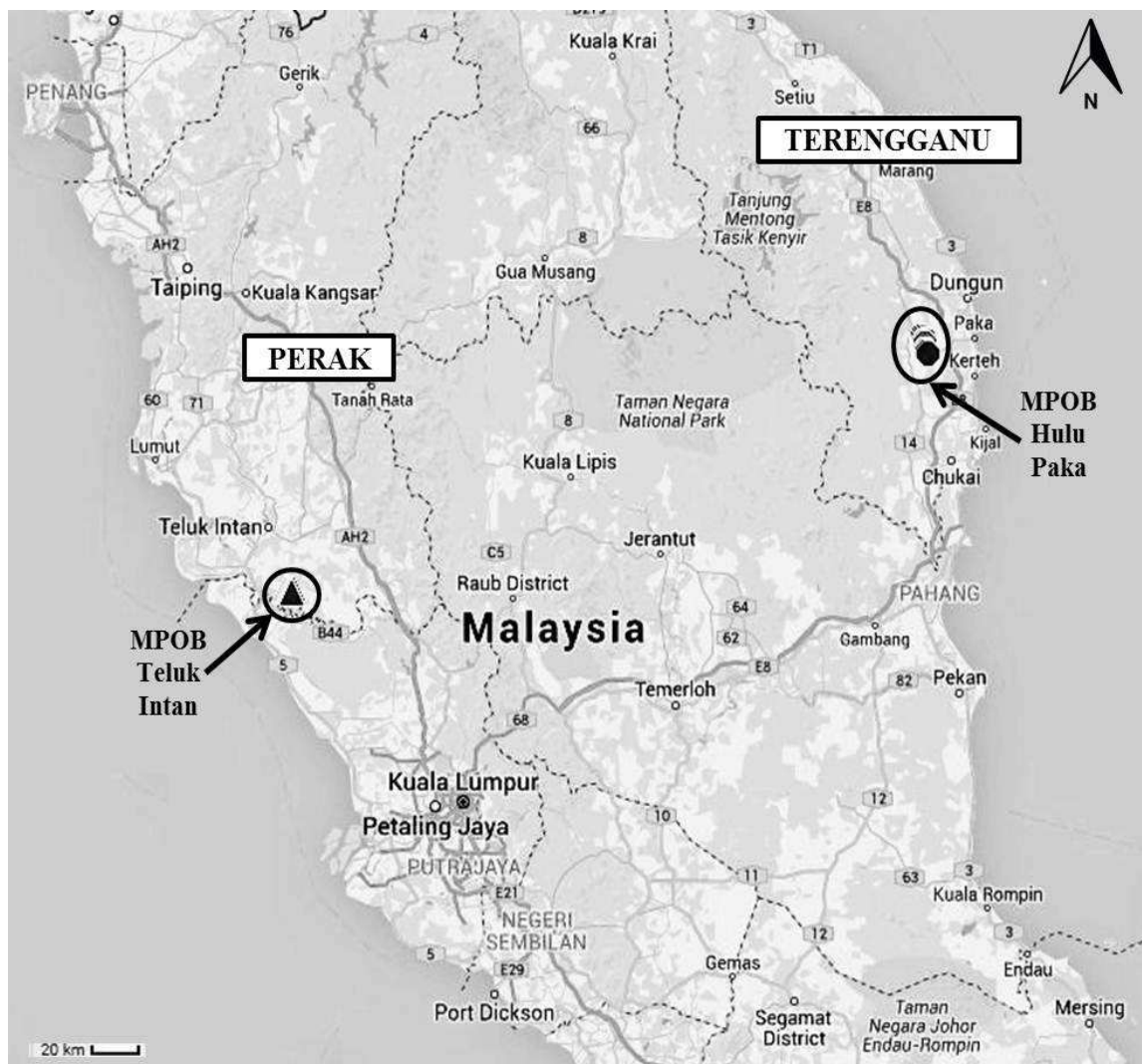


Fig. 1. Map showing location of sampling sites for mineral soil from MPOB Research Station Hulu Paka in Terengganu (latitudes $N4^{\circ} 36.478$ and longitudes $E103^{\circ} 16.187$) and peat soil from MPOB Research Station Teluk Intan in Perak (latitudes $N3^{\circ} 49.166$ and longitudes $E101^{\circ} 05.568$)

Isolation of Entomopathogenic Fungi from Soil

About 5 g of soil samples were placed into a 20 mL universal bottle consisting of 10 mL of sterilized distilled water with 0.02% Tween 80 solution. The soil mixture was vigorously shaken for a few seconds and then the bottle was kept stationary for about a minute to allow sedimentation of soil particles. A total of 1 mL of soil suspension was collected and added into a new bottle. The suspension was diluted 10 times by mixing with 9 mL of sterilized distilled water plus 0.02% Tween 80. A total of 100 μ L soil suspension was pipetted onto selective medium plate and spread evenly using a disposable L-shaped plastic spreader. Three plates were used to isolate EPF from each soil sample. Then the plates were placed inside an incubator at temperature of 28°C for ten

consecutive days. The numbers of fungal Colony Forming Unit (CFU) on each plate was counted. The suspected EPF which grew on the selective medium were sub-cultured onto Potato Dextrose Agar (PDA) for isolation and later for identification.

Identification of Entomopathogenic Fungi

Identification of EPF was based on macro and micro characteristics following the taxonomic keys and classification by various researchers (Samson, 1974; Tulloch, 1976; Rombach *et al.*, 1987; Roberts and St. Leger, 2004; Luangsa-Ard *et al.*, 2005; Bischoff *et al.*, 2009). The macro characteristics of EPF were observed by the colour of the culture and texture of the mycelium grown on the PDA. The micro characteristics of EPF were observed by the shapes and dimension of

conidiophores, phialides and conidia. The micro characteristics of the EPF were observed under an advanced compound microscope equipped with the imaging systems (cell-D, Olympus).

Results

Macro and Micro Characteristics of Entomopathogenic Fungi

Two species of EPF isolated from the mineral and peat soils were identified as *Isaria amoenerosea* and *Metarhizium anisopliae*. The identification of *I. amoenerosea* was firstly based on the taxonomic keys by Samson (1974) and later following reclassification by Luangsa-Ard *et al.* (2005). The *I. amoenerosea* was previously known as *Paecilomyces amoenerosus* by Samson (1974). Whereas, the *Metarhizium* sp. in this study was identified as *M. anisopliae* var. *anisopliae* based on Tulloch (1976), but later it was reclassified as *M. anisopliae* (Roberts and St. Leger, 2004; Bischoff *et al.*, 2009).

On the selective medium, the colonies of *I. amoenerosea* were commonly circular and initially white then turned to powdery and pale in colour with numerous formations of conidiophores (Fig. 2). On PDA, the colony was pink in colour at first and turned to reddish when matured. The colony grew slow and produced floccose mycelium with pink in colour (Fig. 3). Vegetative hyphae were hyaline and smooth-walled. The conidiophores had complex branches with three to four phialides (Fig. 4A). Conidia were small, subglobose, some were irregular in shapes with the dimension between 2.0-3.0 μm long \times 1.7-2.0 μm wide (Fig. 4B).

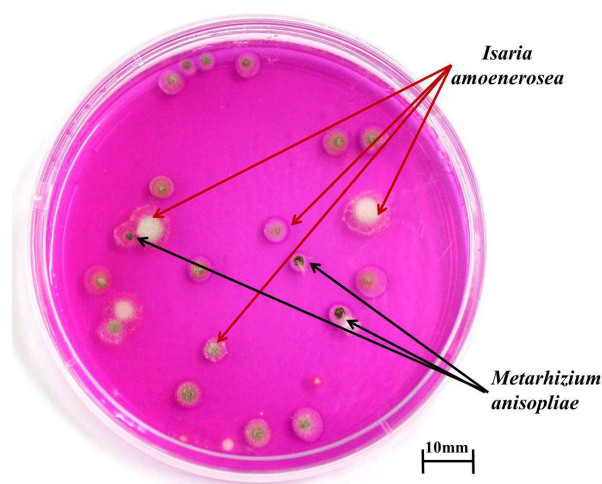


Fig. 2. The morphological appearances of *I. amoenerosea* and *M. anisopliae* on selective medium at ten days after incubation at 28°C

For species *M. anisopliae*, on selective medium, the colonies formed a single green spot with floccose mycelia (Fig. 2). On PDA, the colony was whitish yellow at first and turned to dark green when matured (Fig. 5). The colony was slow growing and produced floccose mycelium with yellow in colour. Vegetative hyphae were hyaline and smooth-walled. The conidiophores have one to two cylindrical phialides (Fig. 6A). Conidia were cylindrical with the dimension ranging from 6.0-7.0 μm long \times 2.0-2.8 μm wide (Fig. 6B).

Occurrence of Entomopathogenic Fungi in Mineral and Peat Soils

The occurrence of EPF *I. amoenerosea* and *M. anisopliae* isolated from mineral and peat soils was shown in Fig. 7. In both soil types, the *I. amoenerosea* was more dominant than the *M. anisopliae*. In the mineral soil, out of 30 soil samples, the *I. amoenerosea* was successfully isolated from 25 soil samples, which contributed to occurrence of 83%. On the other hand, the occurrence of *M. anisopliae* was only 50% (Fig. 7). In the peat soil, the occurrences of *I. amoenerosea* and *M. anisopliae* were relatively low as compared to mineral soil. Out of 36 peat soil samples, only 26 samples or 72% samples were found with fungus *I. amoenerosea*, while *M. anisopliae* was found in eight samples only or 22% occurrence (Fig. 7).

The population density of *I. amoenerosea* and *M. anisopliae* varied depending on the soil types and sampling points (Fig. 8). In mineral soil, the highest CFU of *I. amoenerosea* was recorded in sample AWG-S5, which was approximately 1400 CFU/g, whereas the lowest CFU of *I. amoenerosea* was found in sample AWG-S2 at about 23 CFU/g (Fig. 8A). For *M. anisopliae*, the highest population was recorded in sample BLN-4-S1, which was at 730 CFU/g, whereas the lowest CFU of *M. anisopliae* was found in sample AWG-S2 at about 8 CFU/g (Fig. 8A).

In peat soil, the highest population of *I. amoenerosea* was found in the soil sample 1B1 at about 95 CFU/g and the lowest population was recorded in sample 1B2 at about 18 CFU/g (Fig. 8B). For *M. anisopliae*, the highest CFU was found in soil sample FASA 2 which was at about 1800 CFU/g and the lowest CFU was in sample 4B1, which was 18 CFU/g (Fig. 8B). In the mineral soil, there were 18 samples with *I. amoenerosea* less than 400 CFU/g and 13 samples with *M. anisopliae* less than 400 CFU/g. In the peat soil, 16 samples were found with *I. amoenerosea* of less than 400 CFU/g, five samples with *M. anisopliae* were less than 400 CFU/g. There were two samples in peat, samples of FASA 2 and 5A, recorded higher population of *M. anisopliae* for approximately 1800 CFU/g (Fig. 8B).

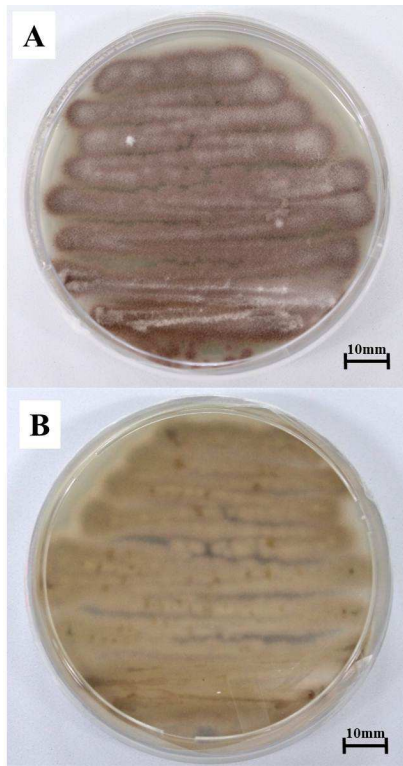


Fig. 3. The appearance of pure culture of fungus *I. amoenerosea* on PDA. (A) Front view and (B) Reverse view

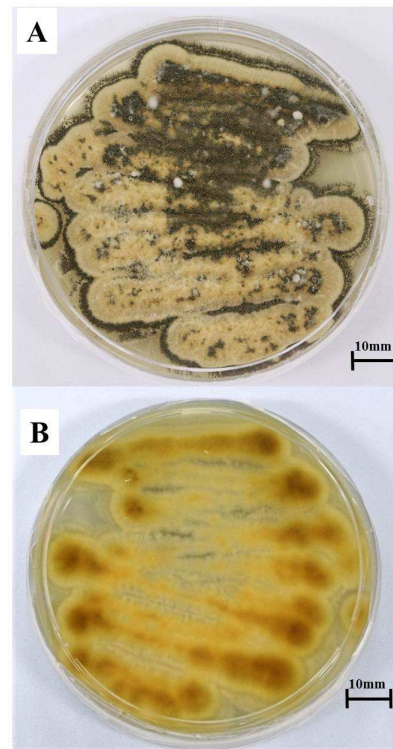


Fig. 5. The appearance of pure culture of fungus *M. anisopliae* on PDA. (A) Front view and (B) Reverse view

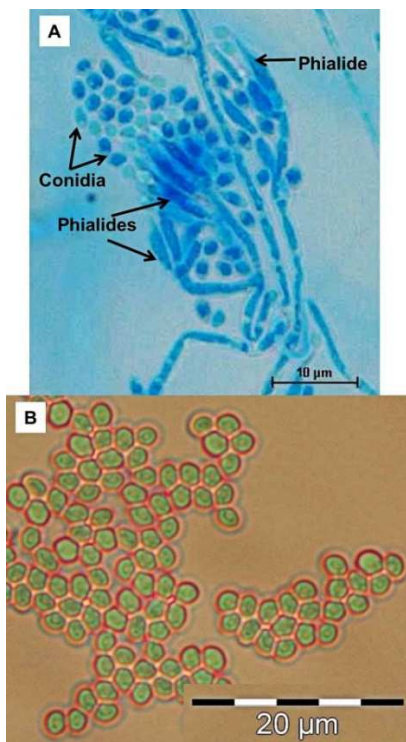


Fig. 4. Microscopic characteristics of *I. amoenerosea* showing the arrangement of structures. (A) Conidiophores and phialides and (B) conidia at 1000× magnifications

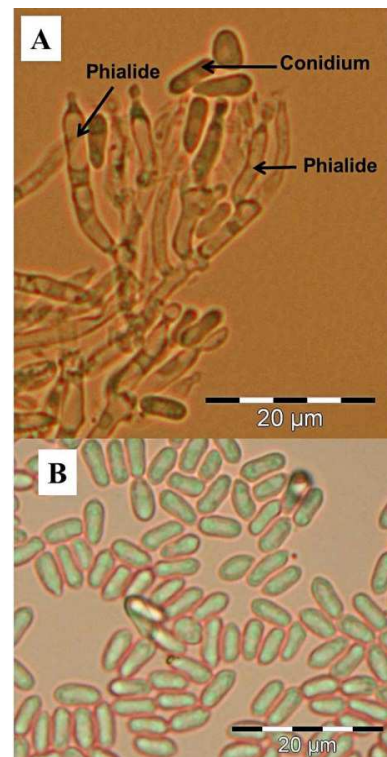


Fig. 6. Microscopic characteristics of *M. anisopliae* showing the arrangement of structures. (A) Conidiophores and phialides and (B) conidia at 1000× magnifications

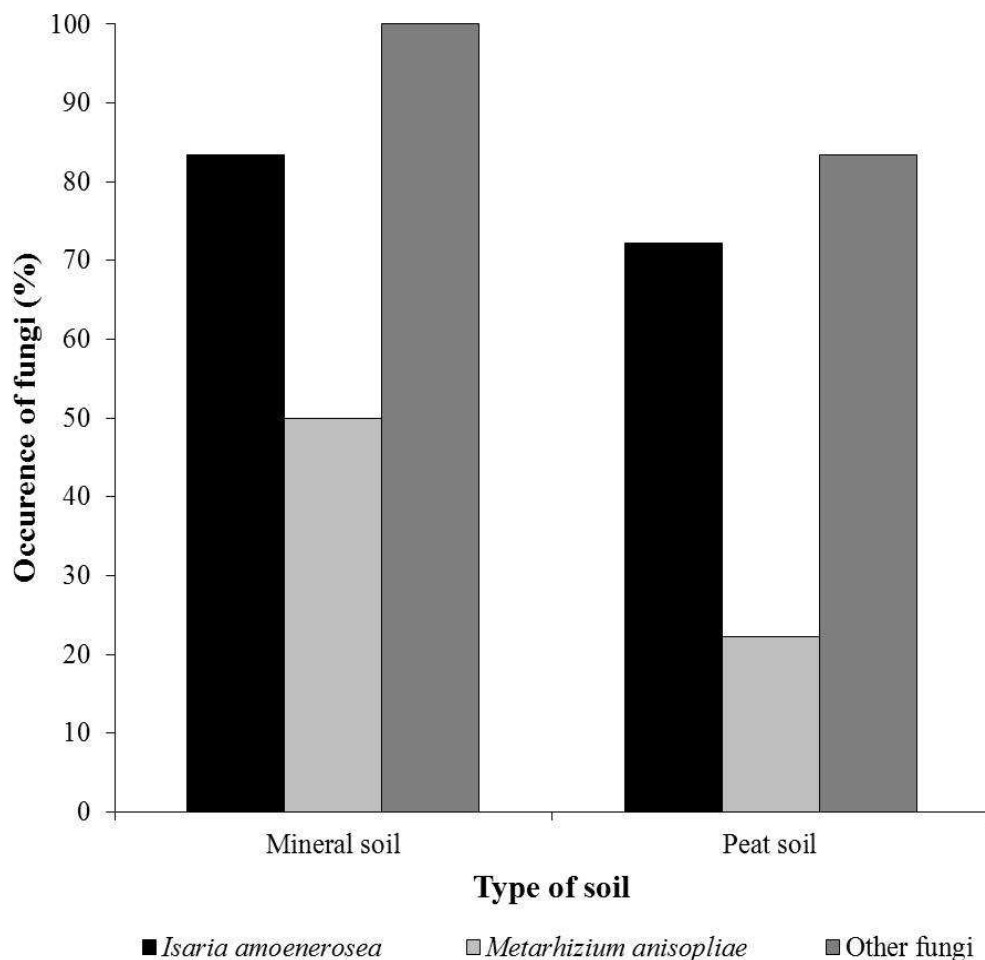


Fig. 7. The occurrence of entomopathogenic and other fungi (*Penicillium* sp., *Aspergillus* sp. and *Tricoderma* sp.) on mineral soil sampled from the MPOB Research Station Hulu Paka in Terengganu and peat soil sampled from the MPOB Research Station Teluk Intan in Perak

In addition, other fungi such as *Penicillium* sp., *Aspergillus* sp. and *Tricoderma* sp. were also isolated from both types of soils. Our data show that the occurrence of these three species either in combination of three, two or single species were higher than the EPF in both types of soil (Fig. 7). These fungi were present in all samples of mineral soil, while in peat it was only 83.3%.

The Effect of Soil Physico-Chemical Parameters on Occurrence of EPF in Soils

The physico-chemical parameters such as pH, water content, total carbon, total nitrogen and C/N ratio for both types of soils are shown in Table 1 and 2. The acidity for mineral soil was ranging from pH 3.38 to 4.35, slightly less acidic than peat soil that ranging from 3.00 to 4.01 pH. The water content between two types of soils was distinctly different. Peat soil had

much higher water content than the mineral soil. For peat soil the percentage of water content was ranging from 79.37 to 425.32%, while for mineral soil it was ranging from 12.01 to 50.57% (Table 2).

The contents of carbon and nitrogen for mineral soil were ranging from 0.81 to 2.41% and from 0.34 to 0.61%, respectively and with the C/N ratio ranging from 2 to 4. Peat soil had high carbon content, which was ranging from 34.87 to 52.45%, low in nitrogen content of 1.67 to 2.12% and high C/N ratio ranging from 21 to 30 (Table 2).

Correlation analysis was performed to determine if any relationship between population density of both types of EPF and the soil physico-chemical parameters. The result shows that very weak correlation was observed between soil parameters such as soil pH, water content and C/N ratio with the population density of *I. amoenerosea* and *M. anisopliae*.

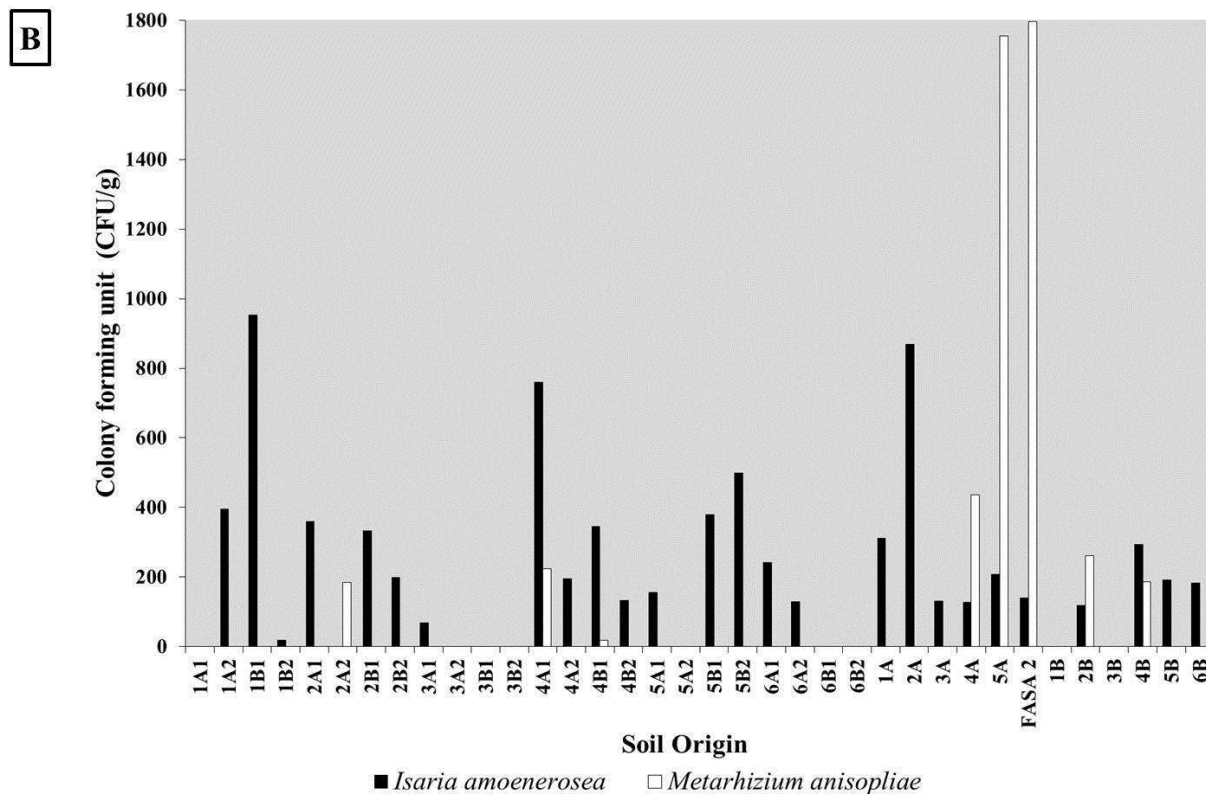
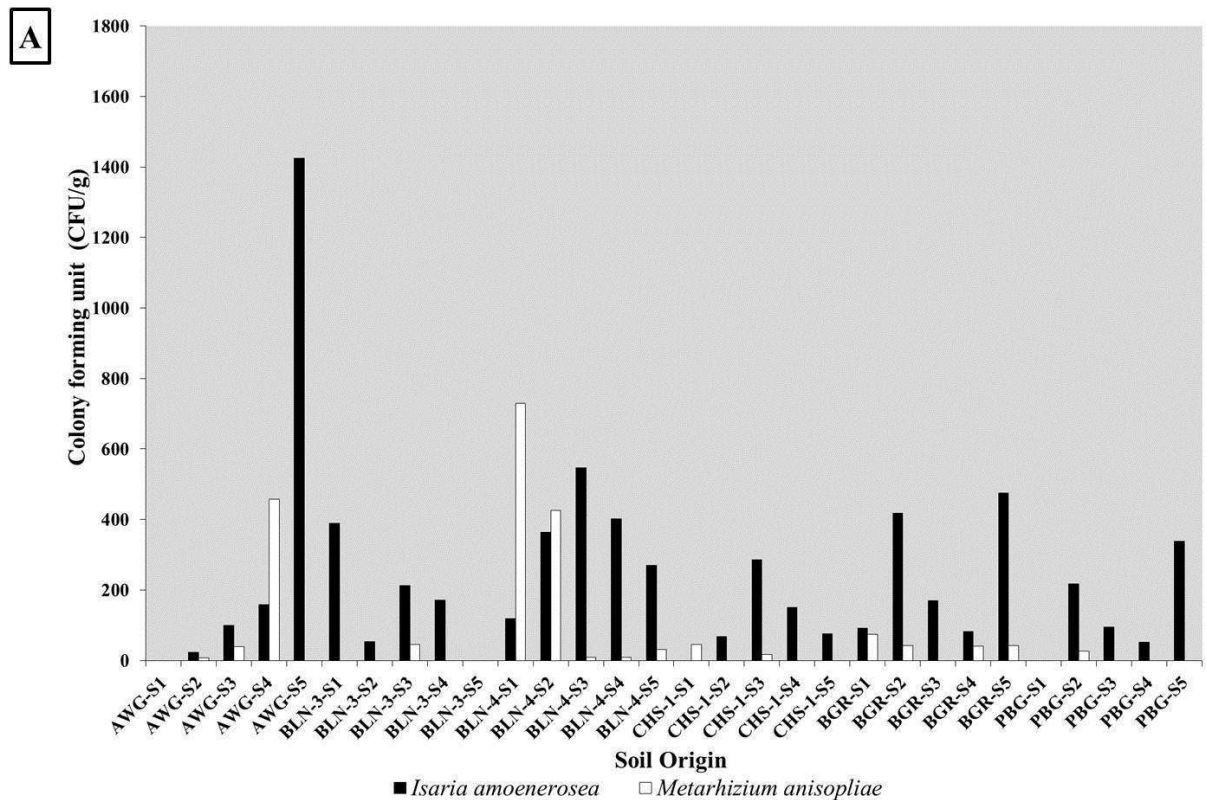


Fig. 8. The population density of *I. amoenerosea* and *M. anisopliae* isolated from (A) mineral soil and (B) peat soil

Table 1. Physico-chemical parameters of mineral soil collected from the MPOB Research Station Hulu Paka in Terengganu

Sample number	pH value	Water content (%)	Total carbon (%)	Total nitrogen (%)	C/N ratio
Awg S1	3.64	20.59	0.94	0.39	2
Awg S2	4.08	19.10	1.22	0.41	3
Awg S3	3.73	50.57	2.01	0.48	4
Awg S4	3.66	40.88	1.91	0.49	4
Awg S5	3.79	29.27	1.03	0.34	3
BT 8/3 S1	4.35	13.02	2.04	0.48	4
BT 8/3 S2	3.73	35.02	1.72	0.49	4
BT 8/3 S3	4.18	14.44	1.05	0.43	2
BT 8/3 S4	4.34	28.52	1.21	0.42	3
BT 8/3 S5	4.30	24.99	1.59	0.41	4
BT 8/4 S1	3.50	12.01	1.02	0.41	2
BT 8/4 S2	3.86	16.31	1.01	0.38	3
BT 8/4 S3	3.49	30.70	1.17	0.49	2
BT 8/4 S4	3.67	31.60	1.42	0.52	3
BT 8/4 S5	3.86	16.42	2.01	0.52	4
BGR S1	3.70	25.13	1.02	0.41	2
BGR S2	3.53	25.84	1.38	0.47	3
BGR S3	3.46	21.53	1.41	0.45	3
BGR S4	3.38	24.87	2.22	0.52	4
BGR S5	3.55	25.53	1.05	0.45	2
CHS S1	3.40	15.48	1.91	0.52	4
CHS S2	3.42	27.10	1.70	0.50	3
CHS S3	3.85	26.37	1.40	0.50	3
CHS S4	3.43	26.13	0.81	0.42	2
CHS S5	3.82	14.61	1.36	0.54	3
PBG S1	3.77	36.09	1.89	0.52	4
PBG S2	3.77	31.19	2.41	0.61	4
PBG S3	3.85	29.11	1.57	0.52	3
PBG S4	3.59	31.94	0.97	0.48	2
PBG S5	3.60	21.64	1.18	0.53	2

Table 2. Physico-chemical parameters of peat soil collected from the MPOB Research Station Teluk Intan in Perak

Sample number	pH value	Water content (%)	Total carbon (%)	Total nitrogen (%)	C/N ratio
1A1	3.11	321.44	48.69	1.93	25
1A2	3.06	196.22	49.83	2.01	25
1B1	3.08	260.17	49.25	1.84	27
1B2	3.20	180.92	52.22	1.78	29
2A1	3.15	157.42	51.39	1.71	30
2A2	3.09	246.69	51.34	1.78	29
2B1	3.37	179.90	50.47	1.75	29
2B2	3.01	276.42	52.28	1.84	28
3A1	3.00	416.64	50.98	1.76	29
3A2	3.23	185.53	50.71	1.76	29
3B1	3.14	366.87	51.46	1.82	28
3B2	3.24	220.67	51.52	1.94	27
4A1	3.23	237.32	50.96	1.83	28
4A2	3.33	195.78	49.31	1.9	26
4B1	3.20	174.36	49.56	1.93	26
4B2	3.14	186.64	49.04	1.85	26
5A1	3.07	135.65	52.45	1.83	29
5A2	3.12	267.31	50.82	1.84	28
5B1	3.05	422.52	52.10	1.79	29
5B2	3.54	187.82	49.76	1.83	27
6A1	3.30	131.38	48.74	1.82	27
6A2	4.01	177.63	45.92	1.83	25
6B1	3.00	238.94	50.62	1.73	29
6B2	3.12	211.98	50.68	1.77	29
1A	3.41	234.07	49.88	1.91	26
2A	3.10	118.87	48.62	1.83	27
3A	3.05	425.32	51.48	1.74	30
4A	3.20	173.42	47.00	1.88	25
5A	3.24	124.73	34.87	1.67	21
FASA 2	3.72	200.98	47.76	2.12	23
1B	3.46	204.48	47.30	1.78	27
2B	3.62	79.37	48.55	1.94	25
3B	3.05	242.19	50.47	1.79	28
4B	3.14	301.06	51.34	1.83	28
5B	3.29	310.30	50.46	1.81	28
6B	3.26	206.74	37.60	1.74	22

Discussion

Two species of EPF, the *I. amoenerosea* and *M. anisopliae* were isolated from mineral and peat soils. Based on taxonomic keys by Samson (1974), there are two species of *Isaria* that have pink colonies which are *I. fumosorosea* and *I. amoenerosea*. However, the sizes of conidia for these two species were different. The size of conidia of *I. fumosorosea* is longer than *I. amoenerosea* with the dimension between 3.0-4.0 μm long \times 1.0-2.0 μm wide. While for *I. amoenerosea*, the size of conidia is between 2.5-3.5 μm long \times 1.7-2.2 μm wide. Based on Samson (1974), the conidia dimension of *I. amoenerosea* found in this study which was between 2.0-3.0 μm long \times 1.7-2.0 μm wide was closely similar with those species of *I. amoenerosea*. Based on molecular phylogenetic study by Luangsa-Ard *et al.* (2005), this species was named as *I. amoenerosea*. Based on taxonomic keys by Tulloch (1976) and Rombach *et al.* (1987), the isolated *Metarhizium* was classified as *M. anisopliae* var. *anisopliae* or well known as short-spored isolates (Fig. 4). Later, based on classification and molecular phylogenetic study (Roberts and St. Leger, 2004; Bischoff *et al.*, 2009), this species was only called as *M. anisopliae*.

Another species of EPF that has received a great interest for research was *Beauveria* sp. This species has a wide hosts, infecting numerous insect pests and its can be isolated either from infected insect cadavers or soil (Oduor *et al.*, 2000; Meyling and Eilenberg, 2006; Quesada-Moraga *et al.*, 2007; Thakur and Sandhu, 2010). However, unlike *Metarhizium* sp., none of the samples from both types of soil was found with *Beauveria* sp. High occurrence of *Metarhizium* sp. was also found by Vega *et al.* (2012), who reported that the *Metarhizium* sp. were easily isolated from soil in tropical countries as compared to *Beauveria* sp. that was more commonly found in temperate countries. Thus, this indicated that the *Beauveria* sp. is more tolerant to cooler environment as claimed by various workers (Keller *et al.*, 2003; Meyling and Eilenberg, 2006; Medo and Cagáñ, 2011).

It is well known that the *I. fumosorosea* and *I. farinosa* are commonly isolated from the soil and pathogenic to a wide range of insects, but not the *I. amoenerosea* (Meyling and Eilenberg, 2006; Sun and Liu, 2008; Medo and Cagáñ, 2011). Although the reason for this is still unknown, but it was possibly due to the differences in selective medium compositions and geographical localities of the samples.

In general, there are two methods that can be used to isolate EPF from soil, either using a selective medium or insect-baiting method using the larvae of *Galleria mellonella* or *Tenebrio molitor*. Isolating EPF using a selective medium is relatively difficult due to the presence of fast growing saprophytic fungi living in the soil. Besides the selective medium is complex and expensive, but yet this method has been widely used by

many researchers as it is found effective in isolating various species of EPF (Strasser *et al.*, 1996; Fernandes *et al.*, 2010; Rangel *et al.*, 2010). In this study, the developed selective medium was partially inhibited the growth of saprophytic fungi. Although some of them were still growing, but they grew at relatively slower rate, thus making the isolation of EPF from the same plate was possible.

In the development of selective media, dodine have been commonly used in several previous studies (Beilharz *et al.*, 1982; Chase *et al.*, 1986; Liu *et al.*, 1993; Strasser *et al.*, 1996; Rangel *et al.*, 2010). Other studies have shown that fungicides such as thiabendazole, benomyl and cupric sulphate can also be used in selective media for isolating EPF from soils (Luz *et al.*, 2007; Fernandes *et al.*, 2010). However, these substances are expensive and hardly to find and therefore it has been replaced with much cheaper substance such as CTAB. Findings in this study and other previous studies have shown that CTAB is cost-effective materials which can be used in selective medium for isolating EPF from soil (Mohan *et al.*, 1982; Ramle *et al.*, 1999; Posadas *et al.*, 2012).

Findings in this study demonstrate that the high occurrence of fungi in soil under the oil palm plantation suggest that the sufficient availability of nutrients and organic matters for the fungi to grow (Ingham, 2000). In the respect of EPF, their occurrence was relatively higher in the mineral soil as compared to peat soil (Fig. 7). In the mineral soil, the occurrence of *I. amoenerosea* and *M. anisopliae* were 83% and 50%, respectively, while in peat soil was 72% for *I. amoenerosea* and 22% for *M. anisopliae*. High occurrence of EPF in mineral soil could be associated with the soil physico-chemical factors such pH, water content and C/N ratio.

The pH value for mineral soil was between pH 3.38 to 4.35, slightly less acidic than peat soil of between 3.00 to 4.01 pH. Study by Quesada-Moraga *et al.* (2007) found that the EPF, especially the *M. anisopliae* was commonly found in slightly acidic soil with pH less than 7. High in water content in peat possibly made the temperature of peat soil cooler than mineral soil, thus make the soil unfavorable to EPF, as compared to mineral soil with higher temperatures (Vänninen *et al.*, 2000). Therefore, findings in this study showed that the occurrence of EPF in peat soil was slightly lower than mineral soil. Vänninen *et al.* (2000) supported this finding, who documented that fungi such as *M. anisopliae* are likely to grow more in soil with high temperatures.

Another factor that associated with high occurrence of EPF in mineral soil was the low C/N ratio as compared to peat soil. Heritage *et al.* (1999) and Paramanathan (2012) reported that mineral soil was generally low in organic matter content, thus contributed low C/N ratio. A study by Miller (2000) found that mineral soil with low C/N ratio was more favorable for

microbiological activity such as bacteria and fungi. Peat soil has high organic carbon and organic matter but low in total nitrogen, in which contributed to high C/N ratio (Paramanathan, 2012; MPOB, 2015b). As reported by Satrio *et al.* (2009), high amount of organic matter and organic carbon can cause the soil to have insufficient of nitrogen for decomposition process and nutrients for microbiological activity. Nitrogen availability in soils is important component for fungal growth (Rousk and Baath, 2007). Other studies also reported that low occurrence of EPF in soil with high organic matter content was due to the presence of high activity of antagonistic microorganisms in the soil (Studdert and Kaya, 1990; Vänninen *et al.*, 2000; Kessler *et al.*, 2003).

Conclusion

In this study, using a selective medium, two types of EPF were successfully isolated and identified as *I. amoenerosea* and *M. anisopliae*. The developed selective medium was effective for isolating EPF from mineral and peat soils. The occurrence of EPF was relatively higher in mineral soil as compared to peat soil and this was due to low water content, high soil temperature, high pH value and low in C/N ratio. The occurrence of the species *I. amoenerosea* was more dominant in both soil types than the species of *M. anisopliae*. Further work need to be carried out particularly on genetic variation among species and their pathogenicity against the oil palm insect pests.

Acknowledgement

The authors would like to thank the Director General of Malaysian Palm Oil Board for supporting this research and permission to publish this paper. We also wish to thank the staff of Insect Biopesticides Research Group of MPOB, MPOB Research Station Hulu Paka in Terengganu, MPOB Research Station Teluk Intan in Perak and staffs of Universiti Malaysia Terengganu (UMT) whom involved in assisting in this study.

Funding Information

The authors would like to thank the Malaysian Palm Oil Board (MPOB) and Universiti Malaysia Terengganu (UMT) for funding the study.

Author's Contributions

Pong Kuan Kin: She carried out most of the laboratory and field works, preparation of samples, statistical analysis, interpreted data and writing the manuscript.

Wahizatul Afzan Azmi: She gave supervision and ideas to perform this study and reviewed the manuscript.

Norman Kamarudin and Siti Ramlah Ahmad Ali: They reviewed on this manuscript.

Ramle Moslim: He gave supervision to perform the study, interpreted results, edited and reviewed the manuscript.

Ethics

The authors confirmed that this manuscript is an original work and do not contain any conflict of interest.

References

- Andriessse, J.P., 1988. Nature and management of tropical peat soils. Food and Agricultural Organizations of the United Nations, Rome, Italy.
- Asensio, L., T. Carbonell, J.L. Jimenez and L.L. Llorca, 2003. Entomopathogenic fungi in soils from Alicante province. Spanish J. Agric. Res., 1: 37-45. DOI: 10.5424/sjar/2003013-33
- Beilharz, V.C., D.G. Parbery and H.J. Swart, 1982. Dodine: A selective agent for certain soil fungi. Trans. Brit. Mycol. Society, 79: 507-511. DOI: 10.1016/S0007-1536(82)80043-0
- Bischoff, J.F., S.A. Rehner and R.A. Humber, 2009. A multilocus phylogeny of the *Metarhizium anisopliae* Lineage. Mycologia, 101: 512-530. DOI: 10.3852/07-202
- Carlile, M.J., S.C. Watkinson and W.G. Graham, 2001. The Fungi. 2nd Edn., San Diego, Academic Press, ISBN-10: 9780127384450, pp: 460.
- Chandler, D., D. Hay and A.P. Reid, 1997. Sampling and occurrence of entomopathogenic fungi and nematodes in UK soils. Applied Soil Ecol., 5: 133-141. DOI: 10.1016/S0929-1393(96)00144-8
- Charnley, A.K. and S.A. Collins, 2007. Entomopathogenic Fungi and their Role in Pest Control. In: Environmental and Microbial Relationships, Esser, K., C.P. Kubicek and I.S. Druzhinina (Eds.), Springer Science & Business Media, Berlin, ISBN-10: 3540718397, pp: 159-187.
- Chase, A.R., L.S. Osborne and V.M. Ferguson, 1986. Selective isolation of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* from an artificial potting medium. Florida Entomol., 69: 285-292. DOI: 10.2307/3494930
- Fernandes, É.K.K., C.A. Keyser, D.E.N. Rangel, R.N. Foster and D.W. Roberts, 2010. CTC medium: A novel dodine-free selective medium for isolating entomopathogenic fungi, especially *Metarhizium acridum*, from soil. Biol. Control, 54: 197-205. DOI: 10.1016/j.biocontrol.2010.05.009
- Heritage, J., E.G.V. Evans and R.A. Killington, 1999. Microbiology in Action. 1st Edn., Cambridge University Press, Cambridge, ISBN-10: 0511019580, pp: 3.

- Ingham, E. 2000. Soil Fungi. In: Soil Biology Primer. Tugel, A.J., A.M. Lewandowski and D. Happe-Vonarb (Eds.), Soil and Water Conservation Society, Iowa, pp: D1-D4.
- Inglis, G.D., M.S. Goettel, T.M. Butt and H. Strasser, 2001. Use of Hyphomycetous Fungi for Managing Insect Pests. In: Fungi as Biocontrol Agents. Progress, Problems and Potential, Butt, T. M., C. Jackson and N. Magan, (Eds.), CABI Publishing, Allingford, ISBN-10: 0851993567, pp: 23-70.
- Jabbour, R. and M.E. Barbercheck, 2009. Soil management effects on entomopathogenic fungi during the transition to organic agriculture in a feed grain rotation. *Biol. Control*, 51: 435-443. DOI: 10.1016/j.biocontrol.2009.08.004
- Jarmul-Pietraszczyk, J., D. Tumialis, J. Igras, K. Klimaszewski and M. Kamionek *et al.*, 2012. The occurrence of entomopathogenic fungi in two catchment basins of the Zagozdzonka and Zwolenka Rivers in Masovian Province, Poland. *Ann. Warsaw Univ. Life Sci. SGGW, Anim. Sci.*, 51: 59-63.
- Kauffman, S., W. Sombroek and S. Mantel, 1998. Soils of Rainforests Characterization and Major Constraints of Dominant Forest Soils in Humid Tropics. In: Soils of Tropical Forest Ecosystems: Characteristics, Ecology and Management, Schulte, A. and D. Ruhayat (Eds.), Springer Science & Business Media, Berlin, ISBN-10: 3540636072, pp: 9-20.
- Keller, S., P. Kessler and C. Schweizer, 2003. Distribution of insect pathogenic soil fungi in Switzerland with special reference to *Beauveria brongniartii* and *Metarhizium anisopliae*. *BioControl*, 48: 307-319. DOI: 10.1023/A:1023646207455
- Kessler, P., H. Matzke and S. Keller, 2003. The effect of application time and soil factors on the occurrence of *Beauveria brongniartii* applied as a biological control agent in soil. *J. Invertebrate Pathol.*, 84: 15-23. DOI: 10.1016/j.jip.2003.08.003
- Klingen, I., J. Eilenberg and R. Meadow, 2002. Effects of farming system, field margins and bait insect on the occurrence of insect pathogenic fungi in soils. *Agric. Ecosyst. Environ.*, 91: 191-198. DOI: 10.1016/S0167-8809(01)00227-4
- Liu, Z.Y., R.J. Milner, C.F. Mcrae and G.G. Lutton, 1993. The use of dodine in selective media for isolation of *Metarhizium* spp. from soil. *J. Invertebrate Pathol.*, 62: 248-251. DOI: 10.1006/jipa.1993.1107
- Luangsa-Ard, J.J., N.L. Hywel-Jones, L. Manoch and R.A. Samson, 2005. On the relationships of *Paecilomyces* sect. *Isarioidea* species. *Mycol. Res.*, 109: 581-589. DOI: 10.1017/S0953756205002741
- Luz, C., M.C.B. Netto and L.F.N. Rocha, 2007. *In vitro* susceptibility to fungicides by invertebrate-pathogenic and saprobic fungi. *Mycopathologia*, 164: 39-47. DOI: 10.1007/s11046-007-9020-0
- Medo, J. and L. Cagaň, 2011. Factors affecting the occurrence of entomopathogenic fungi in soils of Slovakia as revealed using two methods. *Biol. Control*, 59: 200-208. DOI: 10.1016/j.biocontrol.2011.07.020
- Meyling, N. and J. Eilenberg, 2006. Occurrence and distribution of soil-borne entomopathogenic fungi within a single organic agroecosystem. *Agric. Ecosyst. Environ.*, 113: 336-341. DOI: 10.1016/j.agee.2005.10.011
- Meyling, N.V., K. Thorup-Kristensen and J. Eilenberg, 2011. Below- and above ground abundance and distribution of fungal entomopathogens in experimental conventional and organic cropping systems. *Biol. Control*, 59: 180-186. DOI: 10.1016/j.biocontrol.2011.07.017
- Miller, C., 2000. Understanding the carbon-nitrogen ratio. *Acres USA*, 30: 20-20.
- Mohan, K.S., G.B. Pillai, J.C.R. Pereira, O.D. Dhingra and G.M. Chaves, 1982. A selective medium for isolation of *Metarhizium anisopliae* from cattle dung. *Trans. British Mycol. Society*, 78: 181-182. DOI: 10.1016/S0007-1536(82)80094-6
- MPOB, 2015a. Malaysian Oil Palm Statistics 2014. Malaysian Palm Oil Board (MPOB), Bangi.
- MPOB, 2015b. Tropical Peat Ecosystem. In: The Frequently Asked Questions, Mohd Haniff, H., K. Norman, S. Paramanathan and M.C. Lau (Eds.), Malaysian Palm Oil Board, Bangi, ISBN-10: 9789679612127, pp: 1-9.
- MPOB, 2016. Oil palm planted area by state as at December 2015 (hectares). Retrieved from
- Kamarudin, N.H. and M.B. Wahid 2007. Status of common oil palm insect pests in relation to technology adoption. *Planter*, 83: 371-385.
- Oddsóttir, E.S., C. Nielsen, R. Sen, S. Harding and J. Eilenberg *et al.*, 2010. Distribution patterns of soil entomopathogenic and birch symbiotic ectomycorrhizal fungi across native woodland and degraded habitats in Iceland. *Icelandic Agric. Sci.*, 23: 37-49.
- Oduor, G.I., S.M. Smith, E.A. Chandi, L.W. Karanja and J.O. Agano *et al.*, 2000. Occurrence of *Beauveria bassiana* on insect pests of stored maize in Kenya. *J. Stored Products Res.*, 36: 177-185. DOI: 10.1016/S0022-474X(99)00041-7
- Paramanathan, S., 2012. Keys to the Identification of Malaysian Soils using Parent Materials. 2nd Edn., Selangor, Malaysia.
- Patiram, B., T.N.S. Azad and T. Ramesh, 2007. Soil Testing and Analysis: Plant, Water and Pesticide Residues. 1st Edn., New India Publishing, New Delhi, ISBN-10: 8189422707, pp: 238.
- Posadas, J.B., R.M. Comerio, J.I. Mini, A.L. Nussenbaum and R.E. Lecuona, 2012. A novel dodine-free selective medium based on the use of Cetyl Trimethyl Ammonium Bromide (CTAB) to isolate *Beauveria bassiana*, *Metarhizium anisopliae* sensu lato and *Paecilomyces lilacinus* from soil. *Mycologia*, 104: 974-980. PMID: 22314588

- Quesada-Moraga, E., J.A. Navas-Cortés, E.A.A. Maranhao, A. Ortiz-Urquiza and C. Santiago-Álvarez, 2007. Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. *Mycol. Res.*, 111: 947-966.
DOI: 10.1016/j.mycres.2007.06.006
- Ramlah, S.A.A., M. Ramle and M.W. Basri, 1994. The characteristics of indigenous entomopathogenic fungi isolated from insect pests of oil palm. *J. Oil Palm Res.*, 6: 6-13.
- Ramle, M., M.W. Basri, K. Norman, S. Mukesh and S.A.A. Ramlah, 1999. Impact of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) applied by wet and dry inoculum on oil palm rhinoceros beetles, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). *J. Oil Palm Res.*, 2: 25-40.
- Rangel, D.E., S.J. Dettenmaier, É.K. Fernandes and D.W. Roberts, 2010. Susceptibility of *Metarhizium* spp. and other entomopathogenic fungi to dodine-based selective media. *Biocontrol Sci. Technol.*, 20: 375-389. DOI: 10.1080/09583150903518370
- Roberts, D.W. and R.J. St. Leger, 2004. *Metarhizium* spp., cosmopolitan insect-pathogenic fungi: Mycological aspects. *Adv. Applied Microbiol.*, 54: 1-70. DOI: 10.1016/S0065-2164(04)54001-7
- Rombach, M.C., R.A. Humber and H.C. Evans, 1987. *Metarhizium album*, a fungal pathogen of leaf and planthoppers of rice. *Trans. British Mycol. Society*, 88: 451-459. DOI: 10.1016/S0007-1536(87)80028-1
- Rousk, J. and E. Baath, 2007. Fungal and bacterial growth in soil with plant materials of different C/N ratios. *FEMS Microbiol. Ecol.*, 62: 258-267.
DOI: 10.1111/j.1574-6941.2007.00398.x
- Samson, R.A., 1974. *Paecilomyces* and some allied Hyphomycetes. *Stud. Mycol.*, 6: 1-119.
- Satrio, A.E., S. Gandaseca, O.H. Ahmed and A.M. Nik Muhamad, 2009. Influence of chemical properties on soil carbon storage of a tropical peat swamp forest. *Am. J. Applied Sci.*, 6: 1969-1972.
DOI: 10.3844/ajabssp.2009.1969.1972
- Shalina, A.T.N., A.A.S. Ramlah, A.B. Shamsilawani and K.N. Erlina, 2010. Effect of *Beauveria brongniartii* and *B. bassiana* on oil palm bagworm, *Pteroma pendula* (Joannis). *J. Oil Palm Res.*, 22: 729-735.
- Strasser, H., A. Forer and F. Schinner, 1996. Development of media for the selective isolation and maintenance of virulence of *Beauveria brongniartii*. *Proceedings of the 3rd International Workshop on Microbial Control of Soil Dwelling Pests (DSP' 96)*, AgResearch, Lincoln, pp: 125-130.
- Studdert, J.P. and H.K. Kaya, 1990. Water potential, temperature and soil type on the formation of *Beauveria bassiana* soil colonies. *J. Invertebrate Pathol.*, 56: 380-386.
DOI: 10.1016/0022-2011(90)90125-P
- Sun, B.D. and X.Z. Liu, 2008. Occurrence and diversity of insect-associated fungi in natural soils in China. *Applied Soil Ecol.*, 39: 100-108.
DOI: 10.1016/j.apsoil.2007.12.001
- Thakur, R. and S.S. Sandhu, 2010. Distribution, occurrence and natural invertebrate hosts of indigenous entomopathogenic fungi of Central India. *Ind. J. Microbiol.*, 50: 89-96.
DOI: 10.1007/s12088-010-0007-z
- Tulloch, M., 1976. The genus *Metarhizium*. *Trans. British Mycol. Society*, 66: 407-411.
DOI: 10.1016/S0007-1536(76)80209-4
- Van Reeuwijk, L.P., 1986. *Procedures for Soil Analysis*. 1st Edn., ISRIC, Wageningen, ISBN-10: 9066720190, pp: 106.
- Vänninen, I., J. Tyni-Juslin and H. Hokkanen, 2000. Persistence of augmented *Metarhizium anisopliae* and *Beauveria bassiana* in Finnish agricultural soils. *Biocontrol*, 45: 201-222.
DOI: 10.1023/A:1009998919531
- Vega, F.E., N.V. Meyling, J.J. Luangsa-Ard and M. Blackwell, 2012. *Fungal Entomopathogens*. In: *Insect Pathology*, Vega, F.E. and H.K. Kaya (Eds.), Academic Press, Oxford, ISBN-10: 0123849845, pp: 171-220.
- Vega, F.E., M.S. Goettel, M. Blackwell, D. Chandler and M.A. Jackson *et al.*, 2009. *Fungal entomopathogens: new insights on their ecology*. *Fungal Ecol.*, 2: 149-159. DOI: 10.1016/j.funeco.2009.05.001
- Wraight, S.P., G.D. Inglis and M.S. Goettel, 2007. *Fungi*. In: *Field Manual of Techniques in Invertebrate Pathology: Application and Evaluation of Pathogens for Control of Insects and other Invertebrate Pests*, Lacey, L.A. and H.K. Kaya (Eds.), Springer Science & Business Media, Dordrecht, ISBN-10: 1402059310, pp: 223-248.
- Wright, A.F. and J.S. Bailey, 2001. Organic carbon, total carbon and total nitrogen determinations in soils of variable calcium carbonate contents using a Leco CN-2000 dry combustion analyzer. *Commun. Soil Sci. Plant Analysis*, 32: 3243-3258.
DOI: 10.1081/css-120001118