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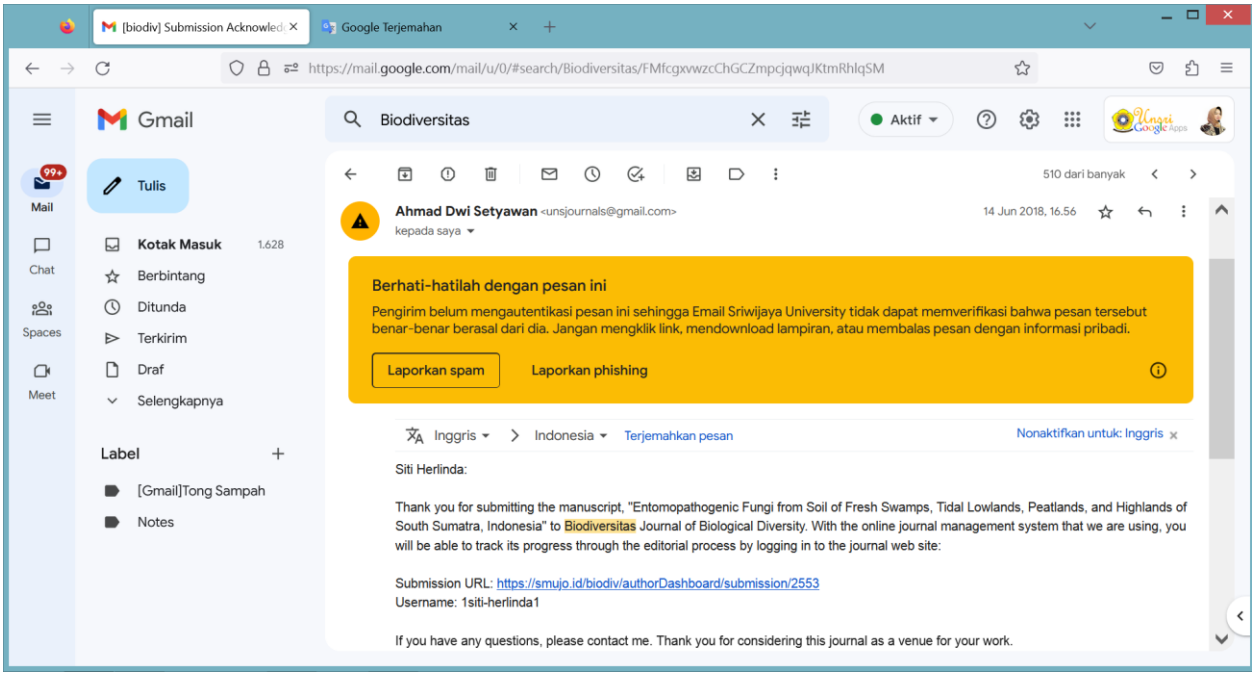

COVERING LETTER

Dear **Editor-in-Chief,**

I herewith enclosed a research article,

Title:

Entomopathogenic Fungi from Soil of Fresh Swamps, Tidal Lowlands, Peatlands, and Highlands of South Sumatra, Indonesia

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For possibility publication on the journal:

Biodiversitas

Novelty:

This study highlights several findings, such as the successful findings of entomopathogenic fungal species, *Beauveria bassiana* and *Metarhizium anisopliae* from soils of wetlands (fresh swamps, tidal lowlands, and peatlands) and highlands in South Sumatra, Indonesia. From highland soils, we found that the entomopathogenic fungi have the highest potential inoculum among those from soils of other ecosystems. Other finding is from peat soils still obtained the entomopathogenic fungi having still high potential inoculum. The findings will make an important contribution to the biological control for insect pests in Indonesia because 30 isolates belonging to the fungi originated and adapted from lowland to highland ecosystems.

Statements:

This manuscript has not been published and is not under consideration for publication to any other journal or any other type of publication (including web hosting) either by me or any of my co-authors.
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Place and date:

Indralaya, 14 June 2018

Sincerely yours,

Siti Herlinda

Entomopathogenic fungi from soils of fresh swamps, tidal lowlands, peatlands, and highlands of South Sumatra, Indonesia

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ABSTRACT. Ecosystems of lowlands (fresh swamps, tidal lowlands, and peatlands) and highlands in South Sumatra, Indonesia have specific characteristics of soils and vegetation that can affect availability of entomopathogenic fungi. This study aimed to identify species and to determine inoculum potentials of the entomopathogenic fungi from soils of fresh swamps, tidal lowlands, peatlands, and highlands. Baiting of entomopathogenic fungi on soil samples was performed using *Tenebrio molitor*. The entomopathogenic fungi species found were *Beauveria bassiana* and *Metarhizium anisopliae*. The highest percentage of inoculum potentials of the fungi was found on soils planted with cabbage (9.33%) in Talang Patai, Pagaralam in the highlands. Percentage of inoculum potentials of the fungi from the fresh swamps, tidal lowlands and peatlands was not significantly affected by locations and vegetations grown in those ecosystems. However, in highlands, percentage of the inoculum potentials was affected by the locations and vegetations. The highest inoculum potentials of the fungi was found in the highlands (4.04%) and not significantly different than those in the tidal lowlands (3.85%), but significantly higher than those of the fresh swamps and peatlands. These fungi will make an important contribution to the biological control for insect pests in Indonesia because 30 isolates of the fungi could be adapted in lowland to highland ecosystems.

Key words: *Beauveria bassiana*, lowlands, *Metarhizium anisopliae*, peat soils, wetlands

Running title: Entomopathogenic Fungi from Soils

INTRODUCTION

Lowlands and highlands in Indonesia usually used for agriculture. Lowlands which consisted of fresh swamps, tidal lowlands, and peatlands are the ecosystems having water saturated condition for the whole year (Sudana 2005). The lowland ecosystems for agricultural purposes in Indonesia are distributed in Sumatra, Kalimantan, and Papua Islands covering areas of 11 million hectares of tidal lowlands, 9.2 million hectares of fresh swamps, and 14.9 million hectares of peatlands, respectively (Mulyani and Sarwani 2013). The highland ecosystem managed for agriculture with magnitude of 16.15 million hectares was distributed in all islands of Indonesia (Center for Agriculture Data and Information System, Secretariat General 2017).

Soils in lowlands and highlands in Indonesia usually have differences, especially in terms of soil moisture, texture, and acidity (pH). Soil moisture in fresh swamp and tidal lowlands can achieve 60%, and 80 to 500% in peatlands (Maftu'ah and Susanti 2009) and in the range of 16 to 50% in highlands (Utomo *et al.* 2013). Soil texture in fresh swamp was dominated by silt due to sedimentation from river flow and had balance clay and sand fractions (Kartika *et al.* 2018); tidal lowlands contain silt from sedimentation mixture of river water and sea water with balance clay and sand fractions (Marlina *et al.* 2016); peat soil has no clay, sand and silt content, but it had organic matter (Sudana 2005), whereas balance soil texture between clay, sand and silt fractions was found in highland ecosystems (Supriadi *et al.* 2016). Soil pH in fresh swamp was in the ranges of 4 to 4.5 (Kodir and Juwita 2016), pH ranges of 4.17 to 5.35 in tidal lowlands (Marlina *et al.* 2016), pH ranges of 3.60 to 3.95 in peatlands (Utami *et al.* 2009), and pH ranges of 5 to 6 in highlands (Supriadi *et al.* 2016), respectively. The variation of pH values of soils at each location can affect adaptation capability of entomopathogenic fungi surviving (Bugeme *et al.* 2008).

Specific characteristics of soils at four typhologies of ecosystems consisting of fresh swamp, tidal lowlands, peatlands, and highland are closely related to specific cultivated vegetations or crops. Rice usually is cultivated at fresh swamps (Herlinda et al. 2018; Lakitan, et al. 2018a; Lakitan, et al. 2018b) as well as at tidal lowlands; however, rice cultivation at tidal lowlands was more intensive (with two to three planting indices) than that of fresh swamps (one planting index) (Herlinda *et al.* 2018). Only small part of soils at peatlands that can be cultivated with seasonal crops and most peatlands in Indonesia was utilized for forestry and conservation areas (Suriadikarta and Sutriadi 2007). Highlands area usually planted with various types of seasonal and annual crops. Specific vegetation or crop species could affect the soil microorganisms associated with plant and crop roots (El-Ghany 2015).

Microorganisms, such as fungi had been found from fresh swamps and highlands of South Sumatra, Indonesia that can be used to control insect pest called entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* (Herlinda

et al. 2008, 2010). However, there was no complete information for fungi from tidal lowlands and peatlands in South Sumatra. *B. bassiana* and *M. anisopliae* that had been found by previous researchers which kill insect pest were consisted of *Crocidolomia pavonana* Fabricius (Hasyim *et al.* 2009), *Aphis gossypii* (Herlinda 2010), *Plutella xylostella* (Loc and Chi 2007), *Lygus* spp. (Leland *et al.* 2005), and *Oryctes rhinoceros* (Moslim *et al.* 2009). Entomopathogen, such as entomopathogenic fungi or entomopathogenic bacteria can be explored from soils (El-Ghany 2015). Certain entomopathogen species have adaptation capability in certain soils (Bugeme *et al.* 2008). Entomopathogen that had already adapted in fresh swamp soils or highland soils generally has specific advantage, i.e. it can adapt more effective at these soils environment (Erler and Ates 2015). Eksploration of entomopathogen starting from lowland to highland ecosystems will produce high variation of species and genetics that can be utilized at extended area and specific location. The purposes of this study were to identify species and to determine inoculum potentials of the entomopathogenic fungi from soils of fresh swamps, tidal lowlands, peatlands, and highlands in South Sumatra.

MATERIALS AND METHODS

Study area

The selected study sites were agricultural center with specific typhology ecosystems of South Sumatra consisting of fresh swamps, tidal lowlands, peatlands and highlands, respectively (Figure 1 and Table 1). Those ecosystems chosen for explorations of entomopathogenic fungi has objective to obtain the fungi that had adapted in the soils from lowlands to highlands. The selected fresh swamp locations were Gandus; Musi 2, Palembang; Rambutan, Banyuasin; and Pemulutan, Ogan Ilir. The selected locations in tidal lowlands were Mulya Sari, Banyuasin; Telang Sari, Banyuasin; and Muara Sungsang, Banyuasin. The selected locations in peatlands were Talang Dabok, Ogan Komering Ilir; Sepucuk, Ogan Komering Ilir; and Kedaton, Ogan Komering Ilir. The selected locations in highlands were Lematang. Lahat; Tanjung Payang, Lahat; Pulau Pinang, Lahat; Rimau, Pagar Alam; and Talang Patai, Pagar Alam.

Soil sampling was carried out in December 2017 and baiting of entomopathogenic fungi was done by using *Tenebrio molitor*, fungal isolation, purification, and identification conducted from January to March 2018 at Laboratory of Entomology, Department of Plant Pest and Disease, College of Agriculture, Universitas Sriwijaya, Indralaya, Indonesia. Supporting data were recorded in term of soil sampling period, village or city names, coordinate points, and vegetation types or crop species for each exploration locations. Value of pH belonging to soil samples were measured by using a method of Kartika et al. (2018).

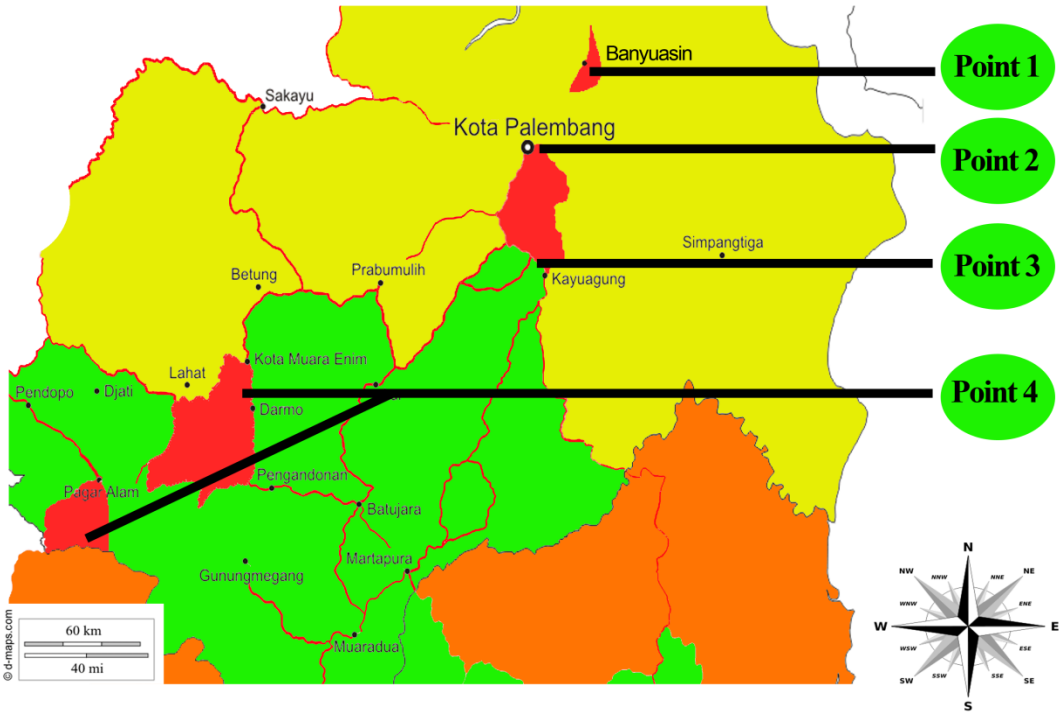

Figure 1. Locations of exploration for entomopathogenic fungi from soils in ecosystems of South Sumatra, Indonesia: point 1 tidal lowlands (S 02°40.866' E 104°44.298'), point 2 fresh swamps (S 03°02.581' E 104°51.231'), point 3 peatlands (S 03°24.840' E 104°53.362'), and point 4 highlands (S 03°48.063' E 103°32.072' and S 03°50.174' E 103°31.293').

Procedures

Observation of Inoculum Potentials of Entomopathogenic Fungi in the Soil

Inoculum potentials are energy of pathogens to infect insect hosts which is measured according to percentage of infected insect hosts by pathogens after infested to inoculum source from soils (El-Ghany 2015). Inoculum potentials observation of the entomopathogenic fungi in this research was started by exploration of fungi in soils of the fresh swamps, tidal lowlands, peatlands and highlands, respectively. Entomopathogenic fungi exploration was done by modifying the methods of Zimmermann (1986) and Anwar *et al.* (2015), i.e. using *T. molitor* as insect bait (*Tenebrio* bait method) which was infested on fungal inoculum of soil samples. Subsequently, three to four soil sampling locations consisting of the fresh swamps, tidal lowlands, peatlands, and highlands were taken from each ecosystem of exploration.

For soil samples sites, several vegetations or crop species population were taken from each location at diagonal position. Soil samples with weight of 1000 g were obtained by digging soil at depth of 5-15 cm in the vicinity of crop roots. Subsequently, soil samples were put into plastic pouch and labelled containing information of soil sampling period and vegetation types or crop species. The soil samples position for each treatment in the laboratory were constructed by using completely randomized design.

Soil samples were previously cleaned of crop roots and sieved by using 5 mesh siever. Then, samples were put into plastic tray (size of 32 cm x 25 cm x 5 cm) containing 1000 g of soil sample for each tray. Soil was subsequently moisted with sterile aquadest until soil moisture of 80-90% using the method of Chen et al. (2014). Then, 30 larvae of the newly-moulting-third instar of *T. molitor* were sterilized with 70% alcohol, and put on soil-sample surface in plastic tray. Each treatment was repeated five times. The body of larvae was sprinkled with one layer of soil sample having thickess of about 5 mm. Subsequently, plastic tray containing soil samples were covered with black cloth and was sprayed with sterile aquadest in order to maintain humidity of soil samples. Larvae were incubated within soil sample for 7 days to provide enough time for entomopathogenic fungi infecting them. Then, dead larvae infected by the entomopathogenic fungi were recorded daily to determine inoculum potential.

Entomopathogen Isolation

The dead *Tenebrio* bait was subsequently isolated and purified by using the methods of Herlinda (2010). Entomopathogenic fungi infecting and growing on integument of *Tenebrio* bait were isolated and grown on SDA (Sabouraud Dextrose Agar) medium. The integument surface of larvae infected by the entomopathogenic fungi was previously sterilized using modified method of Nuraini et al. (2017) with 1% natrium hypochlorite for 3 minutes and subsequently was rinsed 3 times with sterile aquadest and air dried on sterile filter paper. Larvae was put into petridish containing sterile humid tissue paper and then incubated in order to stimulate the growth of entomopathogenic fungi. Conidia of entomopathogenic fungi emerging from the dead larvae body was taken by using sterile ose needle and moved into petridisk containing SDA medium, and incubated for 7 days at constant temperature of 25 ^{o}C within incubator.

Identification of Entomopathogen Fungi

The purified fungi were identified according to macroscopic and microscopic characteristics. Fungi that had been grown on SDA media with area of 1 cm^2 was taken by using ose needle and put into preparations containing SDA media and incubated for three days and then microscopically observed. Subsequently, its morphology was identified macroscopically and microscopically by using the method of Samson et al. (1988). Furthermore, species of fungi were identified by using books of Humber (2005) and El-Ghany (2015).

Data Analysis

Data of inoculum potentials based on *Tenebrio* bait percentage infected by the entomopathogenic fungi among treatments was analyzed by using analysis of variance (ANOVA). If there were differences among treatments, then Honestly Significant Different (HSD) test at 5% was conducted by using program software of SAS University Edition 2.7 9.4 M5.

RESULTS AND DISCUSSION

Insect Characteristics Infected by Entomopathogenic Fungi

Identification based on morphology for entomopathogenic fungi found in this study were consisted of two species, *B. bassiana* and *M. anisopliae.* There was 30 isolates of the entomopathogenic fungi found from these two fungal species

(Table 2). *Tenebrio* bait infected by the entomopathogenic fungi showed symptoms and characteristics which could be used to determine a fungal species. Sick or dead *Tenebrio* bait infected by *B. bassiana* showed symptoms as follows: insect body was dry and wrinkle, its outer integument was coated by white mycelia similar to silk, rigid, easily broken and no smell (Figure 2). The *Tenebrio* bait attacked by *M. anisopliae* showed symptoms as follows: dry and wrinkle, no smell, brittle and easily broken, but its exterior integument was coated by mycelia having greenish white to dark green or dark colour (Figure 3).

Pure isolate was obtained from dead *Tenebrio* bait body which was attacked by entomopathogenic fungi with colony characteristics for each species as follows. Colony of *B. bassiana* had white colour similar to cotton colour, but gradually its colour changed into yellowish white as fungi become older. Colony of *M. anisopliae* initially had white colour similar to colour of *B. bassiana*, and then the colour changed into greenish and dark green or dark as fungi become older (Figure 4). Conidia of *B. bassiana* and *M. anisopliae* with specific characteristics were obtained from each colony species of entomopathogenic fungi. Conidia of *B. bassiana* had single cell and round shape, whereas conidia of *M. anisopliae* had single cell but with cylindrical shape (Figure 5). Mycelia of *B. bassiana* and *M. anisopliae* insulated with upright, branches and layers of conidiofores.

Inoculum Potentials of Entomopathogenic Fungi in the Soil of South Sumatra

Inoculum potentials of entomopathogenic fungi in this research which measured according to percentage of infected *Tenebrio* bait was in the range of 0.67 to 3.33% in lowland swamp soil (Table 3). This research not only found entomopathogenic fungi, but also bacteria. Inoculum potentials of the entomopathogens either fungi or bacteria from fresh swamp soil was in the range of 4.67 to 14.67%. Inoculum potentials of entomopathogens (fungi and bacteria) from the fresh swamp soil was not significantly different among survey locations. Inoculum potentials of the entomopathogens (fungi and bacteria) from the tidal lowlands was not significantly different among survey locations. Inoculum potentials of entomopathogenic fungi found in the tidal lowlands was also not significantly different among survey locations (Table 4). Inoculum potentials of entomopathogenic fungi from tidal lowlands was in the range of 2 to 7.33%, whereas inoculum potentials of both entomopathogens (fungi and bacteria) from tidal lowlands was in the range of 6 to 20.67%. Inoculum potentials of entomopathogenic fungi (2−5.33) found in peat soils was not significantly different among survey locations of peatlands (Table 5). Inoculum potentials of the entomopathogenic fungi and bacteria was also not significantly different (6−8.67%) among survey locations of peatlands. Inoculum potentials of the entomopathogenic fungi and bacteria was not significantly different (4−19.33%) among survey locations of highlands (Table 6). However, the highest percentage for inoculum potentials of entomopathogenic fungi was found in Talang Patai, Pagaralam on cabbage vegetation (9.33%) which was significantly higher than those on rubber and coffee vegetations C in Pulau Pinang, Lahat (1.33%). Therefore, this research showed that inoculum potentials of entomopathogenic fungi from soils of fresh swamps, tidal lowlands and peatlands was not affected by locations and vegetations grown in the ecosystems. However, in highland soils, inoculum potentials of entomopathogenic fungi was affected by locations and vegetations grown in the ecosystems. Thus, highland soil planted with cabbage had more fungal inoculum potentials than that in other locations.

Based on ecosystems classification observed in this research which consisted of the fresh swamps, tidal lowlands, peatlands and highlands, the results showed that inoculum potentials of both entomopathogens (fungi and bacteria) was significantly different among ecosystems; this was also apply for inoculum potentials of entomopathogenic fungi (Table 7). The highest inoculum potentials of entomopathogens (fungi and bacteria) was found in tidal lowlands and it was not significantly different than that in fresh swamps and highlands. The lowest inoculum potentials of the both entomopathogen (fungi and bacteria) was found in peatlands and it was significantly different than that of other ecosystems. The highest inoculum potentials of entomopathogenic fungi was found in highlands (4.04%) and it was not significantly different than that of in tidal lowlands (3.85%), but it was significantly higher than that of in the fresh swamps and peatlands. Therefore, more abundant inoculum potentials of the entomopathogenic fungi was occured in the soils of the highlands and tidal lowlands.

Figure 2. *Tenebrio* bait infected by *Beauveria bassiana* (a) and healthy *Tenebrio* (b)

Figure 3. *Tenebrio* bait infected by *Metarhizium anisopliae* (a) and healthy *Tenebrio* (b)

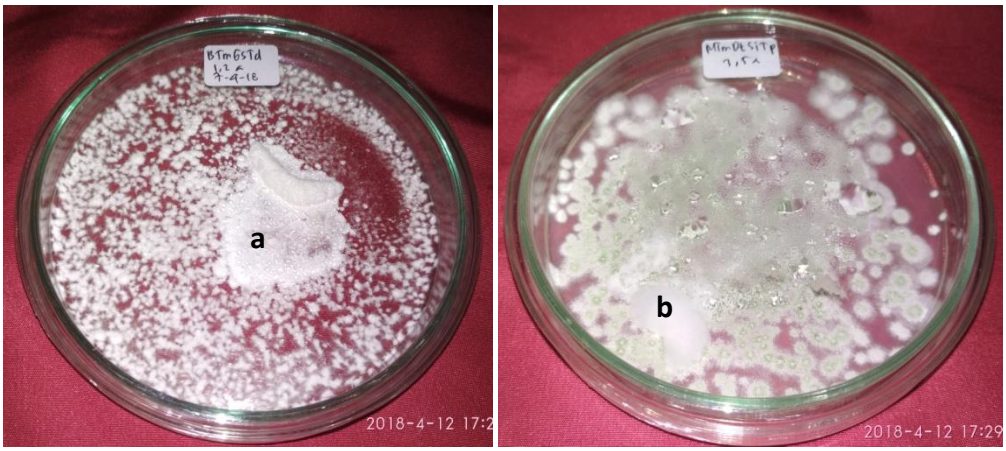

Figure 4. Colony of *Beauveria bassiana* (a) and *Metarhizium anisopliae* (b)

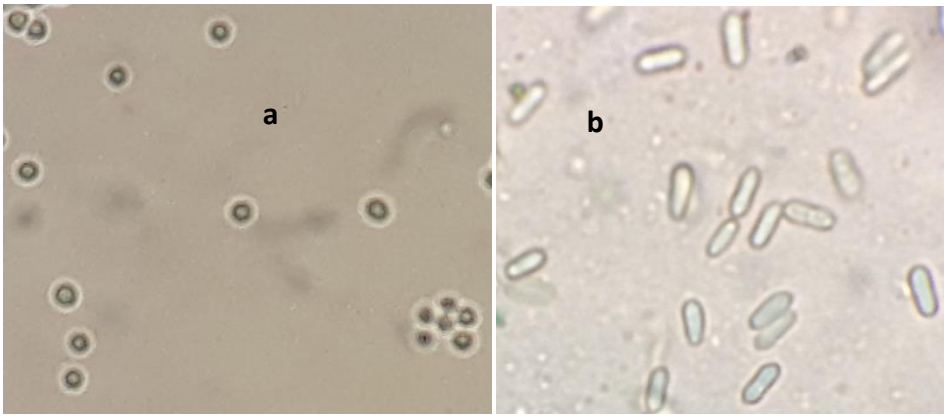

Figure 5. Conidia of *Beauveria bassiana* (a) and *Metarhizium anisopliae* (b) (400x magnification)

Table 1. Locations of exploration for entomopathogenic fungi in South Sumatra, Indonesia.

Ecosystems	**Village or city**	**Vegetation or crop species**	**Height from sea level (m)**
Fresh swamps	Gandus, Palembang	Rice	12
	Musi Dua, Palembang	Rice	16.67
	Rambutan, Banyuasin	Rice	15
	Pemulutan, Ogan Ilir	Rice	22
Tidal lowlands	Mulya Sari, Banyuasin	Corn, rice, watermelon	16.33
	Telang Sari, Banyuasin	Corn, coconut, corn + coconut	18.33
	Muara Sungsang, Banyuasin	Coconut, banana, pineapple	15
Peatlands	Talang Dabok, Ogan Komering Ilir	Palm, rubber, pineapple	24
	Sepucuk, Ogan Komering Ilir	Oil palm, rubber, pineapple	23
	Kedaton, Ogan Komering Ilir	Oil palm	19.67
Highlands	Lematang, Lahat	Rice	121.3

	Tanjung Payang, Lahat	Rubber	121
	Pulau Pinang, Lahat	Rubber + coffee	161
	Rimau, Pagaralam	Tea	1,326
	Talang Patai, Pagaralam	Cabbage	170

Note: + intercropping

Table 2. Species and isolates of entomopathogenic fungi found in South Sumatra, Indonesia.

Ecosystems	Species of fungi	Isolate codes	Vegetation or crop species	Village or city
Fresh swamps	*Metarhizium anisopliae*	MPdB	Rice	Banyuasin
Fresh swamps	*Metarhizium anisopliae*	MPdR1	Rice	Rambutan
Fresh swamps	*Metarhizium anisopliae*	MPdR2	Rice	Rambutan
Fresh swamps	*Metarhizium anisopliae*	MPdPe	Rice	Pemulutan
Fresh swamps	*Beauveria bassiana*	BPdR	Rice	Rambutan
Tidal lowlands	*Metarhizium anisopliae*	MJgMs1	Corn	Mulya Sari
Tidal lowlands	*Metarhizium anisopliae*	MJgMs2	Corn	Mulya Sari
Tidal lowlands	*Metarhizium anisopliae*	MPdMs1	Rice	Mulya Sari
Tidal lowlands	*Metarhizium anisopliae*	MPdMs2	Rice	Mulya Sari
Tidal lowlands	*Metarhizium anisopliae*	MPdMs3	Rice	Mulya Sari
Tidal lowlands	*Metarhizium anisopliae*	MJgKeTs	Corn + coconut	Telang Sari
Tidal lowlands	*Metarhizium anisopliae*	MPdMs4	Rice	Mulya Sari
Tidal lowlands	*Metarhizium anisopliae*	MJgTs1	Corn	Telang Sari
Tidal lowlands	*Metarhizium anisopliae*	MJgTs2	Corn	Telang Sari
Tidal lowlands	*Beauveria bassiana*	BJgTs	Corn	Telang Sari
Tidal lowlands	*Beauveria bassiana*	BSmMs	Watermelon	Mulya Sari
Peatlands	*Beauveria bassiana*	BSwTd1	Oil palm	Talang Dabok
Peatlands	*Beauveria bassiana*	BSwTd2	Oil palm	Talang Dabok
Peatlands	*Beauveria bassiana*	BSwTd3	Oil palm	Talang Dabok
Peatlands	*Beauveria bassiana*	BSwTd4	Oil palm	Talang Dabok
Highlands	*Metarhizium anisopliae*	MKbTp1	Cabbage	Talang Patai
Highlands	*Metarhizium anisopliae*	MSwTp1	Mustard	Talang Patai
Highlands	*Metarhizium anisopliae*	MSwTp2	Mustard	Talang Patai
Highlands	*Metarhizium anisopliae*	MSwTp3	Mustard	Talang Patai
Highlands	*Metarhizium anisopliae*	MSwTp4	Mustard	Talang Patai
Highlands	*Metarhizium anisopliae*	MKKPp1	Rubber + coffee	Pulau Pinang
Highlands	*Metarhizium anisopliae*	MKbTp2	Cabbage	Talang Patai
Highlands	*Metarhizium anisopliae*	MKbTp3	Cabbage	Talang Patai
Highlands	*Beauveria bassiana*	BKKPp2	Rubber + coffee	Pulau Pinang
Highlands	*Beauveria bassiana*	BKbTp	Cabbage	Talang Patai

Note: + intercropping

Table 3. Inoculum potentials of entomopathogenic fungi in the fresh swamp soils of South Sumatra.

Village or city	Vegetation or crop species	GPS (coordinat)	Inoculum potentials (%)		
			Fungi	Bacteria	Total
Rambutan, Kabupaten Banyuasin	Rice	S 03°00.401' E 104°42.380'	0.67	10.00	10.67
Rambutan, Kabupaten Banyuasin	Rice	S 03°00.632' E 104°42.532'	2.67	12.00	14.67
Rambutan, Kabupaten Banyuasin	Rice	S 03°00.632' E 104°42.801'	1.33	10.00	11.33
Gandus, Kota Palembang	Rice	S 03°02.120' E 104°43.021'	3.33	6.67	10.00
Gandus, Kota Palembang	Rice	S 03°02.150' E 104°43.612'	1.33	10.67	12.00
Gandus, Kota Palembang	Rice	S 03°02.510' E 104°43.120'	2.67	6.00	8.67
Musi 2, Kota Palembang	Rice	S 03°02.581' E 104°51.231'	2.00	10.00	12.00
Musi 2, Kota Palembang	Rice	S 03°02.591' E 104°51.217'	3.33	10.67	14.00
Musi 2, Kota Palembang	Rice	S 03°02.586' E 104°51.201'	2.67	8.00	10.67
Pemulutan, Kabupaten Ogan Ilir	Rice	S 03°03.148' E 104°46.230'	1.33	6.00	7.33
Pemulutan, Kabupaten Ogan Ilir	Rice	S 03°03.115' E 104°46.218'	1.33	3.34	4.67
Pemulutan, Kabupaten Ogan Ilir	Rice	S 03°03.113'	2.67	12.00	14.67

	E 104°46.201'		
ANOVA F-value	0.50ns	0.71ns	0.624ns
P value (0.05)	0.89	0.72	0.799
Tukey's HSD test	-	-	-

Note: ns = not significantly different; values within a column followed by the same letters are not significantly different at P < 0.05 according to HSD test.

Table 4. Inoculum potentials of entomopathogenic fungi in the tidal lowland soils of South Sumatra

Village or city	Vegetation or crop species	GPS (coordinat)	Inoculum potentials (%)		
			Fungi	Bacteria	Total
Mulya Sari, Kabupaten Banyuasin	Corn	S 02°40.866' E 104°44.298'	4.00	16.67	20.67
Mulya Sari, Kabupaten Banyuasin	Rice	S 02°40.944' E 104°44.621'	3.33	4.00	7.33
Mulya Sari, Kabupaten Banyuasin	Watermelon	S 02°40.896' E 104°44.676'	3.33	7.34	10.67
Telang Sari, Kabupaten Banyuasin	Corn	S 02°38.842' E 104°45.369'	4.00	6.67	10.67
Telang Sari, Kabupaten Banyuasin	Coconut	S 02°38.813' E 104°45.801'	2.00	7.33	9.33
Telang Sari, Kabupaten Banyuasin	Corn + coconut	S 02°38.875' E 104°44.495'	7.33	6.00	13.33
Muara Sungsang, Kabupaten Banyuasin	Coconut	S 02°21.736' E 104°50.635'	5.33	6.67	12.00
Muara Sungsang, Kabupaten Banyuasin	Banana	S 02°21.823' E 104°50.632'	2.67	3.33	6.00
Muara Sungsang	Pineapple	S 02°22.542' E 104°50.324'	2.67	6.00	8.67
ANOVA F-value			0.77ns	0.78ns	0.415ns
P value (0.05)			0.62	0.63	0.904
Tukey's HSD test			-	-	-

Note: + intercropping, ns = not significantly different; values within a column followed by the same letters are not significantly different at P < 0.05 according to HSD test.

Table 5. Inoculum potentials of entomopathogenic fungi in the peat soils of South Sumatra

Village or city	Vegetation or crop species	GPS (coordinat)	Inoculum potentials (%)		
			Fungi	Bacteria	Total
Talang Dabok, Kabupaten Ogan Komering Ilir	Oil palm	S 03°23.570' E 104°51.498'	4.67	3.33	8.00
Talang Dabok, Kabupaten Ogan Komering Ilir	Rubber	S 03°25.673' E 104°53.258'	4.00	3.33	7.33
Talang Dabok, Kabupaten Ogan Komering Ilir	Pineapple	S 03°25.280' E 104°52.940'	2.67	6.00	8.67
Sepucuk, Kabupaten Ogan Komering Ilir	Oil palm	S 03°24.840' E 104°53.362'	3.33	4.67	8.00
Sepucuk, Kabupaten Ogan Komering Ilir	Rubber	S 03°23.715' E 104°52.275'	5.33	2.00	7.33
Sepucuk, Kabupaten Ogan Komering Ilir	Pineapple	S 03°23.535' E 104°51.780'	2.67	3.33	6.00
Kedaton, Kabupaten Ogan Komering Ilir	Oil palm	S 03°23.308' E 104°51.487'	3.33	5.34	8.67
Kedaton, Kabupaten Ogan Komering Ilir	Rubber	S 03°23.277' E 104°51.398'	2.00	4.67	6.67
Kedaton, Kabupaten Ogan Komering Ilir	Pineapple	S 03°23.204' E 104°51.459'	2.00	3.33	5.33
ANOVA F-value			0.49ns	0.51ns	0.160ns
P value (0.05)			0.85	0.84	0.994
Tukey's HSD test			-	-	-

Note: ns = not significantly different; values within a column followed by the same letters are not significantly different at P < 0.05 according to HSD test.

Table 6. Inoculum potentials of entomopathogenic fungi in the highland soils of South Sumatra

Village or city	Vegetation or	GPS (coordinat)	Inoculum potentials (%)

	crop species		Fungi	Bacteria	Total
Pulau Pinang, Lahat	Rubber + coffee (A)	S 03°48.063' E 103°32.072'	2.67 abc	8.66	11.33
Pulau Pinang, Lahat	Rubber + coffee (B)	S 03°48.051' E 103°32.069'	3.33 abc	4.00	7.33
Pulau Pinang, Lahat	Rubber + coffee (C)	S 03°48.020' E 103°32.064'	1.33 a	2.67	4.00
Tanjung Payang, Lahat	Rubber (A)	S 03°48.094' E 103°32.145'	2.00 abc	3.33	5.33
Tanjung Payang, Lahat	Rubber (B)	S 03°48.122' E 103°32.162'	6.00 abc	2.67	8.67
Tanjung Payang, Lahat	Rubber (C)	S 03°48.177' E 103°32.166'	4.67 abc	5.33	10.00
Lahat	Rice	S 03°48.819' E 103°32.677'	2.00 abc	6.00	8.00
Lahat	Rice	S 03°48.852' E 103°32.698'	7.33 abc	6.67	14.00
Lahat	Rice	S 03°48.883' E 103°32.688'	2.67 abc	5.33	8.00
Talang Patai, Pagaralam	Cabbage	S 04°02.161' E 103°10.484'	8.67 bc	10.66	19.33
Talang Patai, Pagaralam	Cabbage	S 04°02.144' E 103°10.487'	2.00 abc	11.33	13.33
Talang Patai, Pagaralam	Cabbage	S 04°02.136' E 103°10.485'	9.33 c	10.00	19.33
Rimau, Pagaralam	Tea	S 03°50.180' E 103°31.325'	4.00 abc	14.00	18.00
Rimau, Pagaralam	Tea	S 03°50.174' E 103°31.313'	2.67 abc	5.33	8.00
Rimau, Pagaralam	Tea	S 03°50.174' E 103°31.293'	2.00 abc	7.33	9.33
ANOVA F-value			3.31*	1.41ns	1.49ns
P value (0.05)			0.00	0.18	0.14
Tukey's HSD test			11.69	-	-

Note: + intercropping; * = significantly different; ns = not significantly different; values within a column followed by the same letters are not significantly different at $P < 0.05$ according to HSD test.

Table 7. Inoculum potentials of entomopathogenic fungi in the soil of South Sumatra

Ecosystems	Inoculum potentials (%)		
	Fungi	Bacteria	Total
Fresh Swamps	2.11 a	8,78 b	10.89 b
Tidal Lowlands	3.85 b	7,11 b	10.96 b
Peatlands	3.33 a	4,00 a	7.33 a
Highlands	4.04 b	6,89 b	10.93 b
ANOVA F-value	4.54*	8.90*	3,73*
P value (0.05)	0.02	0.00	0.03
Tukey's HSD test	1.49	2.43	3.40

Note: * = Significantly different; values within a column followed by the same letters are not significantly different at $P < 0.05$ according to HSD test.

Discussion

Species entomopathogenic found in this research were *B. bassiana* dan *M. anisopliae*. Morphological characteristics of both entomopathogenic fungi found in this research matched to identification results by several researchers as follows. Humber (2005) stated that mycelia of *B. bassiana* appear from exosceleton of hosts insect, and cover all part of exterior surface of host integument so that host body has white colour, reverse pale to yellow colony, hyaline or colourless, single cell, and globular and conidia as well as insulate hypha. *M. anisopliae* causes integument having colours of whitish green to dark green because its mycelia cover exosceleton of hosts insect; it has green to yellow conidia, single cell and cylindrical conidia as well as insulate hypha (Driver *et al.* 2000; Humber 2005).

B. bassiana and *M. anisopliae* have parasitic and saprophytic phases during the killing process of their host insect (Augustyniuk-Kram and Kram 2007; El-Ghany 2015). Parasitic phase starts viz, the fungal conidia attach to the host insect cuticle, and then the conidia germinate on the host cuticle (El-Ghany 2015). The fungal penetration into the insect cuticle can be performed in producing specific infection hyphae originating at appressoria of the fungus (Fernandes et al. 2007; El-Ghany 2015). Butt et al. (1994) reported that the both fungi could produce germ tubes growing over the surface

of the insect cuticle until the tubes contact weakness area of cuticle where penetration can easily be achieved. After the fungus penetrates successfully, then micelia distribute into the haemolymph by formation of blastospores (El-Ghany 2015). Finally, the host insect will die within 4 days of penetration (Butt *et al.* 1994). Saprophytic phase starts viz, the fungus grows mechanically in the dead insect body, retrieve nutrients from the insect body, and then the fungus produces toxins (El-Ghany 2015).

The success of both fungi in conducting process of parasitic and saprophytic phases was affected by several external factors such as moisture, pH, temperature, ultra violet (UV) radiation, and vegetation (El-Ghany 2015). This research showed that highland soil planted with cabbage in Talang Patai, Pagaralam had more inoculum potentials of entomopatogenic fungi than other locations because cabbage usually has host insects dominated by Lepidoptera, such as *Plutella xylostella*, *Crocidolomia binotalis*, *Spodoptera litura* and *Chrysodeixis chalcites*. Larvae of Lepidoptera are the most suitable hosts for entomopathogic fungi (Godonou et al. 2009; Nunilahwati et al. 2012). This survey results showed a lot of sick host insects hung above of mustard, cabbage, and carrot canopy. Results of this research found that host insects attacked by entomopathogic fungi on cabbage at highlands, South Sumatra generally dominated by *P. xylostella*, *S. litura* and *C. chalcites.* Symptoms of sick insects were dry and stiff condition, white or greenish white colour and attached on upper surface of cabbage leaves. The sick insects which showed the symptoms of dry and stiff condition as mummy and covered with white fungal mycelia were insects which attacked by *B. bassiana*, whereas insect body covered by fungal mycelia having greenish white or dark green colour were symptoms of insects which attacked by *M. anisopliae.* Symptoms of insects attacked by *B. bassiana* and *M. anisopliae* in this research matched to the symptoms reported by El-Ghany (2015) and Mora et al. (2017). Inoculum source from the sick insects was important factor which affect high percentage of *Tenebrio* bait from soil in Talang Patai, Pagaralam.

In this research, more inoculum potential of the entomopathogenic fungi was found in highlands and tidal lowlands than that in fresh swamps and peatlands because it was affected by soil pH and moisture. Groden and Lockwood (1991) reported that soil pH had more significant role in determining the existence of fungal propagules within soils than that of soil texture and organic matter. However, Inubushi et al. (2003) had stated that soil moisture is one of the most important controlling factors for biological reactions in soil. Soil pH in this research was in the range of 4 to 4.5 in fresh swamps, 4.3 to 5 in tidal lowlands, 3.60 to 4 in peatlands and 5 to 6.7 in highlands, respectively. Conidial viability of entomopathogenic fungi, such as *B. bassiana* and *M. anisopliae* were affected by pH of in-vitro medium for entomopathogenic fungi. Rizkie et al. (2017) reported that high acidity (pH < 4) of media in-vitro medium for fungus growing significantly decrease conidial viability of *B. bassiana* and *M. anisopliae*. Therefore, inoculum potentials of the entomopathogenic fungi from peatland soil and fresh swamp soil was lower than that from soil in the tidal lowlands and highlands.

In addition to soil pH and moisture, soil texture also determines the existence and distribution of fungal propagules. Soil texture having low clay content and sandy soil texture tends to have low capability in maintaining the existence of fungal propagules (El-Ghany 2015). Water saturated soil also tend to have low capability in maintaining the existence of fungal propagules (Garrido-Jurado et al. 2011). Soils from fresh swamps and peatlands in South Sumatra had lower clay content and in water saturated condition (Marlina et al. 2016; Kartika et al. 2018). Low existence of fungal propagules finding at lowland swamp and peatland soils was due to both factors. Soils in fresh swamps are water saturated for more than 6 to 7 months per year which is usually occurred from November to April (Herlinda et al. 2018). Peatlands has soil pore saturated with water all year long resulting in anaerobic condition of soil and coupled with high organic matter finally can decrease pH of soil (Utami et al. 2009). Rizkie et al. (2017) confirmed that pH < 4 within media in-vitro for growing fungi can decrease ability to live of fungal propagules. Higher inoculum potentials in highlands in this research was due to balance or higher of clay texture and organic soils than that of fresh swamps and peatlands. The balance or higher of clay texture and organic soils are capable to maintain the existence of fungal propagules (Garrido-Jurado et al. 2011). Lewis and Melvin (1996) stated that soil in ecosystems which apply composted manure or no synthetic fertilizer had higher propagules content of *B. bassiana* than that of soil in ecosystem which apply synthetic fertilizers. In addition, application of synthetic pesticides is capable to decrease the existence of entomopathogenic fungi within soil (Mietkiewski et al. 2010). Local farmers in fresh swamp and peatland areas of South Sumatra usually do not apply synthetic pesticides, whereas many local farmers in tidal lowland and highland areas apply synthetic pesticides (Herlinda et al. 2018). Although, no synthetic pesticides was applied in fresh swamp and peatland areas, fungal propagules or inoculum potentials in these areas was lower than that of tidal lowlands and highlands areas. In this study no evidence that no synthetic pesticide application in fresh swamp and peatland areas can cause high existence of fungal propagules. However, soil pH and soil texture have more effect on the existence of propagules of *B. bassiana* and *M. anisopliae* at lowland swamp and peatland ecosystems.

This research has shown that from soils in South Sumatra, the species of entomopatogenic fungi found were *B. bassiana* and *M. anisopliae*. The highest percentage of the inoculum potentials belonging to the both fungi was occured in the highland ecosystems, and not significantly different from those in the tidal lowland ecosystems. The lowest percentage of the inoculum potentials of the fungi was found in the peatland ecosystems, and not significantly different from those in the fresh swamp ecosystems. In highland ecosystems, percentage of the inoculum potentials was affected by the the locations and the vegetations or the crop species. These fungi will make an important contribution to the biological control for insect pests in Indonesia because 30 isolates of the fungi could be adapted in lowland to highland ecosystems.

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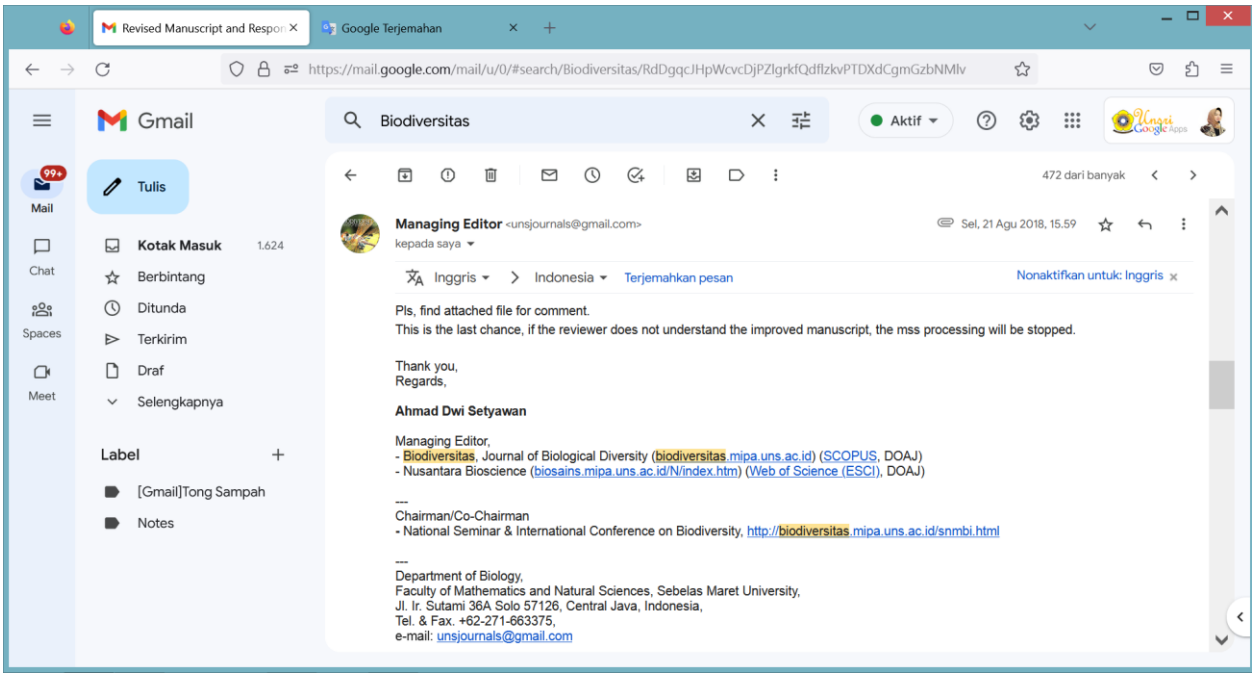

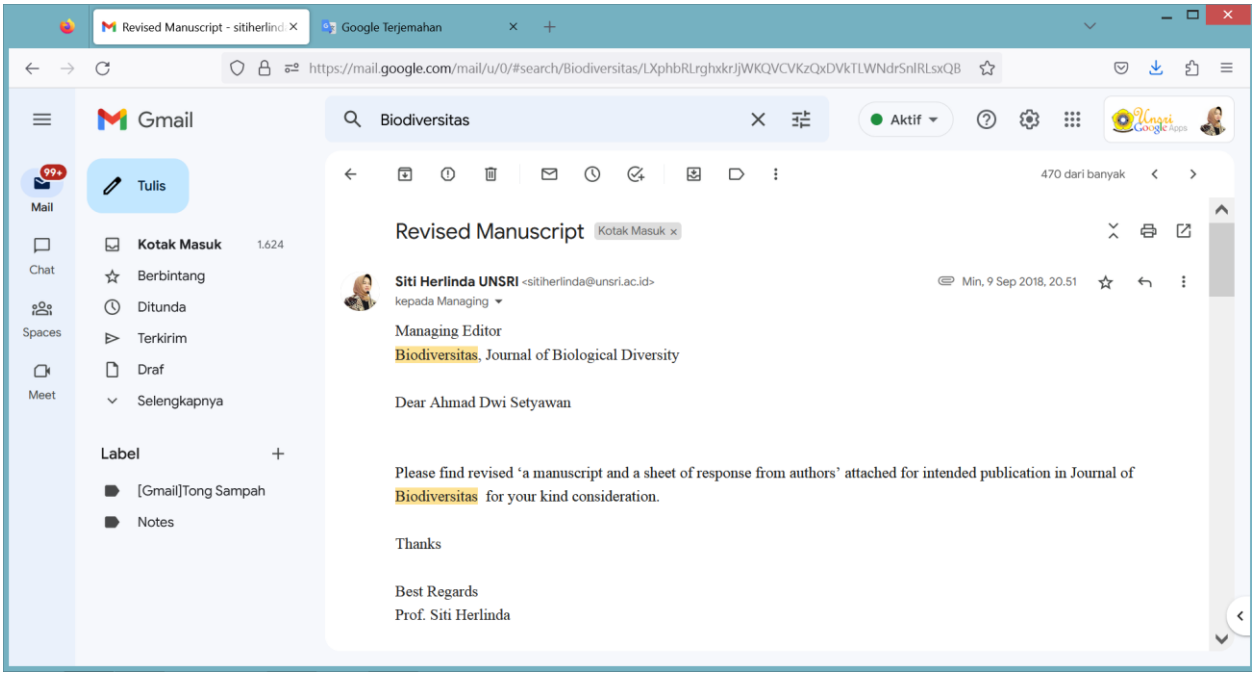

Entomopathogenic fungi from soils of freshwater swamps, tidal lowlands, peatlands, and highlands of South Sumatra, Indonesia

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Abstract. Ecosystems of lowlands and highlands in South Sumatra, Indonesia have specific characteristics of soils and vegetations that can affect the availability of entomopathogenic fungi. This study aimed to identify species and to determine inoculum potentials of the entomopathogenic fungi from soils of freshwater swamps, tidal lowlands, peatlands, and highlands. Baiting of entomopathogenic fungi on soil samples used the larvae of *Tenebrio molitor*. The entomopathogenic fungi species found were *Beauveria bassiana* and *Metarhizium anisopliae*. The number of the fungal isolates found were 30 isolates consisted of 9 isolates of *B. bassiana* and 21 isolates of *M. anisopliae.* The highest number of isolates were found on highland ecosystem (11 isolates) and the lowest one were found on peatland ecosystem (4 isolates). However, the highest percentage of inoculum potentials of the fungi was found on tidal lowland ecosystem (0.74%) and the lowest one was found on tidal lowland ecosystem (0.28%). The peatsoil in Talang Dabok, Ogan Komering Ilir planted with oil palm had higher inoculum potentials (2.67%), as well as the highland soils in Talang Patai, Pagaralam planted with mustard (2.67%) . These fungi will make an important contribution to the biological control for insect pests in lowland to highland ecosystems in Indonesia.

Key words: *Beauveria bassiana*, lowlands, *Metarhizium anisopliae*, peat soils, wetlands

Running title: Entomopathogenic Fungi from Soils

INTRODUCTION

Many parts in the lowlands and highlands of Indonesia are used for agriculture. Lowlands consisting of freshwater swamps, tidal lowlands, and peatlands are the ecosystems having water saturated condition for the whole year (Sudana 2005). The lowland ecosystems for agricultural purposes in Indonesia are distributed in Sumatra, Kalimantan, and Papua Islands covering areas of 11 million ha of tidal lowlands, 9.2 million ha of freshwater swamps, and 14.9 million ha of peatlands, respectively (Mulyani and Sarwani 2013). While the highland ecosystems managed for agriculture with the area of 16.15 million ha are distributed in all Indonesia islands (Center for Agriculture Data and Information System, Secretariat General 2017).

Soils between lowlands and highlands in Indonesia have different characteristics, especially in the moisture, texture, and acidity (pH). The specific characteristics of lowland and highland soils at the four typhologies of ecosystems consist of the freshwater swamp, tidal lowlands, peatlands, and highland are closely related to specific cultivated vegetations or crops. Rice usually is cultivated at freshwater swamps (Herlinda et al. 2018; Lakitan, et al. 2018a; Lakitan, et al. 2018b). Only small part of soils at peatlands that can be cultivated with seasonal crops and most peatlands in Indonesia was utilized for forestry and conservation areas (Suriadikarta and Sutriadi 2007). Highlands area usually planted with various types of seasonal and annual crops. Specific vegetation or crop plants could affect the soil microorganisms associated with plant and crop roots (El-Ghany 2015).

Microorganisms such as fungi had been found from freshwater swamps and highlands of South Sumatra and can be used to control insect pest called entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* (Herlinda *et al.* 2008, 2010). The entomopathogenic fungi had proven to be an effective agents to control some insect pests (Chinniah et al. 2016), and also not harmful toward natural enemies of insect pests (Gholamzadeh-Chitgar et al. 2017). However, there was no complete information for fungi from tidal lowlands and peatlands in that region. *B. bassiana* and *M. anisopliae* had been found by previous researchers killing insect pest such as *Crocidolomia pavonana* (Hasyim *et al.* 2009), *Aphis gossypii* (Herlinda 2010), *Plutella xylostella* (Loc and Chi 2007), *Lygus* spp. (Leland *et al.* 2005), and *Oryctes rhinoceros* (Moslim et al. 2009). Entomopathogen, such as entomopathogenic fungi can be explored from soils (El-Ghany 2015). Certain entomopathogen species have adaptation capability in certain soils (Bugeme *et al.* 2008). Entomopathogen that had already adapted in freshwater swamp soils or highland soils generally has the specific advantage, i.e., it can adapt more effective at these soils environment (Erler and Ates 2015). Exploration of entomopathogen starting from lowland to highland ecosystems will produce the high variation of species and genetics that can be utilized at the extended area and specific location. The purposes of this study were to identify species and to determine inoculum potentials of the entomopathogenic fungi from soils of freshwater swamps, tidal lowlands, peatlands, and highlands in South Sumatra.

MATERIALS AND METHODS

Study area

The study sites were selected in the agricultural center with specific typhology ecosystems in some locations of South Sumatra (Figure 1 and Table 1). The sites consisted of freshwater swamps, tidal lowlands, peatlands and highlands Those ecosystems chosen for explorations of entomopathogenic fungi had objective to obtain the fungi that had adapted in the soils from lowlands to highlands. The selected freshwater swamp locations were Gandus; Musi 2, Palembang; Rambutan, Banyuasin; and Pemulutan, Ogan Ilir. The selected locations in tidal lowlands were Mulya Sari, Banyuasin; Telang Sari,

Banyuasin; and Muara Sungsang, Banyuasin. The selected locations in peatlands were Talang Dabok, Ogan Komering Ilir; Sepucuk, Ogan Komering Ilir; and Kedaton, Ogan Komering Ilir. The selected locations in the highlands were Lahat (Lematang, Tanjung Payang, Pulau Pinang), Pagaralam (Rimau and and Talang Patai).

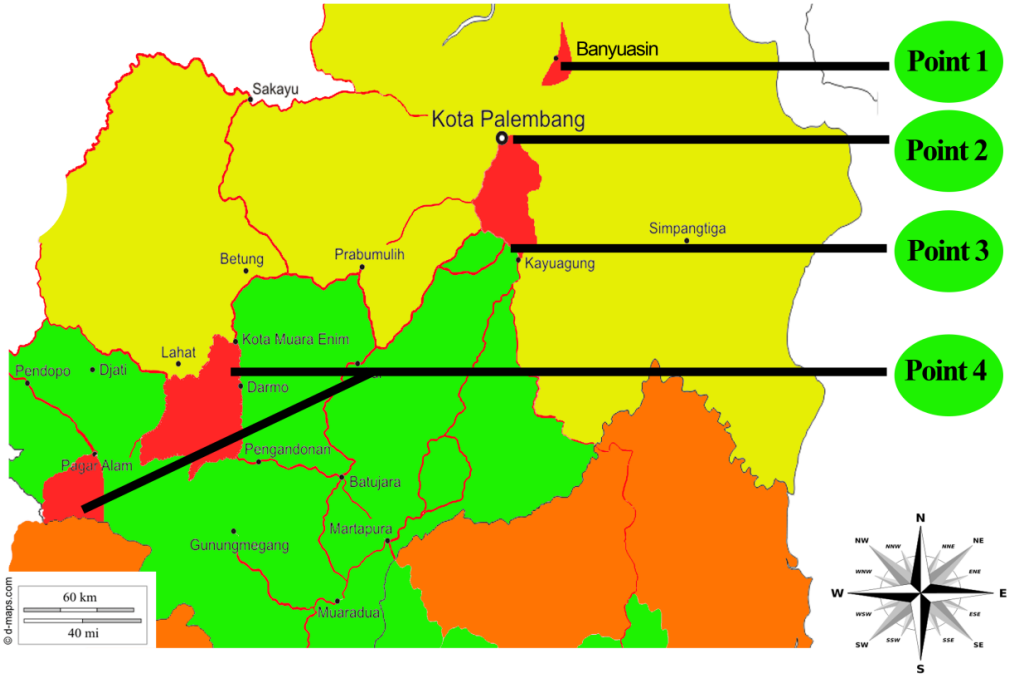

Figure 1. Locations of exploration for entomopathogenic fungi from soils in ecosystems of South Sumatra, Indonesia: point 1 tidal lowlands (S 02°40.866' E 104°44.298'), point 2 freshwater swamps (S 03°02.581' E 104°51.231'), point 3 peatlands (S 03°24.840' E 104°53.362'), and point 4 highlands (S 03°48.063' E 103°32.072' and S 03°50.174' E 103°31.293')
Source: https://d-maps.com/index.php?lang=en

Soil sampling was carried out in December 2017 and baiting of entomopathogenic fungi was done by using *Tenebrio molitor* (Yellow mealworm beetle). Fungal isolation, purification, and identification were conducted from January to March 2018 at Laboratory of Entomology, Department of Plant Pest and Disease, College of Agriculture, Universitas Sriwijaya, Indralaya, Indonesia. Supporting data were recorded in term of soil sampling period, village or city names, coordinate points, and vegetation types or crop plants for each exploration locations (Figure 1 and Table 1). Value of pH belonging to soil samples was measured by using a method of Kartika et al. (2018). In this survey, the application of pesticides in the different ecosystems (freshwater swamps, tidal lowlands, peatlands and highlands) was also monitored.

Procedures

Observation of inoculum potentials of entomopathogenic fungi in the soil

Inoculum potentials observation of the entomopathogenic fungi in this research was conducted by exploration of fungi in soils of the freshwater swamps, tidal lowlands, peatlands, and highlands, respectively. Entomopathogenic fungi exploration was done by modifying the methods of Anwar *et al.* (2015), i.e., using *T. molitor* as insect bait (*Tenebrio* bait method) which was infested on fungal inoculum of soil samples.

For soil samples sites, soil of one up to four crop species (rice in freshwater swamps; corn, rice, coconut, and watermelon in tidal lowlands; oil palm in peatlands; and cabbage, mustard, rubber, and coffee in highlands) were taken from each location at the diagonal position. Three to five soil sampling locations consisting of the freshwater swamps, tidal lowlands, peatlands, and highlands were taken from each ecosystem of exploration. Soil samples with the weight of 5000 g were obtained by digging soil at the depth of 5–15 cm in the vicinity of crop roots. Subsequently, soil samples were put into the plastic pouch and labeled with the information of soil sampling period and crop species. The soil samples arrangement for each treatment in the laboratory were constructed by using completely randomized design.

Previously, soil samples were sieved by using 5 mesh siever to separate from crop roots. Then, samples were put into plastic tray (size of 32 cm x 25 cm x 5 cm) containing 5000 g of soil sample for each tray, and yielded 1000 g of finer soil sample. The finer soil sample was subsequently moisted with sterile aquadest until soil moisture of 80–90% using the method of Chen et al. (2014). Then, 30 larvae of the newly-moulting-third instar of *T. molitor* were sterilized with 70%

alcohol, and put on soil-sample surface in a plastic tray. Each treatment (or each location) was repeated five times (a total of 150 larvae per location) (Table 1). The body of larvae was sprinkled with soil sample with thickness of about 5 mm. Subsequently, plastic tray containing soil samples were covered with black cloth and was sprayed with sterile aquadest in order to maintain humidity of soil samples. Larvae were incubated within soil sample for seven days to provide enough time for entomopathogenic fungi infecting them. Then, dead larvae infected by the entomopathogenic fungi were recorded daily to determine inoculum potential. Inoculum potentials of entomopathogenic fungi in this research were measured according to percentage of infected *tenebrio* bait or hosts (Hofgaard et al. 2016). The inoculum potential (IP) was calculated based on the equation below:

$$\text{IP} = \frac{ib}{tb} \times 100$$

ib were the number infected *Tenebrio* bait, and *tb* were the total *Tenebrio* baits

Table 1. Locations of exploration for entomopathogenic fungi in South Sumatra, Indonesia

Ecosystems	Village or city	GPS (coordinat)	*Tenebrio* baits (larvae)	Vegetation or crop plants	Height from sea level (m)
Freshwater swamps	Rambutan, Banyuasin	Rambutan, Banyuasin S 03°02.581', E 104°51.231'	150	Rice	15
		Rambutan, Banyuasin S 03°02.591', E 104°51.217'	150	Rice	15
		Rambutan, Banyuasin S 03°02.586', E 104°51.201'	150	Rice	15
	Gandus, Palembang	Gandus, Palembang S 03°00.401', E 104°42.380'	150	Rice	7
		Gandus, Palembang S 03°00.632', E 104°42.532'	150	Rice	14
		Gandus, Palembang S 03°00.632', E 104°42.801'	150	Rice	14
	Musi Dua, Palembang	Musi Dua, Palembang S 03°02.120' E 104°43.021'	150	Rice	17
		Musi Dua, Palembang S 03°02.150' E 104°43.612'	150	Rice	16
		Musi Dua, Palembang S 03°02.510' E 104°43.120'	150	Rice	17
	Pemulutan, Ogan Ilir	Pemulutan, Ogan Ilir S 03°03.148', E 104°46.230'	150	Rice	21
		Pemulutan, Ogan Ilir S 03°03.115', E 104°46.218'	150	Rice	21
		Pemulutan, Ogan Ilir S 03°03.113', E 104°46.201'	150	Rice	20
Total larvae			1800		
Tidal lowlands	Mulya Sari, Banyuasin	Mulya Sari, Banyuasin S 02°40.866', E 104°44.298'	150	Corn	15
		Mulya Sari, Banyuasin S 02°40.944', E 104°44.621'	150	Rice	15
		Mulya Sari, Banyuasin S 02°40.896', E 104°44.676'	150	Watermelon	14
	Telang Sari, Banyuasin	Telang Sari, Banyuasin S 02°38.842', E 104°45.369'	150	Corn	14
		Telang Sari, Banyuasin S 02°38.813', E 104°45.801'	150	Coconut	20
		Telang Sari, Banyuasin S 02°38.875', E 104°44.495'	150	Corn + coconut	19
	Muara Sungsang, Banyuasin	Muara Sungsang, Banyuasin S 02°21.736', E 104°50.635'	150	Coconut	16
		Muara Sungsang, Banyuasin S 02°21.823', E 104°50.632'	150	Banana	15
		Muara Sungsang, Banyuasin S 02°22.542', E 104°50.324'	150	Pineapple	12
Total larvae			1350		
Peatlands	Talang	Talang Dabok, Ogan Komering Ilir	150	Oil palm	24

	Dabok, Ogan Komering Ilir	S 03°23.570', E 104°51.498'			
		Talang Dabok, Ogan Komering Ilir S 03°25.673', E 104°53.258'	150	Rubber	24
		Talang Dabok, Ogan Komering Ilir S 03°25.280', E 104°52.940'	150	Pineapple	22
	Sepucuk, Ogan Komering Ilir	Sepucuk, Ogan Komering Ilir S 03°24.840', E 104°53.362'	150	Oil palm	23
		Sepucuk, Ogan Komering Ilir S 03°23.715', E 104°52.275'	150	Rubber	28
		Sepucuk, Ogan Komering Ilir S 03°23.535', E 104°51.780'	150	Pineapple	19
	Kedaton, Ogan Komering Ilir	Kedaton, Ogan Komering Ilir S 03°23.308', E 104°51.487'	150	Oil palm	18
		Kedaton, Ogan Komering Ilir S 03°23.277', E 104°51.398'	150	Oil palm	18
		Kedaton, Ogan Komering Ilir S 03°23.204', E 104°51.459'	150	Oil palm	24
Total larvae			1350		
Highlands	Pulau Pinang, Lahat	Pulau Pinang, Lahat S 03°48.819', E 103°32.677'	150	Rubber + coffee (A)	155
		Pulau Pinang, Lahat S 03°48.852', E 103°32.698'	150	Rubber + coffee (B)	153
		Pulau Pinang, Lahat S 03°48.883', E 103°32.688'	150	Rubber + coffee (C)	169
	Tanjung Payang, Lahat	Tanjung Payang, Lahat S 03°48.094', E 103°32.145'	150	Rubber (A)	122
		Tanjung Payang, Lahat S 03°48.122', E 103°32.162'	150	Rubber (B)	120
		Tanjung Payang, Lahat S 03°48.177', E 103°32.166'	150	Rubber (C)	117
	Lahat	Lahat S 03°48.063', E 103°32.072'	150	Rice	124
		Lahat S 03°48.051', E 103°32.069'	150	Rice	123
		Lahat S 03°48.020', E 103°32.064'	150	Rice	118
	Talang Patai, Pagaralam	Talang Patai, Pagaralam S 03°50.180', E 103°31.325'	150	Cabbage	155
		Talang Patai, Pagaralam S 03°50.174', E 103°31.313'	150	Cabbage	175
		Talang Patai, Pagaralam S 03°50.174', E 103°31.293'	150	Mustard	193
	Rimau, Pagaralam	Rimau, Pagaralam S 04°02.161', E 103°10.484'	150	Tea	1327
		Rimau, Pagaralam S 04°02.144', E 103°10.487'	150	Tea	1329
		Rimau, Pagaralam S 04°02.136', E 103°10.485'	150	Tea	1327
Total larvae			2250		

Entomopathogen Isolation

The dead *Tenebrio* bait was subsequently isolated and purified by using the methods of Herlinda (2010). Entomopathogenic fungi infecting and growing on integument of *Tenebrio* bait were isolated and grown on SDA (Sabouraud Dextrose Agar) medium. The integument surface of larvae infected by the entomopathogenic fungi was previously sterilized using the modified method of Nuraini et al. (2017) with 1% natrium hypochlorite for 3 minutes and subsequently was rinsed three times with sterile aquadest and air dried on sterile filter paper. Larvae were put into petridish containing sterile humid tissue paper and then incubated in order to stimulate the growth of entomopathogenic fungi. Conidia of entomopathogenic fungi emerging from the dead larvae body were taken by using sterile ose needle and moved into petri disk containing SDA medium, and incubated for seven days at the constant temperature of 25 ^{o}C within the incubator.

Identification of Entomopathogen Fungi

The purified fungi were identified according to macroscopic and microscopic characteristics. Fungi that had been grown on SDA media with the area of 1 cm^2 was taken by using ose needle and put into preparations containing SDA

media and incubated for three days and then microscopically observed. Subsequently, its morphology was identified macroscopically and microscopically by using the method of Guilherme et al. (2015). Furthermore, species of fungi were identified by using books of Humber (2005) and El-Ghany (2015).

Data analysis

Data on inoculum potentials based on *Tenebrio* bait percentage infected by the entomopathogenic fungi among treatments was analyzed descriptively.

RESULTS AND DISCUSSION

Insect Characteristics Infected by Entomopathogenic Fungi

Identification based on morphology for entomopathogenic fungi found in this study was consisted of two species , *B. bassiana* and *M. anisopliae*. There were 30 isolates (obtained from 30 larvae infected by the entomopathogenic fungi among 6750 larvae samples used in all locations) found from these two fungal species (Table 2). The isolates consisted of 9 isolates of *B. bassiana* and 21 isolates of *M. anisopliae. Tenebrio* bait infected by the entomopathogenic fungi showed symptoms and characteristics which could be used to determine a fungal species. Sick or dead *Tenebrio* bait infected by *B. bassiana* showed symptoms as follows: insect body was dry and wrinkle, its outer integument was coated by white mycelia similar to silk, rigid, easily broken and no smell (Figure 2). The *Tenebrio* bait attacked by *M. anisopliae* showed symptoms as follows: dry and wrinkle, no smell, brittle and easily broken, but its outer integument was coated by mycelia having greenish white to dark green or dark colour (Figure 3).

The pure isolate was obtained from dead *Tenebrio* bait body which was attacked by entomopathogenic fungi with colony characteristics for each species as follows; Colony of *B. bassiana* had the white colour similar to cotton, but gradually its colour changed into yellowish white as fungi become older. Colony of *M. anisopliae* initially had white colour similar to colour of *B. bassiana*, but the colour changed into greenish and dark green or dark as fungi become older (Figure 4). Conidia of *B. bassiana* and *M. anisopliae* with specific characteristics were obtained from each colony species of entomopathogenic fungi. Conidia of *B. bassiana* had single cell and round shape, whereas conidia of *M. anisopliae* had single cell but with cylindrical shape (Figure 5). Mycelia of *B. bassiana* and *M. anisopliae* insulated with upright, branches and layers of conidiophores.

Inoculum Potentials of Entomopathogenic Fungi in the Soil of South Sumatra

Inoculum potentials of entomopathogenic fungi in this research were measured according to the percentage of infected *Tenebrio* bait. The inoculum potentials of entomopathogens fungi from the freshwater swamp soil, tidal lowlands, peat soils, and high land each was different among survey locations (Tables 3−6). The value of inoculum potential in freshwater swamp, tidal lowland, pet soil and high land were in the range of 0−1.33%, 0-2.00%, 0-2.67%, and 0-2.67%, respectively.

The percentage of inoculum potentials based on locations or crop species showed that the inoculum potentials from the freshwater swamp soils was found on rice in Rambutan, Banyuasin (2.67% of two locations) and rice in Pemulutan, Ogan Ilir (0.67%) (Table 3). From tidal lowlands, the highest inoculum potentials was found on rice in Mulya Sari, Banyuasin (2%) (Table 4). The peatsoil in Talang Dabok, Ogan Komering Ilir planted with oil palm had higher inoculum potentials (2.67%) (Table 5), as well as the highland soils in Talang Patai, Pagaralam planted with mustard (2.67%) (Table 6). Inoculum potentials of the fungi was found on two locations in the freshwater swamps, six locations in tidal lowlands, one location in peatlands, and four locations in highlands (Table 3-6).

Based on ecosystems classification observed in this research which consisted of the freshwater swamps, tidal lowlands, peatlands and highlands, the results showed that inoculum potentials of entomopathogenic fungi was different among ecosystems (Table 7). The highest percentage of inoculum potentials of the fungi was found on tidal lowland ecosystem (0.74%) and the lowest one was found on tidal lowland ecosystem (0.28%). However, the highest number of isolates were found on highland ecosystem (11 isolates) and the lowest one were found on peatland ecosystem (4 isolates).

Figure 2. *Tenebrio* bait infected by *Beauveria bassiana* (a) and healthy *Tenebrio* (b)

Figure 3. *Tenebrio* bait infected by *Metarhizium anisopliae* (a) and healthy *Tenebrio* (b)

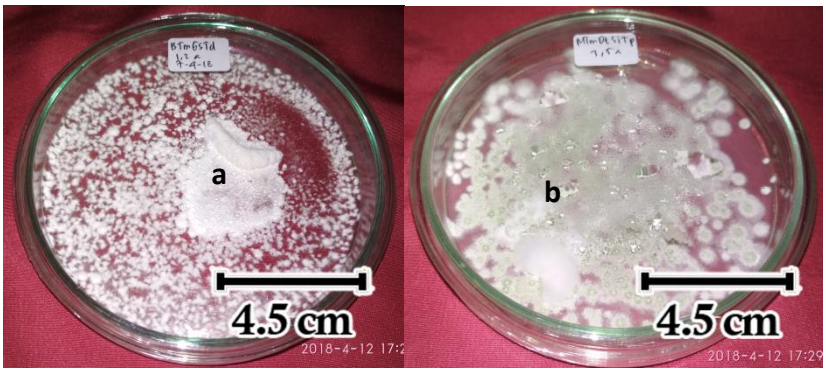

Figure 4. Colony of *Beauveria bassiana* (a) and *Metarhizium anisopliae* (b)

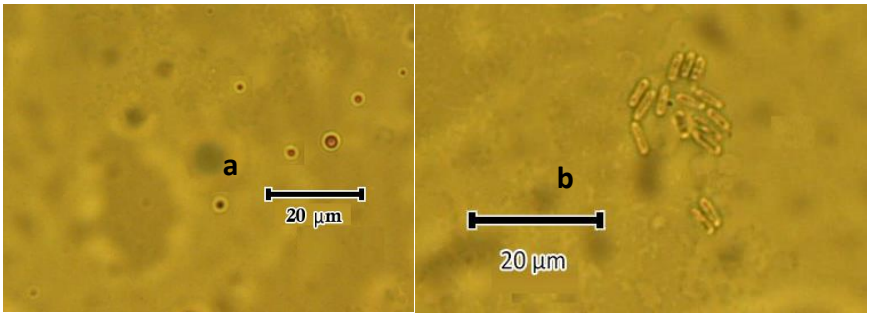

Figure 5. Conidia of *Beauveria bassiana* (a) and *Metarhizium anisopliae* (b) (400x magnification)

Table 2. Species and isolates of entomopathogenic fungi found in South Sumatra, Indonesia.

Ecosystems	**Species of fungi**	**Number of isolate**	**Vegetation or crop plants**	**Village or city**
Freshwater swamps	*Metarhizium anisopliae*	3	Rice	Rambutan
Freshwater swamps	*Metarhizium anisopliae*	1	Rice	Pemulutan
Freshwater swamps	*Beauveria bassiana*	1	Rice	Rambutan
Tidal lowlands	*Metarhizium anisopliae*	2	Corn	Mulya Sari
Tidal lowlands	*Metarhizium anisopliae*	3	Rice	Mulya Sari
Tidal lowlands	*Metarhizium anisopliae*	1	Corn + coconut	Telang Sari
Tidal lowlands	*Metarhizium anisopliae*	2	Corn	Telang Sari
Tidal lowlands	*Beauveria bassiana*	1	Corn	Telang Sari
Tidal lowlands	*Beauveria bassiana*	1	Watermelon	Mulya Sari
Peatlands	*Beauveri abassiana*	4	Oil palm	Talang Dabok
Highlands	*Metarhizium anisopliae*	4	Cabbage	Talang Patai
Highlands	*Metarhizium anisopliae*	4	Mustard	Talang Patai
Highlands	*Metarhizium anisopliae*	1	Rubber + coffee	Pulau Pinang
Highlands	*Beauveria bassiana*	1	Rubber + coffee	Pulau Pinang
Highlands	*Beauveria bassiana*	1	Cabbage	Talang Patai
Total		30		

Table 3. Inoculum potentials of entomopathogenic fungi in the freshwater swamp soils of South Sumatra

Village or city	Vegetation or crop species	GPS (coordinat)	Inoculum potentials (%)			
			Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (Total)
Rambutan, Banyuasin	Rice	S 03°02.581', E 104°51.231'	0	0.00 (0)	0.00 (0)	0.00 (0)
Rambutan, Banyuasin	Rice	S 03°02.591', E 104°51.217'	2	0.00 (0)	1.33 (2)	1.33 (2)
Rambutan, Banyuasin	Rice	S 03°02.586', E 104°51.201'	2	0.67 (1)	0.67 (1)	1.33 (2)
Gandus, Palembang	Rice	S 03°00.401', E 104°42.380'	0	0.00 (0)	0.00 (0)	0.00 (0)
Gandus, Palembang	Rice	S 03°00.632', E 104°42.532'	0	0.00 (0)	0.00 (0)	0.00 (0)
Gandus, Palembang	Rice	S 03°00.632', E 104°42.801'	0	0.00 (0)	0.00 (0)	0.00 (0)
Musi Dua, Palembang	Rice	S 03°02.120', E 104°43.021'	0	0.00 (0)	0.00 (0)	0.00 (0)
Musi Dua, Palembang	Rice	S 03°02.150', E 104°43.612'	0	0.00 (0)	0.00 (0)	0.00 (0)
Musi Dua, Palembang	Rice	S 03°02.510', E 104°43.120'	0	0.00 (0)	0.00 (0)	0.00 (0)
Pemulutan, Ogan Ilir	Rice	S 03°03.148', E 104°46.230'	0	0.00 (0)	0.00 (0)	0.00 (0)
Pemulutan, Ogan Ilir	Rice	S 03°03.115', E 104°46.218'	0	0.00 (0)	0.00 (0)	0.00 (0)
Pemulutan, Ogan Ilir	Rice	S 03°03.113', E 104°46.201'	1	0.00 (0)	0.67 (1)	0.67 (1)

Note: The total *Tenebrio* baits (*tb*) = 150 larvae per village; data in brackets () = the number infected *Tenebrio* bait (*ib*)

Table 4. Inoculum potentials of entomopathogenic fungi in the tidal lowland soils of South Sumatra

Village or city	Vegetation or crop species	GPS (coordinat)	Inoculum potentials (%)			
			Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (Total)
Mulya Sari, Banyuasin	Corn	S 02°40.866', E 104°44.298'	2	0.00 (0)	1.33 (2)	1.33 (2)
Mulya Sari, Banyuasin	Rice	S 02°40.944', E 104°44.621'	3	0.00 (0)	2.00 (3)	2.00 (3)
Mulya Sari, Banyuasin	Watermelon	S 02°40.896', E 104°44.676'	1	0.67 (1)	0.00 (0)	0.67 (1)
Telang Sari, Banyuasin	Corn	S 02°38.842', E 104°45.369'	2	0.00 (0)	1.33 (2)	1.33 (2)
Telang Sari, Banyuasin	Coconut	S 02°38.813', E 104°45.801'	1	0.67 (1)	0.00 (0)	0.67 (1)
Telang Sari, Banyuasin	Corn + coconut	S 02°38.875', E 104°44.495'	1	0.00 (0)	0.67 (1)	0.67 (1)
Muara Sungsang, Banyuasin	Coconut	S 02°21.736', E 104°50.635'	0	0.00 (0)	0.00 (0)	0.00 (0)
Muara Sungsang, Banyuasin	Banana	S 02°21.823', E 104°50.632'	0	0.00 (0)	0.00 (0)	0.00 (0)
Muara Sungsang	Pineapple	S 02°22.542', E 104°50.324'	0	0.00 (0)	0.00 (0)	0.00 (0)

Note: The total *Tenebrio* baits (*tb*) = 150 larvae per village; data in brackets () = the number infected *Tenebrio* bait (*ib*)

Table 5. Inoculum potentials of entomopathogenic fungi in the peatland soils of South Sumatra

Village or city	Vegetation or crop plants	GPS (coordinat)	Inoculum potentials (%)			
			Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (Total)
Talang Dabok, Ogan Komering Ilir	Oil palm	S 03°23.570', E 104°51.498'	4	2.67 (4)	0.00 (0)	2.67 (4)
Talang Dabok, Ogan Komering Ilir	Rubber	S 03°25.673', E 104°53.258'	0	0.00 (0)	0.00 (0)	0.00 (0)
Talang Dabok, Ogan Komering Ilir	Pineapple	S 03°25.280', E 104°52.940'	0	0.00 (0)	0.00 (0)	0.00 (0)
Sepucuk, Ogan Komering Ilir	Oil palm	S 03°24.840', E 104°53.362'	0	0.00 (0)	0.00 (0)	0.00 (0)
Sepucuk, Ogan Komering Ilir	Rubber	S 03°23.715', E 104°52.275'	0	0.00 (0)	0.00 (0)	0.00 (0)
Sepucuk, Ogan Komering Ilir	Pineapple	S 03°23.535', E 104°51.780'	0	0.00 (0)	0.00 (0)	0.00 (0)
Kedaton, Ogan Komering Ilir	Oil palm	S 03°23.308', E 104°51.487'	0	0.00 (0)	0.00 (0)	0.00 (0)
Kedaton, Ogan Komering Ilir	Oil palm	S 03°23.277', E 104°51.398'	0	0.00 (0)	0.00 (0)	0.00 (0)
Kedaton, Ogan Komering Ilir	Oil palm	S 03°23.204', E 104°51.459'	0	0.00 (0)	0.00 (0)	0.00 (0)

Note: The total *Tenebrio* baits (*tb*) = 150 larvae per village; data in brackets () = the number infected *Tenebrio* bait (*ib*)

Table 6. Inoculum potentials of entomopathogenic fungi in the highland soils of South Sumatra

Village or city	Vegetation or crop plants	GPS (coordinat)	Inoculum potentials (%)			
			Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (Total)
Pulau Pinang, Lahat	Rubber + coffee (A)	S 03°48.819', E 103°32.677'	2	0.67 (1)	0.67 (1)	1.33 (2)
Pulau Pinang, Lahat	Rubber + coffee (B)	S 03°48.852', E 103°32.698'	0	0.00 (0)	0.00 (0)	0.00 (0)
Pulau Pinang, Lahat	Rubber + coffee	S 03°48.883', E 103°32.688'	0	0.00 (0)	0.00 (0)	0.00 (0)

	(C)					
Tanjung Payang, Lahat	Rubber (A)	S 03°48.094', E 103°32.145'	0	0.00 (0)	0.00 (0)	0.00 (0)
Tanjung Payang, Lahat	Rubber (B)	S 03°48.122', E 103°32.162'	0	0.00 (0)	0.00 (0)	0.00 (0)
Tanjung Payang, Lahat	Rubber (C)	S 03°48.177', E 103°32.166'	0	0.00 (0)	0.00 (0)	0.00 (0)
Lahat	Rice	S 03°48.063', E 103°32.072'	0	0.00 (0)	0.00 (0)	0.00 (0)
Lahat	Rice	S 03°48.051', E 103°32.069'	0	0.00 (0)	0.00 (0)	0.00 (0)
Lahat	Rice	S 03°48.020', E 103°32.064'	0	0.00 (0)	0.00 (0)	0.00 (0)
Talang Patai, Pagaralam	Cabbage	S 03°50.180', E 103°31.325'	2	0.00 (0)	1.33 (2)	1.33 (2)
Talang Patai, Pagaralam	Cabbage	S 03°50.174', E 103°31.313'	3	0.67 (1)	1.33 (2)	2.00 (3)
Talang Patai, Pagaralam	Mustard	S 03°50.174', E 103°31.293'	4	0.00 (0)	2.67 (4)	2.67 (4)
Rimau, Pagaralam	Tea	S 04°02.161', E 103°10.484'	0	0.00 (0)	0.00 (0)	0.00 (0)
Rimau, Pagaralam	Tea	S 04°02.144', E 103°10.487'	0	0.00 (0)	0.00 (0)	0.00 (0)
Rimau, Pagaralam	Tea	S 04°02.136', E 103°10.485'	0	0.00 (0)	0.00 (0)	0.00 (0)

Note: The total *Tenebrio* baits (*tb*) = 150 larvae per village; data in brackets () = the number infected *Tenebrio* bait (*ib*)

Table 7. Inoculum potentials of entomopathogenic fungi in the soil of South Sumatra

Ecosystems	**Inoculum potentials (%)**			
	Number of isolate	***B. bassiana***	***M. anisoplia***	**Fungi (Total)**
Freshwater Swamps	5	0.06 (1)	0.22 (4)	0.28 (5)
Tidal Lowlands	10	0.15 (2)	0.59 (8)	0.74 (10)
Peatlands	4	0.30 (4)	0.00 (0)	0.30 (4)
Highlands	11	0.09 (2)	0.40 (9)	0.49 (11)

Note: *tb* freshwater swamps = the total *Tenebrio* baits (1800 larvae) per ecosystem; *tb* tidal lowlands = the total *Tenebrio* baits (1350 larvae) per ecosystem; peatlands = the total *Tenebrio* baits (1350 larvae) per ecosystem; *tb* highlands = the total *Tenebrio* baits (2250 larvae) per ecosystem; data in brackets () = the number infected *Tenebrio* bait (*ib*)

Discussion

Entomopathogenic species found in this research were *B. bassiana* and *M. anisopliae*. Morphological characteristics of both entomopathogenic fungi found in this research matched to the previous studies. Humber (2005) stated that mycelia of *B. bassiana* appear from exosceleton of hosts insect, and cover all part of exterior surface of host integument so that host body has white colour, reverse pale to yellow colony, hyaline or colourless, single cell, and globular and conidia as well as insulate hypha. *M. anisopliae* causes integument having colours of whitish green to dark green because its mycelia cover exosceleton of hosts insect; it has green to yellow conidia, single cell and cylindrical conidia as well as insulate hypha (Driver *et al.* 2000; Humber 2005).

Beauveria bassiana and *M. anisopliae* have parasitic and saprophytic phases during the killing process of their host insect (Augustyniuk-Kram and Kram 2012; El-Ghany 2015). Parasitic phase starts viz, the fungal conidia attach to the host insect cuticle, and then the conidia germinate on the host cuticle (El-Ghany 2015). The fungal penetration into the insect cuticle can be performed in producing specific infection hyphae originating at appressoria of the fungus (Fernandes et al. 2007; El-Ghany 2015). Gürlek et al. (2018) reported that both species could produce germ tubes growing over the surface of the insect cuticle until the tubes contact weakness area of cuticle where penetration can easily be achieved. After the fungus successfully penetrates, then micelia distribute into the hemolymph by the formation of blastospores (El-Ghany 2015). Finally, the host insect will die within four days of penetration (Gürlek et al. 2018). Saprophytic phase starts viz, the fungus grows mechanically in the dead insect body, retrieve nutrients from the insect body, and then the fungus produces toxins (El-Ghany 2015).

The success of both fungi in conducting the process of parasitic and saprophytic phases was affected by several external factors such as moisture, pH, temperature, ultraviolet (UV) radiation, and vegetation (El-Ghany 2015). This research showed that highland soil planted with cabbage and mustard in Talang Patai, Pagaralam had more inoculum potentials of entomopathogenic fungi than other locations because cabbage were usually as the host of insects pest dominated by Lepidoptera, such as *Plutella xylostella*, *Crocidolomia binotalis*, *Spodoptera litura*, and *Chrysodeixis chalcites*. While the larvae of Lepidoptera are the most suitable hosts for entomopathogic fungi (Godonou et al. 2009; Nunilahwati et al. 2012). This study also found that a lot of sick insects pest larvae hung above of mustard and cabbage canopy. Host insects attacked by entomopathogic fungi on cabbage canopy at highlands, South Sumatra generally dominated by *P. xylostella*, *S. litura* and *C. chalcites.* Symptoms of sick insects larvae were dry and in the stiff condition,

white or greenish white in colour and attached on upper surface of cabbage leaves. The sick larvae insects which showed the symptoms of dry and stiff condition as mummy and covered with white fungal mycelia were insects which attacked by *B. bassiana*, whereas insect body covered by fungal mycelia having greenish white or dark green colour were symptoms of insects which attacked by *M. anisopliae*. Symptoms of insects attacked by *B. bassiana* and *M. anisopliae* in this research matched to the symptoms reported by El-Ghany (2015) and Mora et al. (2017).

In this research, more inoculum potential of the entomopathogenic fungi was found in the highlands and tidal lowlands than that in freshwater swamps and peatlands because it was affected by soil pH and moisture. Zhong et al. (2010) reported that soil pH had more significant role in determining the existence of fungal propagules within soils than that of soil texture and organic matter. However, Inubushi et al. (2003) had stated that soil moisture is one of the most important controlling factors for biological reactions in the soil. Soil pH in this research was in the range of 4 to 4.5 in freshwater swamps, 4.3 to 5 in tidal lowlands, 3.60 to 4 in peatlands and 5 to 6.7 in highlands, respectively. Kodir and Juwita (2016) stated that the pH value of soil in freshwater swamps in Indonesia are in the ranges of 4 to 4.5, pH 4.17 to 5.35 in tidal lowlands (Marlina *et al.* 2016), and 3.60 to 3.95 in peatlands (Utami *et al.* 2009), and 5 to 6 in highlands (Supriadi *et al.* 2016). The variation of pH values of soils at each location can affect adaptation capability of entomopathogenic fungi surviving (Bugeme *et al.* 2008). The conidial viability of entomopathogenic fungi, such as *B. bassiana* and *M. anisopliae* were affected by pH of in-vitro medium for entomopathogenic fungi. Rizkie et al. (2017) reported that high acidity (pH < 4) of in-vitro medium for fungus growing significantly decrease conidial viability of *B. bassiana* and *M. anisopliae*. Therefore, inoculum potentials of the entomopathogenic fungi from peatland soil and freshwater swamp soil were lower than that from the soil in the tidal lowlands and highlands.

In addition to soil pH and moisture, soil texture also determines the existence and distribution of fungal propagules. Soil texture has low clay content, and sandy soil texture tends to have the low capability in maintaining the existence of fungal propagules (El-Ghany 2015). Water saturated soil also tend to have low capability in maintaining the existence of fungal propagules (Garrido-Jurado et al. 2011). Soils from freshwater swamps and peatlands in South Sumatra had lower clay content and in water saturated condition (Marlina et al. 2016; Kartika et al. 2018). The low existence of fungal propagules finding at freshwater swamp and peatland soils was due to both factors. Soils in freshwater swamps are water saturated for more than 6 to 7 months per year which is usually occurred from November to April (Herlinda et al. 2018) and soil moisture in the peatlands can reach 500% (Maftu'ah and Susanti 2009).

Peatlands have soil pore saturated with water all year long resulting in the anaerobic condition of soil. Peat soil has no clay, sand, and silt content, but it had organic matter (Sudana 2005). High organic matter finally can decrease the pH of soil (Utami et al. 2009). Rizkie et al. (2017) confirmed that pH < 4 within media in-vitro for growing fungi can decrease the ability to live of fungal propagules. In highland ecosystems, the portion of the soil texture among clay, sand, and silt fractions was found in the same composition or balance (Utomo et al. 2013: Supriadi *et al.* 2016). While tidal lowlands contain silt from sedimentation mixture of river water and seawater with balance clay and sand fractions (Marlina et al. 2016). Higher inoculum potentials in highlands and tidal lowlands in this research was due to balance or higher of clay texture and organic soils than that of freshwater swamps and peatlands.

The balance or higher of clay texture and organic soils are capable of maintaining the existence of fungal propagules (Garrido-Jurado et al. 2011). Zhong et al. (2010) stated that soil in ecosystems which apply composted manure or no synthetic fertilizer had higher propagules content of *B. bassiana* than that of soil in the ecosystem which applies synthetic fertilizers. Also, application of synthetic pesticides is capable of decreasing the existence of entomopathogenic fungi within the soil (Mietkiewski et al. 2010). Local farmers in freshwater swamp and peatland areas of South Sumatra usually do not apply synthetic pesticides, whereas many local farmers in tidal lowland and highland areas apply synthetic pesticides (Herlinda et al. 2018). Although no synthetic pesticides were applied in freshwater swamp and peatland areas, fungal propagules or inoculum potentials in these areas was lower than that of tidal lowlands and highlands areas. In this study no evidence that no synthetic pesticide application in freshwater swamp and peatland areas can cause high existence of fungal propagules. However, soil pH and soil texture have more effect on the existence of propagules of *B. bassiana* and *M. anisopliae* at lowland swamp and peatland ecosystems.

Higher inoculum potentials in highlands and tidal lowlands in this research was closely related to specific cultivated vegetations or crops. Most peatlands in Indonesia was utilized for forestry and conservation areas (Suriadikarta and Sutriadi 2007). Rice generally is cultivated at freshwater swamps (Herlinda et al. 2018; Lakitan, et al. 2018a; Lakitan, et al. 2018b) as well as in tidal lowlands; however, rice cultivation at tidal lowlands was more intensive (with two to three planting indices) than that of freshwater swamps (one planting index). Thus, diversity species of vegetation or crop plants could affect abundance and diversity species of the soil microorganisms associated with plant and crop roots (El-Ghany 2015).

This research found two species of entomopatogenic fungi from soils in South Sumatra i.e., *B. bassiana* and *M. anisopliae*. The highest percentage of the inoculum potentials of both fungi was occured in the highland ecosystems and the lowest percentage of the inoculum potentials of the fungi was found in the peatlands ecosystems. In highland ecosystems, percentage of the inoculum potentials was affected by the the locations and the vegetations or the crop plants. These fungi will make an important contribution to the biological control for insect pests in lowland to highland ecosystems in Indonesia.

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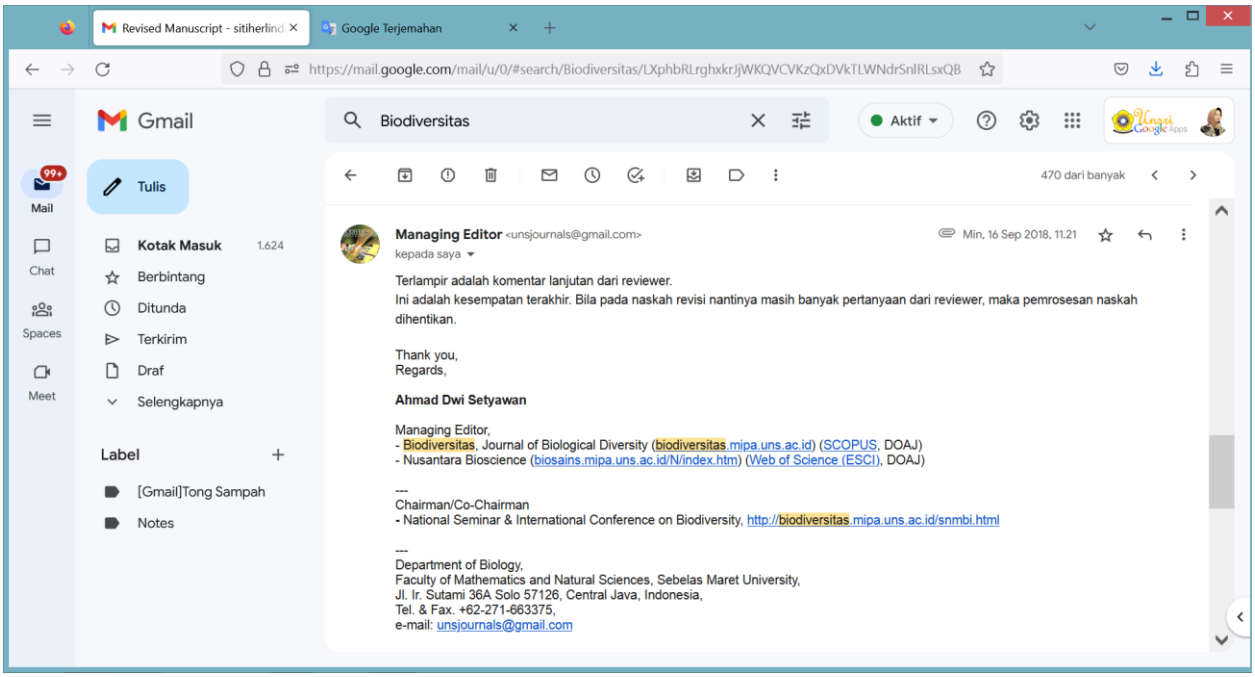
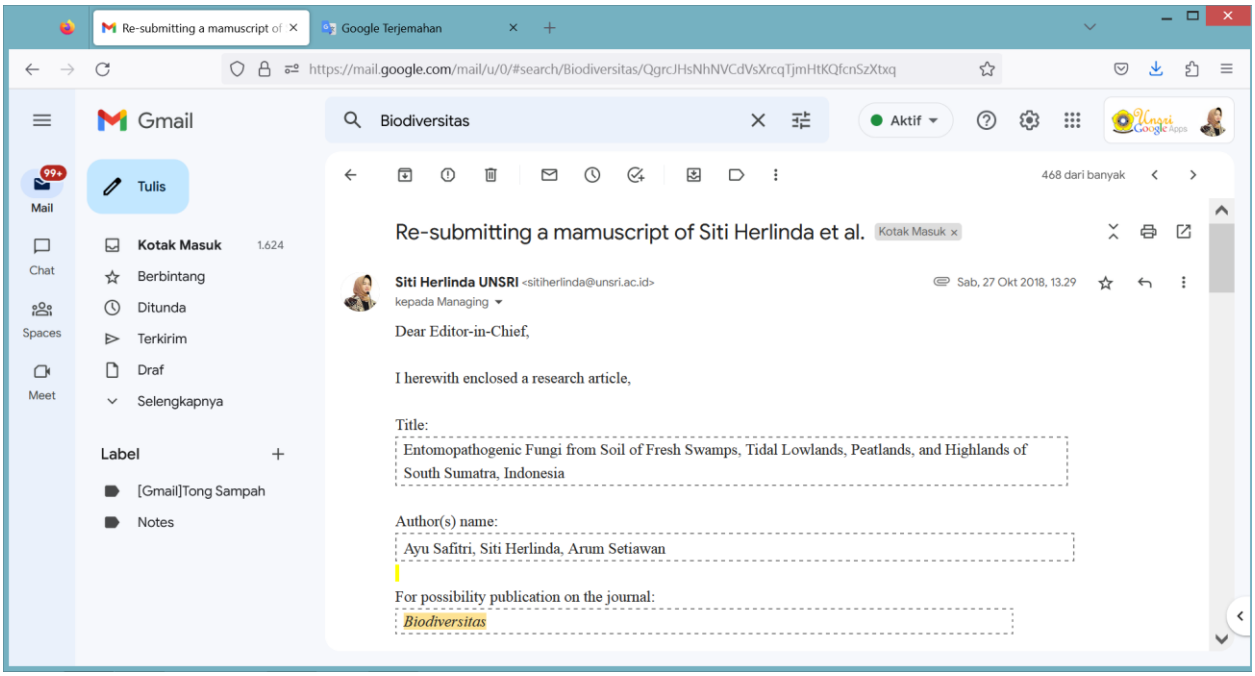
3. Bukti konfirmasi review kedua dan hasil revisi kedua





COVERING LETTER

Dear Editor-in-Chief,

I herewith enclosed a research article,

Title:

Entomopathogenic Fungi from Soil of Fresh Swamps, Tidal Lowlands, Peatlands, and Highlands of South Sumatra, Indonesia

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For possibility publication on the journal:
Biodiversitas

Novelty:
This study highlights several findings, such as the successful findings of entomopathogenic fungal species, *Beauveria bassiana* and *Metarhizium anisopliae* from soils of wetlands (fresh swamps, tidal lowlands, and peatlands) and highlands in South Sumatra, Indonesia. From highland soils, we found that the entomopathogenic fungi have the highest potential inoculum among those from soils of other ecosystems. Other finding is from peat soils still obtained the entomopathogenic fungi having still high potential inoculum. The findings will make an important contribution to the biological control for insect pests in Indonesia because 30 isolates belonging to the fungi originated and adapted from lowland to highland ecosystems.

Statements:
This manuscript has not been published and is not under consideration for publication to any other journal or any other type of publication (including web hosting) either by me or any of my co-authors.
Author(s) has been read and agree to the Ethical Guidelines.

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Place and date:
Indralaya, 27 October 2018

Sincerely yours,
Siti Herlinda

Entomopathogenic fungi from soils of freshwater swamps, tidal lowlands, peatlands, and highlands of South Sumatra, Indonesia

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Abstract. Ecosystems of lowlands and highlands in South Sumatra have specific characteristics of soils and vegetations that can affect the availability of entomopathogenic fungi. This study aimed to explore and identify species and to determine inoculum potentials of the entomopathogenic fungi from soils of freshwater swamps, tidal lowlands, peatlands, and highlands. Baiting of entomopathogenic fungi on soil samples used the larvae of *Tenebrio molitor*. The entomopathogenic fungi species found were *Beauveria bassiana* and *Metarhizium anisopliae*. The number of the fungal isolates found were 30 isolates consisted of nine isolates of *B. bassiana* and 21 isolates of *M. anisopliae*. The highest number of isolates were found on highland ecosystem (11 isolates) and the lowest one were found on peatland ecosystem (4 isolates). The highest percentage of inoculum potentials of the fungi was found on high land ecosystem (4.04%) and the lowest one was found on freshwater swamps ecosystem (2.11%). The soil of tidal lawlands in Telang Sari, Banyuasin planted with coconut had higher inoculum potentials (7.33%), as well as the highland soils in Talang Patai, Pagaralam planted with mustard (9.33%) . These fungi will make an important contribution to the biological control for insect pests in lowland to highland ecosystems in Indonesia.

Key words: *Beauveria bassiana*, lowlands, *Metarhizium anisopliae*, peat soils, wetlands

Running title: Entomopathogenic Fungi from Soils

INTRODUCTION

Many parts in the lowlands and highlands of Indonesia are used for agriculture. Lowlands consisting of freshwater swamps, tidal lowlands, and peatlands are the ecosystems having water saturated condition for the whole year (Sudana 2005). The lowland ecosystems for agricultural purposes in Indonesia are distributed in Sumatra, Kalimantan, and Papua Islands covering areas of 11 million ha of tidal lowlands, 9.2 million ha of freshwater swamps, and 14.9 million ha of peatlands, respectively (Mulyani and Sarwani 2013). While the highland ecosystems managed for agriculture with the area of 16.15 million ha are distributed in all Indonesia islands (Center for Agriculture Data and Information System, Secretariat General 2017).

Soils between lowlands and highlands in Indonesia have different characteristics, especially in the moisture, texture, and acidity (pH). The specific characteristics of lowland and highland soils at the four typhologies of ecosystems consist of the freshwater swamp, tidal lowlands, peatlands, and highland are closely related to specific cultivated vegetations or crops. Rice usually is cultivated at freshwater swamps (Herlinda et al. 2018; Lakitan, et al. 2018a; Lakitan, et al. 2018b). Only small part of soils in peatlands that can be cultivated with seasonal crops and most peatlands in Indonesia was utilized for forestry and conservation areas (Suriadikarta and Sutriadi 2007). Highlands area usually planted with various types of seasonal and annual crops. Specific vegetation or crop plants could affect the soil microorganisms associated with plant and crop roots (El-Ghany 2015).

Microorganisms such as fungi had been found from freshwater swamps and highlands of South Sumatra and can be used to control insect pest called entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* (Herlinda *et al.* 2008, 2010). The entomopathogenic fungi had proven to be an effective agents to control some insect pests (Chinniah et al. 2016), and also not harmful toward natural enemies of insect pests (Gholamzadeh-Chitgar et al. 2017). However, there was no complete information for fungi from tidal lowlands and peatlands in that region. *B. bassiana* and *M. anisopliae* had been found by previous researchers killing insect pest such as *Crocidolomia pavonana* (Hasyim *et al.* 2009), *Aphis gossypii* (Herlinda 2010), *Plutella xylostella* (Loc and Chi 2007), *Lygus* spp. (Leland et al. 2005), and *Oryctes rhinoceros* (Moslim et al. 2009). Entomopathogen, such as entomopathogenic fungi can be explored from soils (El-Ghany 2015). Certain entomopathogen species have adaptation capability in certain soils (Bugeme et al. 2008). Entomopathogen that had already adapted in freshwater swamp soils or highland soils generally has the specific advantage, i.e., it can adapt more effective at these soils environment (Erler and Ates 2015). Exploration of entomopathogen starting from lowland to highland ecosystems will produce the high variation of species and genetics that can be utilized at the extended area and specific location. The purposes of this study were to explore and identify species and to determine inoculum potentials of the entomopathogenic fungi from soils of freshwater swamps, tidal lowlands, peatlands, and highlands in South Sumatra.

MATERIALS AND METHODS

Study area

The study sites were selected in the agricultural center with specific typhology ecosystems in some locations of South Sumatra (Figure 1 and Table 1). The sites consisted of freshwater swamps, tidal lowlands, peatlands, and highlands. Those ecosystems chosen for explorations of entomopathogenic fungi had objective to obtain the fungi that had adapted in the soils from lowlands to highlands. The selected freshwater swamp locations were Gandus; Musi 2, Palembang; Rambutan, Banyuasin; and Pemulutan, Ogan Ilir. The selected locations in tidal lowlands were Mulya Sari, Banyuasin; Telang Sari, Banyuasin; and Muara Sungsang, Banyuasin. The selected locations in peatlands were Talang Dabok, Ogan Komering Ilir; Sepucuk, Ogan Komering Ilir; and Kedaton, Ogan Komering Ilir. The selected locations in the highlands were Lahat (Lematang, Tanjung Payang, Pulau Pinang), Pagaralam (Rimau and and Talang Patai).

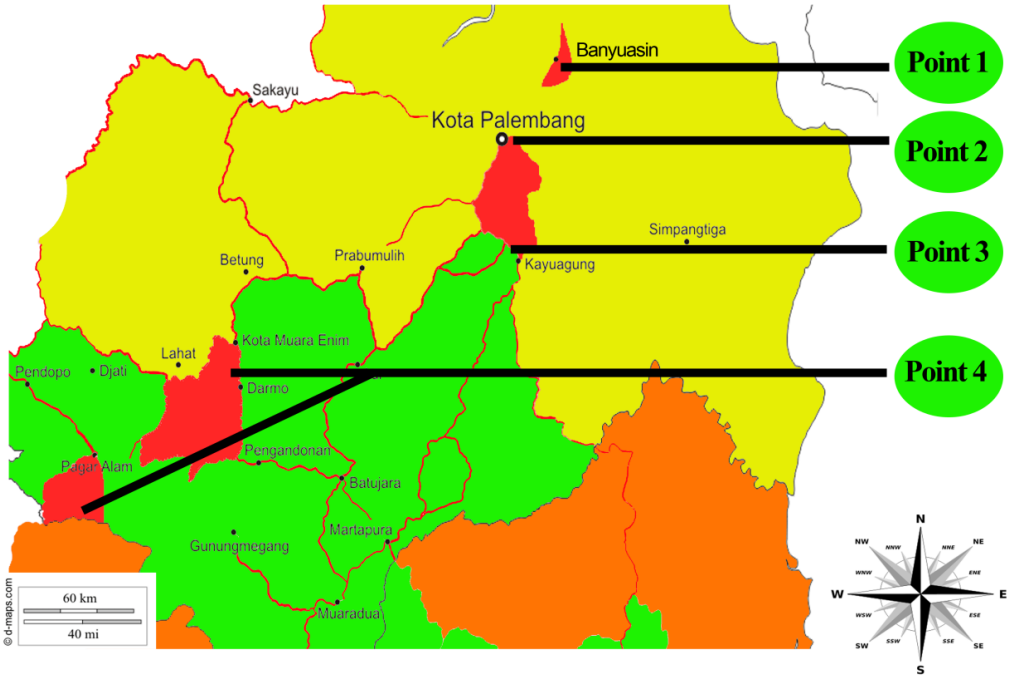

Figure 1. Locations of exploration for entomopathogenic fungi from soils in ecosystems of South Sumatra, Indonesia: point 1 = tidal lowlands (S 02°40.866' E 104°44.298'), point 2 = freshwater swamps (S 03°02.581' E 104°51.231'), point 3 = peatlands (S 03°24.840' E 104°53.362'), and point 4 = highlands (S 03°48.063' E 103°32.072' and S 03°50.174' E 103°31.293')
Source: https://d-maps.com/index.php?lang=en

Soil sampling was carried out in December 2017 and baiting of entomopathogenic fungi was done by using *Tenebrio molitor* (yellow mealworm beetle). Fungal isolation, purification, and identification were conducted from January to March 2018 at Laboratory of Entomology, Department of Plant Pest and Disease, College of Agriculture, Universitas Sriwijaya, Indralaya, Indonesia. Supporting data were recorded in term of soil sampling period, village or city names, coordinate points, and vegetation types or crop plants for each exploration locations (Figure 1 and Table 1). Value of pH belonging to soil samples was measured by using a method of Kartika et al. (2018). In this survey, the application of pesticides in the different ecosystems (freshwater swamps, tidal lowlands, peatlands and highlands) was also monitored.

Procedures

Observation of inoculum potentials of entomopathogenic fungi in the soil

Inoculum potentials observation of the entomopathogenic fungi in this research was conducted by exploration of fungi in soils of the freshwater swamps, tidal lowlands, peatlands, and highlands, respectively. Entomopathogenic fungi exploration was done by modifying the methods of Anwar *et al.* (2015), i.e., using *T. molitor* as insect bait (*Tenebrio* bait method) which was infested on fungal inoculum from soil samples.

For soil samples sites, soil of one up to four crop species (rice in freshwater swamps; corn, rice, coconut, and watermelon in tidal lowlands; oil palm in peatlands; and cabbage, mustard, rubber, and coffee in highlands) were taken

from each location at the diagonal position. Three to five soil sampling locations consisting of the freshwater swamps, tidal lowlands, peatlands, and highlands were taken from each ecosystem of exploration. Soil samples with the weight of 5000 g were obtained by digging soil at the depth of 5–15 cm in the vicinity of crop roots. Subsequently, soil samples were put into the plastic pouch and labeled with the information of soil sampling period and crop species.

Previously, soil samples were sieved by using 5-mesh siever to separate from crop roots. Then, samples were put into plastic tray (size of 32 cm x 25 cm x 5 cm) containing 5000 g of soil sample for each tray, and yielded 1000 g of finer soil sample. The finer soil sample was subsequently moisted with sterile aquadest until soil moisture of 80–90% using the method of Chen et al. (2014). Then, 30 larvae of the newly-moulting-third instar of *T. molitor* were sterilized with 70% alcohol, and put on soil-sample surface in a plastic tray. The larvae used per location were 150 larvae or 5 plastic trays containing 30 larvae per a tray (Table 1). The body of larvae was sprinkled with soil sample with thickness of about 5 mm. Subsequently, the plastic tray containing soil samples were covered with black cloth and was sprayed with sterile aquadest in order to maintain humidity of soil samples. Larvae were incubated within soil sample for seven days to provide enough time for entomopathogenic fungi infecting them. Then, dead larvae infected by the entomopathogenic fungi were recorded daily to determine inoculum potential. Inoculum potentials of entomopathogenic fungi in this research were measured according to percentage of infected *tenebrio* bait or hosts (Hofgaard et al. 2016). The inoculum potential (IP) was calculated based on the equation below:

$$\text{IP} = \frac{ib}{tb} \times 100$$

ib were the number of infected *Tenebrio* bait, and *tb* were the total of *Tenebrio* baits

Table 1. Locations of exploration for entomopathogenic fungi in South Sumatra, Indonesia

Ecosystems	Village or city	Vegetation or crop plants	Height from sea level (m)
Freshwater swamps	Rambutan, Banyuasin	Rice	15
	Gandus, Palembang	Rice	12
	Musi Dua, Palembang	Rice	16.67
	Pemulutan, Ogan Ilir	Rice	22
Tidal lowlands	Mulya Sari, Banyuasin	Corn, rice, watermelon	16.33
	Telang Sari, Banyuasin	Corn, coconut, corn + coconut	18.33
	Muara Sungsang, Banyuasin	Coconut, banana, pineapple	15
Peatlands	Talang Dabok, Ogan Komering Ilir	Palm, rubber, pineapple	24
	Sepucuk, Ogan Komering Ilir	Oil palm, rubber, pineapple	23
	Kedaton, Ogan Komering Ilir	Oil palm	19.67
Highlands	Talang Patai, Pagaralam	Cabbage	170
	Pulau Pinang, Lahat	Rubber + coffee	161
	Tanjung Payang, Lahat	Rubber	121
	Lematang, Lahat	Rice	121.3
	Rimau, Pagaralam	Tea	1,326

Note: + intercropping

Entomopathogen Isolation

The dead *Tenebrio* bait was subsequently isolated and purified by using the methods of Herlinda (2010). Entomopathogenic fungi infecting and growing on integument of *Tenebrio* bait were isolated and grown on SDA (Sabouraud Dextrose Agar) medium. The integument surface of larvae infected by the entomopathogenic fungi was previously sterilized using the modified method of Nuraini et al. (2017) with 1% natrium hypochlorite for 3 minutes and subsequently was rinsed three times with sterile aquadest and air dried on sterile filter paper. Larvae were put into petridish containing sterile humid tissue paper and then incubated in order to stimulate the growth of entomopathogenic fungi. Conidia of entomopathogenic fungi emerging from the dead larvae body were taken by using sterile ose needle and moved into petri disk containing SDA medium, and incubated for seven days at the constant temperature of 25 ^{o}C within the incubator.

Identification of Entomopathogen Fungi

The purified fungi were identified according to macroscopic and microscopic characteristics. Fungi that had been grown on SDA media with the area of 1 cm^2 was taken by using ose needle and put into preparations containing SDA media and incubated for three days and then microscopically observed. Subsequently, its morphology was identified macroscopically and microscopically by using the method of Guilherme et al. (2015). Furthermore, species of fungi were identified by using books of Humber (2005) and El-Ghany (2015).

Data analysis

Data on inoculum potentials based on percentage of *Tenebrio* bait infected by the entomopathogenic fungi among treatments was analyzed descriptively.

RESULTS AND DISCUSSION

Insect Characteristics Infected by Entomopathogenic Fungi

Identification based on morphology for entomopathogenic fungi found in this study was consisted of two species , *B. bassiana* and *M. anisopliae*. There were only 30 isolates found from these two fungal species (Table 2). These 30 isolates isolated from 223 larvae (Table 3-7) of *Tenebrio* bait infected by the entomopathogenic fungi, however a lot of larvae were failed to be isolated due to contamination mostly by aerial fungi and *Trichoderma* spp.

These 30 isolates consisted of nine isolates of *B. bassiana* and 21 isolates of *M. anisopliae. Tenebrio* bait infected by the entomopathogenic fungi showed macroscopic and microscopic characteristics or symptoms which were confirmed to determine the entomopathogenic fungal species. Sick or dead *Tenebrio* bait infected by *B. bassiana* showed symptoms as follows: insect body was dry and wrinkle, its outer integument was coated by white mycelia similar to silk, rigid, easily broken and no smell (Figure 2). The *Tenebrio* bait attacked by *M. anisopliae* showed symptoms as follows: dry and wrinkle, no smell, brittle and easily broken, but its outer integument was coated by mycelia having greenish white to dark green or dark colour (Figure 3).

The pure isolate was obtained from dead *Tenebrio* bait body which was attacked by entomopathogenic fungi with colony characteristics for each species as follows; colony of *B. bassiana* had the white colour similar to cotton, but gradually its colour changed into yellowish white as fungi become older. Colony of *M. anisopliae* initially had white colour similar to colour of *B. bassiana*, but the colour changed into greenish and dark green or dark as fungi become older (Figure 4). Conidia of *B. bassiana* and *M. anisopliae* with specific characteristics were obtained from each colony species of entomopathogenic fungi. Conidia of *B. bassiana* had single cell and round shape, whereas conidia of *M. anisopliae* had single cell but with cylindrical shape (Figure 5). Mycelia of *B. bassiana* and *M. anisopliae* insulated with upright, branches and layers of conidiophores.

Inoculum Potentials of Entomopathogenic Fungi in the Soil of South Sumatra

Inoculum potentials of entomopathogenic fungi in this research were measured according to the percentage of infected *Tenebrio* bait. The inoculum potentials of entomopathogens fungi from the freshwater swamp soil, tidal lowlands, peat soils, and high land each was different among survey locations (Tables 3−6). The value of inoculum potential in soil of freshwater swamps, tidal lowlands, peatlands and high lands were in the range of 0.67−3.33%, 2.67-7.33%, 0-4%, and 1.33-9.33%, respectively.

The inoculum potentials based on locations or crop species showed that the inoculum potentials from the freshwater swamps was only found on soil planted by rice in Rambutan, Banyuasin; in Gandus Palembang; Musi Dua, Palembang; in Pemulutan, Ogan Ilir. The highest inoculum potentials was found on soil planted by rice in Musi Dua, Palembang (3.33%) (Table 3). From the freshwater swamp soils, we only could produced five isolates of the entomopathogenic fungi isolated from 35 infected *Tenebrio* baits. A lot of the infected *Tenebrio* baits were failed to be isolated due to contamination by the aerial fungi and *Trichoderma* spp.

From the tidal lowlands, the inoculum potentials were found on soil planted with corn, rice, and watermelon in Mulya Sari, Banyuasin; by corn and coconut in Telang Sari, Banyuasin; by coconut, banana, and pineapple in Muara Sungsang, Banyuasin. The highest inoculum potentials was found on soil planted with coconut in Telang Sari, Banyuasin (7.33%) (Table 4). The inoculum potentials of the entomopathogenic fungi gained were only 10 isolates, the other infected *Tenebrio* baits were contaminated.

The inoculum potentials from peatlands were found on soil planted with oil palm, rubber, and pine apple in Talang Dabok, Ogan Komering Ilir; with oil palm, rubber, and pine apple in Sepucuk, Ogan Komering Ilir; and with oil palm in Kedaton, Ogan Komering Ilir. The peatsoil in Sepucuk, Ogan Komering Ilir planted with rubber had higher inoculum potentials (5.33%) (Table 5).

The inoculum potentials from high lands were found on soil planted with rubber and coffee in Pulau Pinang, Lahat; by rubber in Tanjung Payang, Lahat; with rice in Lematang, Lahat; with cabbage and mustard in Talang Patai, Pagaralam; and with tea in Rimau, Pagaralam. The highland soils in Talang Patai, Pagaralam planted with mustard (9.33%) (Table 6).

Based on ecosystems classification observed in this research which consisted of the freshwater swamps, tidal lowlands, peatlands and highlands, the results showed that inoculum potentials of entomopathogenic fungi was different among ecosystems (Table 7). The highest percentage of inoculum potentials of the fungi was found on high land ecosystem (4.04%) and the lowest one was found on freshwater swamp ecosystem (2.11%). However, the highest number of isolates were found on highland ecosystem (11 isolates) and the lowest one were found on peatland ecosystem (4 isolates).

Figure 2. *Tenebrio* bait infected by *Beauveria bassiana* (a) and healthy *Tenebrio* (b)

Figure 3. *Tenebrio* bait infected by *Metarhizium anisopliae* (a) and healthy *Tenebrio* (b)

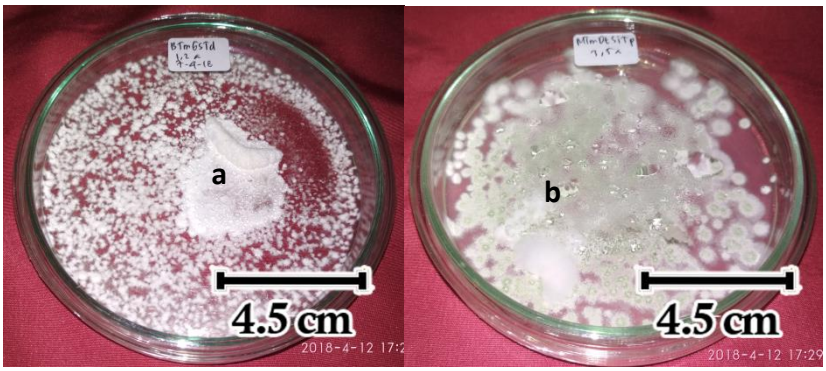

Figure 4. Colony of *Beauveria bassiana* (a) and *Metarhizium anisopliae* (b)

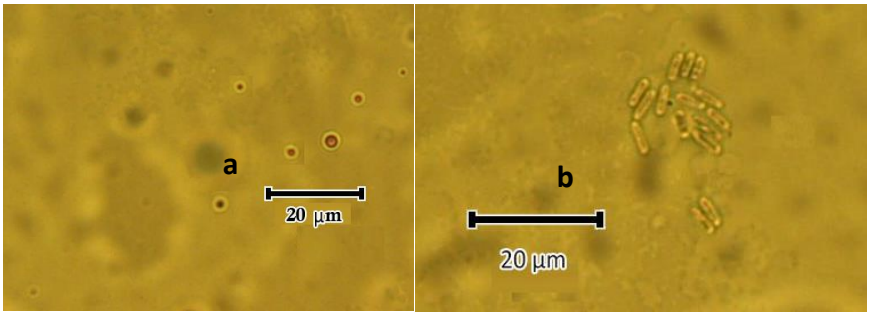

Figure 5. Conidia of *Beauveria bassiana* (a) and *Metarhizium anisopliae* (b) (400x magnification)

Table 2. Species and isolates of entomopathogenic fungi found in South Sumatra, Indonesia.

Ecosystems	**Species of fungi**	**Number of isolate**	**Vegetation or crop plants**	**Village or city**
Freshwater swamps	*Metarhizium anisopliae*	3	Rice	Rambutan
Freshwater swamps	*Metarhizium anisopliae*	1	Rice	Pemulutan
Freshwater swamps	*Beauveria bassiana*	1	Rice	Rambutan
Tidal lowlands	*Metarhizium anisopliae*	1	Corn + coconut	Telang Sari
Tidal lowlands	*Metarhizium anisopliae*	2	Corn	Telang Sari
Tidal lowlands	*Metarhizium anisopliae*	2	Corn	Mulya Sari
Tidal lowlands	*Metarhizium anisopliae*	3	Rice	Mulya Sari
Tidal lowlands	*Beauveria bassiana*	1	Corn	Telang Sari
Tidal lowlands	*Beauveria bassiana*	1	Watermelon	Mulya Sari

Peatlands	*Beauveri abassiana*	4	Oil palm	Talang Dabok
Highlands	*Metarhizium anisopliae*	1	Rubber + coffee	Pulau Pinang
Highlands	*Metarhizium anisopliae*	4	Cabbage	Talang Patai
Highlands	*Metarhizium anisopliae*	4	Mustard	Talang Patai
Highlands	*Beauveria bassiana*	1	Rubber + coffee	Pulau Pinang
Highlands	*Beauveria bassiana*	1	Cabbage	Talang Patai

Note: + intercropping

Table 3. Inoculum potentials of entomopathogenic fungi in the freshwater swamp soils of South Sumatra

Village or city	Vegetation or crop species	GPS (coordinat)	Inoculum potentials (%)			
			Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (Total)
Rambutan, Banyuasin	Rice	S 03°02.581', E 104°51.231'	0	0.00 (0)	0.67 (1)	0.67 (1)
Rambutan, Banyuasin	Rice	S 03°02.591', E 104°51.217'	2	0.67 (1)	2.00 (3)	2.67 (4)
Rambutan, Banyuasin	Rice	S 03°02.586', E 104°51.201'	2	0.67 (1)	0.67 (1)	1.33 (2)
Gandus, Palembang	Rice	S 03°00.401', E 104°42.380'	0	1.33 (2)	2.00 (3)	3.33 (5)
Gandus, Palembang	Rice	S 03°00.632', E 104°42.532'	0	0.67 (1)	0.67 (1)	1.33 (2)
Gandus, Palembang	Rice	S 03°00.632', E 104°42.801'	0	1.33 (2)	1.33 (2)	2.67 (4)
Musi Dua, Palembang	Rice	S 03°02.120', E 104°43.021'	0	0.67 (1)	1.33 (2)	2.00 (3)
Musi Dua, Palembang	Rice	S 03°02.150', E 104°43.612'	0	1.33 (2)	2.00 (3)	3.33 (5)
Musi Dua, Palembang	Rice	S 03°02.510', E 104°43.120'	0	1.33 (2)	1.33 (2)	2.67 (4)
Pemulutan, Ogan Ilir	Rice	S 03°03.148', E 104°46.230'	0	0.67 (1)	0.67 (1)	1.33 (2)
Pemulutan, Ogan Ilir	Rice	S 03°03.115', E 104°46.218'	0	0.67 (1)	0.67 (1)	1.33 (2)
Pemulutan, Ogan Ilir	Rice	S 03°03.113', E 104°46.201'	1	1.33 (2)	1.33 (2)	2.67 (4)

Note: + intercropping; The total of *Tenebrio* baits (*tb*) = 150 larvae per village; data in brackets () = the number of infected *Tenebrio* bait (*ib*)

Table 4. Inoculum potentials of entomopathogenic fungi in the tidal lowland soils of South Sumatra

Village or city	Vegetation or crop species	GPS (coordinat)	Inoculum potentials (%)			
			Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (Total)
Mulya Sari, Banyuasin	Corn	S 02°40.866', E 104°44.298'	2	1.33 (2)	2.67 (4)	4.00 (6)
Mulya Sari, Banyuasin	Rice	S 02°40.944', E 104°44.621'	3	1.33 (2)	2.00 (3)	3.33 (5)
Mulya Sari, Banyuasin	Watermelon	S 02°40.896', E 104°44.676'	1	1.33 (2)	2.00 (3)	3.33 (5)
Telang Sari, Banyuasin	Corn	S 02°38.842', E 104°45.369'	2	1.33 (2)	2.67 (4)	4.00 (6)
Telang Sari, Banyuasin	Coconut	S 02°38.813', E 104°45.801'	1	0.67 (1)	1.33 (2)	2.00 (3)
Telang Sari, Banyuasin	Corn + coconut	S 02°38.875', E 104°44.495'	1	2.00 (3)	5.33 (8)	7.33 (11)
Muara Sungsang, Banyuasin	Coconut	S 02°21.736', E 104°50.635'	0	1.33 (2)	4.00 (6)	5.33 (8)
Muara Sungsang, Banyuasin	Banana	S 02°21.823', E 104°50.632'	0	0.67 (1)	2.00 (3)	2.67 (4)
Muara Sungsang	Pineapple	S 02°22.542', E 104°50.324'	0	0.67 (1)	2.00 (3)	2.67 (4)

Note: + intercropping; The total of *Tenebrio* baits (*tb*) = 150 larvae per village; data in brackets () = the number of infected *Tenebrio* bait (*ib*)

Table 5. Inoculum potentials of entomopathogenic fungi in the peatland soils of South Sumatra

Village or city	Vegetation or crop plants	GPS (coordinat)	Inoculum potentials (%)			
			Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (Total)
Talang Dabok, Ogan Komering Ilir	Oil palm	S 03°23.570', E 104°51.498'	4	3.33 (5)	1.33 (2)	4.67 (7)
Talang Dabok, Ogan Komering Ilir	Rubber	S 03°25.673', E 104°53.258'	0	2.67 (4)	1.33 (2)	4.00 (6)
Talang Dabok, Ogan Komering Ilir	Pineapple	S 03°25.280', E 104°52.940'	0	2.00 (3)	0.67 (1)	2.67 (4)
Sepucuk, Ogan Komering Ilir	Oil palm	S 03°24.840', E 104°53.362'	0	2.00 (3)	1.33 (2)	3.33 (5)
Sepucuk, Ogan Komering Ilir	Rubber	S 03°23.715', E 104°52.275'	0	3.33 (5)	2.00 (3)	5.33 (8)
Sepucuk, Ogan Komering Ilir	Pineapple	S 03°23.535', E 104°51.780'	0	1.33 (2)	1.33 (2)	2.67 (4)
Kedaton, Ogan Komering Ilir	Oil palm	S 03°23.308', E 104°51.487'	0	1.33 (2)	2.00 (3)	3.33 (5)
Kedaton, Ogan Komering Ilir	Oil palm	S 03°23.277', E 104°51.398'	0	1.33 (2)	0.67 (1)	2.00 (3)
Kedaton, Ogan Komering Ilir	Oil palm	S 03°23.204', E 104°51.459'	0	1.33 (2)	0.67 (1)	2.00 (3)

Note: + intercropping; The total of *Tenebrio* baits (*tb*) = 150 larvae per village; data in brackets () = the number of infected *Tenebrio* bait (*ib*)

Table 6. Inoculum potentials of entomopathogenic fungi in the highland soils of South Sumatra

Village or city	Vegetation or crop plants	GPS (coordinat)	Inoculum potentials (%)			
			Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (Total)
Pulau Pinang, Lahat	Rubber + coffee (A)	S 03°48.819', E 103°32.677'	2	1.33 (2)	1.33 (2)	2.67 (4)
Pulau Pinang, Lahat	Rubber + coffee (B)	S 03°48.852', E 103°32.698'	0	1.33 (2)	2.00 (3)	3.33 (5)
Pulau Pinang, Lahat	Rubber + coffee (C)	S 03°48.883', E 103°32.688'	0	0.67 (1)	0.67 (1)	1.33 (2)
Tanjung Payang, Lahat	Rubber (A)	S 03°48.094', E 103°32.145'	0	0.67 (1)	1.33 (2)	2.00 (3)
Tanjung Payang, Lahat	Rubber (B)	S 03°48.122', E 103°32.162'	0	3.33 (5)	2.67 (4)	6.00 (9)
Tanjung Payang, Lahat	Rubber (C)	S 03°48.177', E 103°32.166'	0	2.00 (3)	2.67 (4)	4.67 (7)
Lematang, Lahat	Rice	S 03°48.063', E 103°32.072'	0	0.67 (1)	1.33 (2)	2.00 (3)
Lematang, Lahat	Rice	S 03°48.051', E 103°32.069'	0	3.33 (5)	4.00 (6)	7.33 (11)
Lematang, Lahat	Rice	S 03°48.020', E 103°32.064'	0	1.33 (2)	1.33 (2)	2.67 (4)
Talang Patai, Pagaralam	Cabbage	S 03°50.180', E 103°31.325'	2	4.00 (6)	4.67 (7)	8.67 (13)
Talang Patai, Pagaralam	Cabbage	S 03°50.174', E 103°31.313'	3	0.67 (1)	1.33 (2)	2.00 (3)
Talang Patai, Pagaralam	Mustard	S 03°50.174', E 103°31.293'	4	4.67 (7)	4.67 (7)	9.33 (14)
Rimau, Pagaralam	Tea	S 04°02.161', E 103°10.484'	0	2.00 (3)	2.00 (3)	4.00 (6)
Rimau, Pagaralam	Tea	S 04°02.144', E 103°10.487'	0	1.33 (2)	1.33 (2)	2.67 (4)
Rimau, Pagaralam	Tea	S 04°02.136', E 103°10.485'	0	0.67 (1)	1.33 (2)	2.00 (4)

Note: + intercropping; The total of *Tenebrio* baits (*tb*) = 150 larvae per village; data in brackets () = the number of infected *Tenebrio* bait (*ib*)

Table 7. Inoculum potentials of entomopathogenic fungi in the soil of South Sumatra

Ecosystems	Inoculum potentials (%)			
	Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (Total)
Freshwater Swamps	5	0.89 (16)	1.22 (22)	2.11 (38)
Tidal Lowlands	10	1.18 (16)	2.67 (36)	3.85 (52)
Peatlands	4	2.07 (28)	1.25 (17)	3.33 (45)
Highlands	11	1.87 (42)	2.17 (49)	4.04 (91)

Note: + intercropping; *tb* freshwater swamps = the total of *Tenebrio* baits (1800 larvae) per ecosystem; *tb* tidal lowlands = the total of *Tenebrio* baits (1350 larvae) per ecosystem; peatlands = the total of *Tenebrio* baits (1350 larvae) per ecosystem; *tb* highlands = the total of *Tenebrio* baits (2250 larvae) per ecosystem; data in brackets () = the number of infected *Tenebrio* bait (*ib*)

Discussion

Entomopathogenic species found in this research were *B. bassiana* and *M. anisopliae*. Macroscopic and microscopic characteristics of both entomopathogenic fungi found in this research matched to the previous studies. Humber (2005) stated that mycelia of *B. bassiana* appear from exosceleton of hosts insect, and cover all part of exterior surface of host integument so that host body has white colour, reverse pale to yellow colony, hyaline or colourless, single cell, and globular and conidia as well as insulate hypha. *M. anisopliae* causes integument having colours of whitish green to dark green because its mycelia cover exosceleton of hosts insect; it has green to yellow conidia, single cell and cylindrical conidia as well as insulate hypha (Driver *et al.* 2000; Humber 2005).

Beauveria bassiana and *M. anisopliae* have parasitic and saprophytic phases during the killing process of their host insect (Augustyniuk-Kram and Kram 2012; El-Ghany 2015). Parasitic phase starts viz, the fungal conidia attach to the host insect cuticle, and then the conidia germinate on the host cuticle (El-Ghany 2015). The fungal penetration into the insect cuticle can be performed in producing specific infection hyphae originating at appressoria of the fungus (Fernandes et al. 2007; El-Ghany 2015). Gürlek et al. (2018) reported that both species could produce germ tubes growing over the surface of the insect cuticle until the tubes contact weakness area of cuticle where penetration can easily be achieved. After the fungus successfully penetrates, then micelia distribute into the hemolymph by the formation of blastospores (El-Ghany 2015). Finally, the host insect will die within four days of penetration (Gürlek et al. 2018). Saprophytic phase

starts viz, the fungus grows mechanically in the dead insect body, retrieve nutrients from the insect body, and then the fungus produces toxins (El-Ghany 2015).

The success of both fungi in conducting the process of parasitic and saprophytic phases was affected by several external factors such as moisture, pH, temperature, ultraviolet (UV) radiation, and vegetation (El-Ghany 2015). This research showed that highland soil planted with cabbage and mustard in Talang Patai, Pagaralam had more inoculum potentials of entomopathogenic fungi than other locations because cabbage and mustard observed in this survey were inhabited by insects pests dominated by Lepidoptera, such as *Plutella xylostella*, *Crocidolomia binotalis*, *Spodoptera litura*, and *Chrysodeixis chalcites*. While the larvae of Lepidoptera are the most suitable hosts for entomopathogic fungi (Godonou et al. 2009; Nunilahwati et al. 2012). This study also found that a lot of infected larvae of the insects pests hung above of the mustard and cabbage canopy. Host insects attacked by entomopathogic fungi on the cabbage canopy at highlands, South Sumatra generally dominated by *P. xylostella*, *S. litura* and *C. chalcites.* Symptoms of sick or infected insects larvae were dry and in the stiff condition, white or greenish white in colour and attached on upper surface of cabbage leaves. The sick larvae insects which showed the symptoms of dry and stiff condition as mummy and covered with white fungal mycelia were insects which attacked by *B. bassiana*, whereas insect body covered by fungal mycelia having greenish white or dark green colour were symptoms of insects which attacked by *M. anisopliae.* Symptoms of insects attacked by *B. bassiana* and *M. anisopliae* in this research matched to the symptoms reported by El-Ghany (2015) and Mora et al. (2017).

In this research, more inoculum potential of the entomopathogenic fungi was found in the highlands and tidal lowlands than that in freshwater swamps and peatlands because it was affected by soil pH and moisture. Zhong et al. (2010) reported that soil pH had more significant role in determining the existence of fungal propagules within soils than that of soil texture and organic matter. However, Inubushi et al. (2003) had stated that soil moisture is one of the most important controlling factors for biological reactions in the soil. Soil pH in this research was in the range of 4 to 4.5 in freshwater swamps, 4.3 to 5 in tidal lowlands, 3.60 to 4 in peatlands and 5 to 6.7 in highlands, respectively. Kodir and Juwita (2016) stated that the pH value of soil in freshwater swamps in Indonesia are in the ranges of 4 to 4.5, pH 4.17 to 5.35 in tidal lowlands (Marlina *et al.* 2016), and 3.60 to 3.95 in peatlands (Utami *et al.* 2009), and 5 to 6 in highlands (Supriadi *et al.* 2016). The variation of pH values of soils at each location can affect adaptation capability of entomopathogenic fungi surviving (Bugeme *et al.* 2008). The conidial viability of entomopathogenic fungi, such as *B. bassiana* and *M. anisopliae* were affected by pH of in-vitro medium for entomopathogenic fungi. Rizkie et al. (2017) reported that high acidity (pH < 4) of in-vitro medium for fungus growing significantly decrease conidial viability of *B. bassiana* and *M. anisopliae*. Therefore, inoculum potentials of the entomopathogenic fungi from peatland soil and freshwater swamp soil were lower than that from the soil in the tidal lowlands and highlands.

In addition to soil pH and moisture, soil texture also determines the existence and distribution of fungal propagules. Soil texture has low clay content, and sandy soil texture tends to have the low capability in maintaining the existence of fungal propagules (El-Ghany 2015). Water saturated soil also tend to have low capability in maintaining the existence of fungal propagules (Garrido-Jurado et al. 2011). Soils from freshwater swamps and peatlands in South Sumatra had lower clay content and in water saturated condition (Marlina et al. 2016; Kartika et al. 2018). The low existence of fungal propagules finding at freshwater swamp and peatland soils was due to both factors. Soils in freshwater swamps are water saturated for more than 6 to 7 months per year which is usually occurred from November to April (Herlinda et al. 2018) and soil moisture in the peatlands can reach 500% (Maftu'ah and Susanti 2009).

Peatlands have soil pore saturated with water all year long resulting in the anaerobic condition of soil. Peat soil has no clay, sand, and silt content, but it had organic matter (Sudana 2005). High organic matter finally can decrease the pH of soil (Utami et al. 2009). Rizkie et al. (2017) confirmed that pH < 4 within media in-vitro for growing fungi can decrease the ability to live of fungal propagules. In highland ecosystems, the portion of the soil texture among clay, sand, and silt fractions was found in the same composition or balance (Utomo et al. 2013: Supriadi *et al.* 2016). While tidal lowlands contain silt from sedimentation mixture of river water and seawater with balance clay and sand fractions (Marlina et al. 2016). Higher inoculum potentials in highlands and tidal lowlands in this research was due to balance or higher of clay texture and organic soils than that of freshwater swamps and peatlands.

The balance or higher of clay texture and organic soils are capable of maintaining the existence of fungal propagules (Garrido-Jurado et al. 2011). Zhong et al. (2010) stated that soil in ecosystems which apply composted manure or no synthetic fertilizer had higher propagules content of *B. bassiana* than that of soil in the ecosystem which applies synthetic fertilizers. Also, application of synthetic pesticides is capable of decreasing the existence of entomopathogenic fungi within the soil (Mietkiewski et al. 2010). Local farmers in freshwater swamp and peatland areas of South Sumatra usually do not apply synthetic pesticides, whereas many local farmers in tidal lowland and highland areas apply synthetic pesticides (Herlinda et al. 2018). Although no synthetic pesticides were applied in freshwater swamp and peatland areas, fungal propagules or inoculum potentials in these areas was lower than that of tidal lowlands and highlands areas. In this study no evidence that no synthetic pesticide application in freshwater swamp and peatland areas can cause high existence of fungal propagules. However, soil pH and soil texture have more effect on the existence of propagules of *B. bassiana* and *M. anisopliae* at lowland swamp and peatland ecosystems.

Higher inoculum potentials in highlands and tidal lowlands in this research was closely related to specific cultivated vegetations or crops. Most peatlands in Indonesia was utilized for forestry and conservation areas (Suriadikarta and Sutriadi

2007). Rice generally is cultivated at freshwater swamps (Herlinda et al. 2018; Lakitan, et al. 2018a; Lakitan, et al. 2018b) as well as in tidal lowlands; however, rice cultivation at tidal lowlands was more intensive (with two to three planting indices) than that of freshwater swamps (one planting index). Thus, diversity species of vegetation or crop plants could affect abundance and diversity species of the soil microorganisms associated with plant and crop roots (El-Ghany 2015).

This research found two species of entomopatogenic fungi from soils in South Sumatra i.e., *B. bassiana* and *M. anisopliae*. The highest percentage of the inoculum potentials of both fungi was occured in the highland ecosystems and the lowest percentage of the inoculum potentials of the fungi was found in the peatlands ecosystems. In highland ecosystems, percentage of the inoculum potentials was affected by the the locations and the vegetations or the crop plants. These fungi will make an important contribution to the biological control for insect pests in lowland to highland ecosystems in Indonesia.

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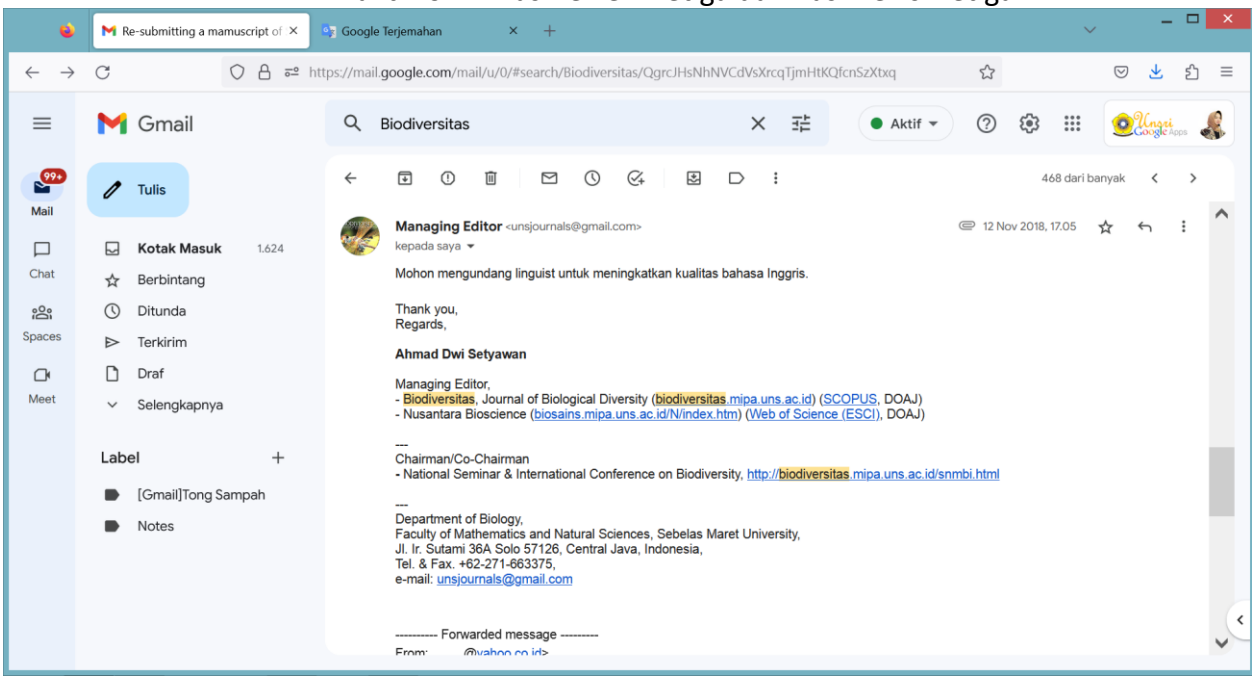
4. Bukti konfirmasi review ketiga dan hasil revisi ketiga



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Entomopathogenic fungi of soils of freshwater swamps, tidal lowlands, peatlands, and highlands of South Sumatra, Indonesia

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Abstract. Ecosystems of lowlands and highlands in South Sumatra have specific characteristics of soils and vegetations that can affect the availability of entomopathogenic fungi. This study aimed to explore and identify species and to determine inoculum potentials of the entomopathogenic fungi from soils of freshwater swamps, tidal lowlands, peatlands, and highlands. Baiting of entomopathogenic fungi on soil samples used the larvae of *Tenebrio molitor*. The entomopathogenic fungi species found in this research were *Beauveria bassiana* and *Metarhizium anisopliae*. The number of the fungal isolates were 30 isolates consisting of nine isolates of *B. bassiana* and 21 isolates of *M. anisopliae.*The highest number of isolates was found in the highland ecosystem (11 isolates) and the lowest was found in peatland ecosystem (4 isolates). The highest percentage average of inoculum potentials of the fungi was found in the high land ecosystem (4.04%) and the lowest one was found in freshwater swamps ecosystem (2.11%). Based on the vegetation type, the soil planted with mustard in Talang Patai-Pagaralam (highland ecosystem) had the highest inoculum potentials (9.33%). These fungi will make an important contribution to the biological control for insect pests in lowland to highland ecosystems in Indonesia.

Keywords: *Beauveria bassiana*, lowlands, *Metarhizium anisopliae*, peat soils, wetlands

Running title: Entomopathogenic fungi of soils in South Sumatra

INTRODUCTION

Many parts of lowlands and highlands of Indonesia are used for agriculture. Lowlands consisting of freshwater swamps, tidal lowlands, and peatlands are the ecosystems having water saturated condition for the whole year (Sudana 2005). The lowland ecosystems for agricultural purposes in Indonesia are distributed in Sumatra, Kalimantan, and Papua Islands covering 11 million ha of tidal lowlands, comprising 9.2 million ha of freshwater swamps, and 14.9 million ha of peatlands, respectively (Mulyani and Sarwani 2013). While the highland ecosystems managed for agriculture with the area of 16.15 million ha are distributed all over Indonesia islands (Center for Agriculture Data and Information System, Secretariat General 2017).

Soils between lowlands and highlands in Indonesia have different characteristics, especially in the moisture, texture, and acidity (pH). The specific characteristics of lowland and highland soils at the four typologies of ecosystems consist of the freshwater swamp, tidal lowlands, peatlands, and highland closely related to specific cultivated vegetations or crops. Paddy usually is cultivated at freshwater swamps (Herlinda et al. 2018; Lakitan, et al. 2018a; Lakitan, et al. 2018b). There is only a small part of soils in peatlands that can be cultivated with seasonal crops and most peatlands in Indonesia are utilized for forestry and conservation areas (Suriadikarta and Sutriadi 2007). Highlands area usually planted with various types of seasonal and annual crops. Specific vegetation or crop plants could affect the soil microorganisms associated with plant and crop roots (El-Ghany 2015).

Microorganisms such as fungi are found in freshwater swamps and highlands of South Sumatra and can be used to control insect pest called entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* (Herlinda et al. 2008; 2010). The entomopathogenic fungi are proven to be an effective agent to control some insect pests (Chinniah et al. 2016), and they are not harmful toward natural enemies of insect pests (Gholamzadeh-Chitgar et al. 2017). However, there is no complete information for fungi of tidal lowlands and peatlands in that region. *B. bassiana* and *M. anisopliae* were found to kill insect pests such as *Crocidolomia pavonana* (Hasyim *et al.* 2009), *Aphis gossypii* (Herlinda 2010), *Plutella xylostella* (Loc and Chi 2007), *Lygus* spp. (Leland et al. 2005), and *Oryctes rhinoceros* (Moslim et al. 2009). Entomopathogen, such as entomopathogenic fungi can be explored in soils (El-Ghany 2015). Certain entomopathogen species have adaptation capability in certain soils (Bugeme et al. 2008). Entomopathogen already adapted in freshwater swamp soils or highland soils generally has the specific advantage, i.e., it can adapt more effectively in this soil environment (Erler and Ates 2015). Exploration of entomopathogen starting from lowland to highland ecosystems will produce the high variation of species and genetics that can be utilized in the extended area and specific location. The purposes of this study were to explore and identify species and to determine inoculum potentials of the entomopathogenic fungi of soils of freshwater swamps, tidal lowlands, peatlands, and highlands in South Sumatra.

MATERIALS AND METHODS

Study area

Soil sampling was carried out in December 2017. The study sites were selected in the agricultural center with specific typology ecosystems in some locations of South Sumatra (Figure 1 and Table 1). The sites consisted of freshwater swamps, tidal lowlands, peatlands, and highlands. Those ecosystems chosen for explorations of entomopathogenic fungi aimed to obtain the fungi adapting in lowland and highland soils. The selected freshwater swamp locations were in Gandus and Musi Subdistricts of Palembang City; Rambutan Subdistrict of Banyuasin District, and Pemulutan Subdistrict of Ogan Ilir District. The selected locations of tidal lowlands were Mulya Sari, Telang Sari, Muara Sungsang Subdistricts of Banyuasin District. The selected peatland locations were in Talang Dabok, Sepucuk, and Kedaton Subdistricts of Ogan Komering Ilir District. The selected highland locations were in Lematang, Tanjung Payang, and Pulau Pinang of Lahat District, and Rimau and Talang Patai of Pagaralam City.

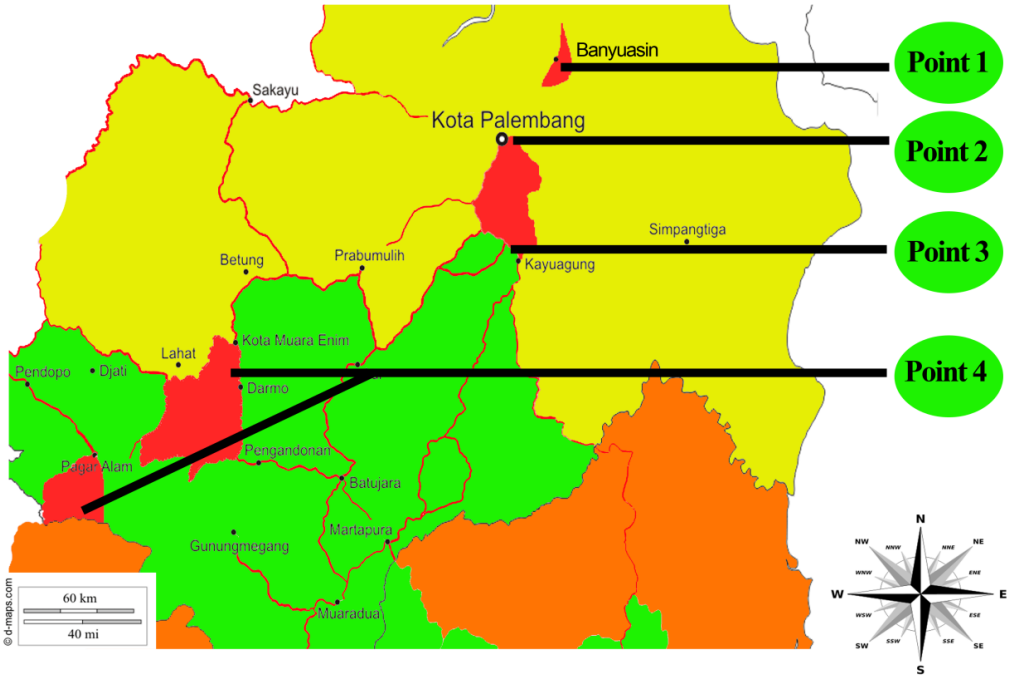

Figure 1. Locations of exploration for entomopathogenic fungi of soils in ecosystems of South Sumatra, Indonesia: point 1 = tidal lowlands (S 02°40.866' E 104°44.298'), point 2 = freshwater swamps (S 03°02.581' E 104°51.231'), point 3= peatlands (S 03°24.840' E 104°53.362'), and point 4 = highlands (S 03°48.063' E 103°32.072' and S 03°50.174' E 103°31.293')
Source: https://d-maps.com/index.php?lang=en

Fungal isolation, purification, and identification were conducted from January to March 2018 in the Laboratory of Entomology, Department of Plant Pest and Disease, College of Agriculture, Sriwijaya University, Indonesia. Supporting data were recorded in terms of soil sampling period, village or city names, coordinate points, and vegetation types or crop plants for each exploration locations (Figure 1 and Table 1). The pH of soil samples was measured by using a method of Kartika et al. (2018). In this survey, the application of pesticides in the sampling locations was also monitored.

Procedures

Observation of inoculum potentials of entomopathogenic fungi in the soil

Inoculum potential observation of the entomopathogenic fungi in this research was conducted by exploration of fungi in soils of the freshwater swamps, tidal lowlands, peatlands, and highlands. Entomopathogenic fungi exploration was carried out by modifying the methods of Anwar et al. (2015), i.e., using newly-moulting-third instar of *Tenebrio molitor* (Yellow mealworm beetle) as insect bait (*Tenebrio* bait method) which was infested on fungal inoculum of soil samples.

The soil samples were collected from some crop fields in the sampling locations (Table 1). Soils were taken from each location at the diagonal position. Soil samples of 5000 g were obtained by digging soil at the depth of 5–15 cm in the

vicinity of crop roots, and then they were put into the plastic pouch and labeled with the information of period of the sampling and crop plants.

Previously, soil samples were sieved by using 5-mesh siever to separate them from crop roots. Then, samples were put into plastic tray (size of 32 cm x 25 cm x 5 cm) and yielded 1000 g of finer soil sample. The finer soil sample was subsequently moisted with sterile aquadest until it obtained the soil moisture of 80–90% using the method of Chen et al. (2014). The number of larvae used per sampling location was 150 larvae (5 plastic trays each containing 30 larvae) (Table 1), so the total larvae per ecosystem type was as follows: in the freshwater swamps it was 1 800 (150 larvae x 12 sampling locations), in tidal lowlands it was 1 350 (150 larvae x 9 sampling locations), in peatlands it was 1 350 (150 larvae x 9 sampling locations), and in highlands it was 2 250 (150 larvae x 15 sampling locations).

The larvae were sterilized with 70% alcohol, and put on soil-sample surface in a plastic tray. The body of larvae was sprinkled using soil sample of about 5 mm thick. Subsequently, the plastic trays containing soil samples were covered with black cloth and sprayed with sterile aquadest to maintain humidity of soil samples. The larvae were incubated within soil sample for seven days to provide enough time for entomopathogenic fungi to infect them. Then, the dead larvae infected by the entomopathogenic fungi were recorded daily to determine inolum potential. The inoculum potentials of entomopathogenic fungi in this research were measured according to the percentage of infected *Tenebrio* bait or hosts (Hofgaard et al. 2016). The inoculum potential (IP) was calculated using the equation below:

$$\text{IP} = \frac{ib}{tb} \times 100$$

ib was the number of infected *Tenebrio* bait, and *tb* was the total number of *Tenebrio* baits

Table 1. Locations of exploration for entomopathogenic fungi in South Sumatra, Indonesia

Ecosystems	Village or city	Vegetation or crop plants	Height from sea level (m)
Freshwater swamps	Rambutan, Banyuasin	Paddy	15
	Gandus, Palembang	Paddy	12
	Musi Dua, Palembang	Paddy	16.67
	Pemulutan, Ogan Ilir	Paddy	22
Tidal lowlands	Mulya Sari, Banyuasin	Corn, paddy, watermelon	16.33
	Telang Sari, Banyuasin	Corn, coconut, corn + coconut	18.33
	Muara Sungsang, Banyuasin	Coconut, banana, pineapple	15
Peatlands	Talang Dabok, Ogan Komering Ilir	Palm, rubber, pineapple	24
	Sepucuk, Ogan Komering Ilir	Oil palm, rubber, pineapple	23
	Kedaton, Ogan Komering Ilir	Oil palm	19.67
Highlands	Talang Patai, Pagaralam	Cabbage	170
	Pulau Pinang, Lahat	Rubber + coffee	161
	Tanjung Payang, Lahat	Rubber	121
	Lematang, Lahat	Paddy	121.3
	Rimau, Pagaralam	Tea	1,326

Entomopathogen Isolation

The dead *Tenebrio* baits were subsequently isolated and purified by using the methods of Herlinda (2010). Entomopathogenic fungi infecting and growing on integument of *Tenebrio* bait were isolated and grown on medium of Sabouraud Dextrose Agar (SDA, Merck). The integument surface of larvae infected by the entomopathogenic fungi was previously sterilized using the modified method of Nuraini et al. (2017) with 1% natrium hypochlorite for 3 minutes and subsequently was rinsed three times with sterile aquadest and air dried on sterile filter paper. The larvae were put into a Petri dish containing sterile humid tissue paper and then incubated in order to stimulate the growth of entomopathogenic fungi. Conidia of entomopathogenic fungi emerging from the dead larvae body were taken by using sterile ose needle and moved into a Petri dish containing SDA medium, and incubated for seven days at the constant temperature of 25 ^{o}C within the incubator.

Identification of Entomopathogen Fungi

The purified fungi were identified according to macroscopic and microscopic characteristics using the method of Guilherme et al. (2015). Fungi growing on SDA medium with the area of 1 cm^2 were taken by using ose needle and put into preparations containing SDA medium, incubated for three days and then microscopically observed. Furthermore, fungi were identified by using books of Humber (2005) and El-Ghany (2015).

Data analysis

Data on inoculum potentials based on percentage of *Tenebrio* bait which was infected by the entomopathogenic fungi among the treatments were analyzed descriptively.

RESULTS AND DISCUSSION

Insect Characteristics Infected by Entomopathogenic Fungi

The fungi obtained in this study were identified as *B. bassiana* and *M. anisopliae*. There were only 30 isolates found from these two fungal species (Table 2). These 30 isolates were isolated from 223 infected larvae of *Tenebrio* bait by the entomopathogenic fungi (Table 3-7); however a lot of larvae were failed to be isolated due to contamination mostly by aerial fungi and *Trichoderma* spp. These 30 isolates consisted of nine isolates of *B. bassiana* and 21 isolates of *M. anisopliae***.**

Tenebrio bait infected by the entomopathogenic fungi showed macroscopic and microscopic characteristics or symptoms that could be used to confirm the determination of the entomopathogenic fungal species. Sick or dead *Tenebrio* bait infected by *B. bassiana* showed symptoms as follows: insect body was dry and wrinkle, its outer integument was coated by white mycelia similar to silk, rigid, easily broken and no smell (Figure 2). The *Tenebrio* bait attacked by *M. anisopliae* showed symptoms as follows: dry and wrinkle, no smell, brittle and easily broken, but its outer integument was coated by mycelia having greenish white to dark green or dark colour (Figure 3).

The pure isolate was obtained from dead *Tenebrio* bait body which was attacked by entomopathogenic fungi with colony characteristics for each species as follows; colony of *B. bassiana* had the white colour similar to cotton, but gradually its colour changed into yellowish white as fungi become older. Colony of *M. anisopliae* initially had white colour similar to colour of *B. bassiana*, but the colour changed into greenish and dark green or dark as fungi become older (Figure 4). Conidia of *B. bassiana* and *M. anisopliae* with specific characteristics were obtained from each colony species of entomopathogenic fungi. Conidia of *B. bassiana* had a single cell and a round shape, whereas conidia of *M. anisopliae* also had a single cell but with a cylindrical shape (Figure 5). Mycelia of *B. bassiana* and *M. anisopliae* insulated with upright, branches, and layers of conidiophores.

Inoculum Potentials of Entomopathogenic Fungi in the Soil of South Sumatra

Inoculum potentials of entomopathogenic fungi in this research were measured according to the percentage of infected *Tenebrio* bait. The inoculum potentials of entomopathogens fungi derived from the freshwater swamp soil, tidal lowlands, peat soils, and high land each was different among the survey locations (Tables 3−6). The value of inoculum potentialin soil of freshwater swamps, tidal lowlands, peatlands and high lands were in the range of 0.67−3.33%, 2.67−7.33%, 0−4%, and 1.33−9.33%, respectively.

From the freshwater swamp soils, we only could obtain five isolates of the entomopathogenic fungi isolated from 35 infected *tenebrio* baits (Table 3). A lot of the infected *Tenebrio* baits were failed to be isolated due to the contamination by other fungi. The inoculum potentials of the entomopathogenic fungi from the tidal lowlands gained were only 10 isolates (Table 4). The peat soils in sepucuk, Ogan Komering Ilir were planted with rubber had higher inoculum potentials (Table 5). The highland soils in Talang Patai, Pagaralam were planted with mustard also had higher inoculum potential (Table 6). The highest percentage of inoculum potentials of the fungi was found in the highland ecosystem and the lowest one was found in the freshwater swamp ecosystem. However, the highest number of isolates was found in highland ecosystem (11 isolates) and the lowest one found in peatland ecosystem (4 isolates) (Table 7).

Figure 2. *Tenebrio* bait infected by *Beauveria bassiana* (a) and healthy *Tenebrio* (b)

Figure 3. *Tenebrio* bait infected by *Metarhizium anisopliae* (a) and healthy *Tenebrio* (b)

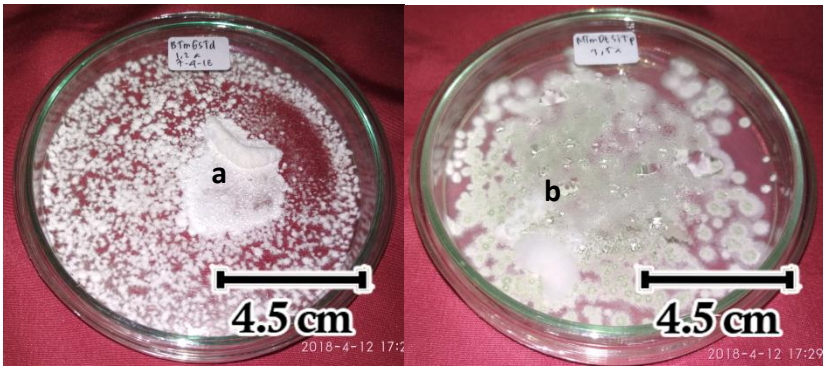

Figure 4. Colony of *Beauveria bassiana* (a) and *Metarhizium anisopliae* (b)

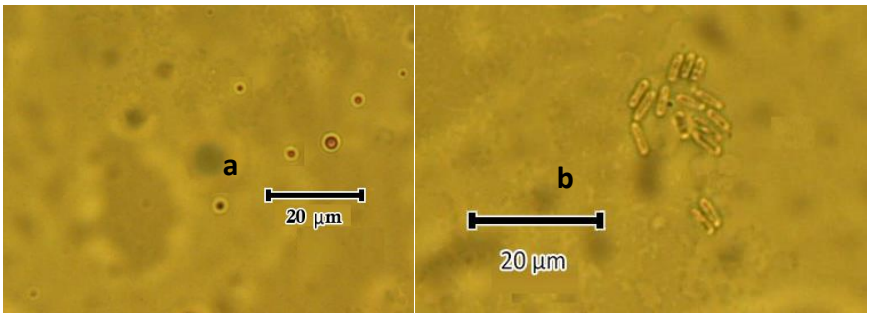

Figure 5. Conidia of *Beauveria bassiana* (a) and *Metarhizium anisopliae* (b) (400x magnification)

Table 2. Species and isolates of entomopathogenic fungi found in South Sumatra, Indonesia.

Ecosystems	**Species of fungi**	**Number of isolate**	**Vegetation or crop plants**	**Village or city**
Freshwater swamps	*M. anisopliae*	3	Paddy	Rambutan
Freshwater swamps	*M. anisopliae*	1	Paddy	Pemulutan
Freshwater swamps	*B. bassiana*	1	Paddy	Rambutan
Tidal lowlands	*M. anisopliae*	1	Corn + coconut	Telang Sari
Tidal lowlands	*M. anisopliae*	2	Corn	Telang Sari
Tidal lowlands	*M. anisopliae*	2	Corn	Mulya Sari
Tidal lowlands	*M. anisopliae*	3	Paddy	Mulya Sari
Tidal lowlands	*B. bassiana*	1	Corn	Telang Sari
Tidal lowlands	*B bassiana*	1	Watermelon	Mulya Sari
Peatlands	*B bassiana*	4	Oil palm	Talang Dabok
Highlands	*M. anisopliae*	1	Rubber + coffee	Pulau Pinang
Highlands	*M. anisopliae*	4	Cabbage	Talang Patai
Highlands	*M anisopliae*	4	Mustard	Talang Patai
Highlands	*B. bassiana*	1	Rubber + coffee	Pulau Pinang
Highlands	*B. bassiana*	1	Cabbage	Talang Patai

Table 3. Inoculum potentials of entomopathogenic fungi in the freshwater swamp soils of South Sumatra

Village or city	**Vegetation or crop**	**GPS (coordinat)**	**Inoculum potentials (%)**			
			Number of	***B. bassiana***	***M. anisoplia***	**Fungi**

	species		isolate			(Total)
Rambutan, Banyuasin	Paddy	S 03°02.581', E 104°51.231'	0	0.00 (0)	0.67 (1)	0.67 (1)
Rambutan, Banyuasin	Paddy	S 03°02.591', E 104°51.217'	2	0.67 (1)	2.00 (3)	2.67 (4)
Rambutan, Banyuasin	Paddy	S 03°02.586', E 104°51.201'	2	0.67 (1)	0.67 (1)	1.33 (2)
Gandus,Palembang	Paddy	S 03°00.401', E 104°42.380'	0	1.33 (2)	2.00 (3)	3.33 (5)
Gandus, Palembang	Paddy	S 03°00.632', E 104°42.532'	0	0.67 (1)	0.67 (1)	1.33 (2)
Gandus,Palembang	Paddy	S 03°00.632', E 104°42.801'	0	1.33 (2)	1.33 (2)	2.67 (4)
Musi Dua, Palembang	Paddy	S 03°02.120', E 104°43.021'	0	0.67 (1)	1.33 (2)	2.00 (3)
MusiDua, Palembang	Paddy	S 03°02.150', E 104°43.612'	0	1.33 (2)	2.00 (3)	3.33 (5)
Musi Dua, Palembang	Paddy	S 03°02.510', E 104°43.120'	0	1.33 (2)	1.33 (2)	2.67 (4)
Pemulutan, Ogan Ilir	Paddy	S 03°03.148', E 104°46.230'	0	0.67 (1)	0.67 (1)	1.33 (2)
Pemulutan, Ogan Ilir	Paddy	S 03°03.115', E 104°46.218'	0	0.67 (1)	0.67 (1)	1.33 (2)
Pemulutan, Ogan Ilir	Paddy	S 03°03.113', E 104°46.201'	1	1.33 (2)	1.33 (2)	2.67 (4)

Table 4. Inoculum potentials of entomopathogenic fungi in the tidal lowland soils of South Sumatra

Village or city	Vegetation or crop species	GPS (coordinat)	Inoculum potentials (%)			
			Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (Total)
Mulya Sari, Banyuasin	Corn	S 02°40.866', E 104°44.298'	2	1.33 (2)	2.67 (4)	4.00 (6)
Mulya Sari, Banyuasin	Paddy	S 02°40.944', E 104°44.621'	3	1.33 (2)	2.00 (3)	3.33 (5)
Mulya Sari, Banyuasin	Watermelon	S 02°40.896', E 104°44.676'	1	1.33(2)	2.00 (3)	3.33 (5)
Telang Sari, Banyuasin	Corn	S 02°38.842', E 104°45.369'	2	1.33 (2)	2.67 (4)	4.00 (6)
Telang Sari, Banyuasin	Coconut	S 02°38.813', E 104°45.801'	1	0.67 (1)	1.33 (2)	2.00 (3)
Telang Sari, Banyuasin	Corn + coconut	S 02°38.875', E 104°44.495'	1	2.00 (3)	5.33 (8)	7.33 (11)
Muara Sungsang, Banyuasin	Coconut	S 02°21.736', E 104°50.635'	0	1.33 (2)	4.00 (6)	5.33 (8)
Muara Sungsang, Banyuasin	Banana	S 02°21.823', E 104°50.632'	0	0.67 (1)	2.00 (3)	2.67 (4)
Muara Sungsang	Pineapple	S 02°22.542', E 104°50.324'	0	0.67 (1)	2.00 (3)	2.67 (4)

Table 5. Inoculum potentials of entomopathogenic fungi in the peatland soils of South Sumatra

Village or city	Vegetation or crop plants	GPS (coordinat)	Inoculum potentials (%)			
			Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (Total)
Talang Dabok, Ogan Komering Ilir	Oil palm	S 03°23.570', E 104°51.498'	4	3.33 (5)	1.33 (2)	4.67 (7)
Talang Dabok, Ogan Komering Ilir	Rubber	S 03°25.673', E 104°53.258'	0	2.67 (4)	1.33 (2)	4.00 (6)
Talang Dabok, Ogan Komering Ilir	Pineapple	S 03°25.280', E 104°52.940'	0	2.00 (3)	0.67 (1)	2.67 (4)
Sepucuk, Ogan Komering Ilir	Oil palm	S 03°24.840', E 104°53.362'	0	2.00 (3)	1.33 (2)	3.33 (5)
Sepucuk, Ogan Komering Ilir	Rubber	S 03°23.715', E 104°52.275'	0	3.33 (5)	2.00 (3)	5.33 (8)
Sepucuk, Ogan Komering Ilir	Pineapple	S 03°23.535', E 104°51.780'	0	1.33 (2)	1.33 (2)	2.67 (4)
Kedaton, Ogan Komering Ilir	Oil palm	S 03°23.308', E 104°51.487'	0	1.33 (2)	2.00 (3)	3.33 (5)
Kedaton, Ogan Komering Ilir	Oil palm	S 03°23.277', E 104°51.398'	0	1.33 (2)	0.67 (1)	2.00 (3)
Kedaton, Ogan Komering Ilir	Oil palm	S 03°23.204', E 104°51.459'	0	1.33 (2)	0.67 (1)	2.00 (3)

Table 6. Inoculum potentials of entomopathogenic fungi in the highland soils of South Sumatra

Village or city	Vegetation or crop plants	GPS (coordinat)	Inoculum potentials (%)			
			Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (Total)
Pulau Pinang, Lahat	Rubber + coffee (A)	S 03°48.819', E 103°32.677'	2	1.33 (2)	1.33 (2)	2.67 (4)
Pulau Pinang, Lahat	Rubber + coffee (B)	S 03°48.852', E 103°32.698'	0	1.33 (2)	2.00 (3)	3.33 (5)
Pulau Pinang, Lahat	Rubber + coffee (C)	S 03°48.883', E 103°32.688'	0	0.67 (1)	0.67 (1)	1.33 (2)
Tanjung Payang, Lahat	Rubber (A)	S 03°48.094', E 103°32.145'	0	0.67 (1)	1.33 (2)	2.00 (3)
Tanjung Payang, Lahat	Rubber (B)	S 03°48.122', E 103°32.162'	0	3.33 (5)	2.67 (4)	6.00 (9)
Tanjung Payang, Lahat	Rubber (C)	S 03°48.177', E 103°32.166'	0	2.00 (3)	2.67 (4)	4.67 (7)

Lematang, Lahat	Paddy	S 03°48.063', E 103°32.072'	0	0.67 (1)	1.33 (2)	2.00 (3)
Lematang, Lahat	Paddy	S 03°48.051', E 103°32.069'	0	3.33 (5)	4.00 (6)	7.33 (11)
Lematang, Lahat	Paddy	S 03°48.020', E 103°32.064'	0	1.33 (2)	1.33 (2)	2.67 (4)
Talang Patai, Pagaralam	Cabbage	S 03°50.180', E 103°31.325'	2	4.00 (6)	4.67 (7)	8.67 (13)
Talang Patai, Pagaralam	Cabbage	S 03°50.174', E 103°31.313'	3	0.67 (1)	1.33 (2)	2.00 (3)
Talang Patai, Pagaralam	Mustard	S 03°50.174', E 103°31.293'	4	4.67 (7)	4.67 (7)	9.33 (14)
Rimau, Pagaralam	Tea	S 04°02.161', E 103°10.484'	0	2.00 (3)	2.00 (3)	4.00 (6)
Rimau, Pagaralam	Tea	S 04°02.144', E 103°10.487'	0	1.33(2)	1.33(2)	2.67 (4)
Rimau, Pagaralam	Tea	S 04°02.136', E 103°10.485'	0	0.67 (1)	1.33(2)	2.00 (4)

Table 7. Inoculum potentials of entomopathogenic fungi in the soil of South Sumatra

Ecosystems	**Inoculum potentials (%)**			
	Number of isolate	***B. bassiana***	***M. anisoplia***	**Fungi (Total)**
Freshwater Swamps	5	0.89 (16)	1.22 (22)	2.11 (38)
Tidal Lowlands	10	1.18 (16)	2.67 (36)	3.85 (52)
Peatlands	4	2.07 (28)	1.25 (17)	3.33 (45)
Highlands	11	1.87 (42)	2.17 (49)	4.04 (91)

Discussion

Entomopathogenic species found in this research were *B. bassiana* and *M. anisopliae*. Macroscopic and microscopic characteristics of both entomopathogenic fungi found in this research matched the previous studies. Humber (2005) stated that mycelia of *B. bassiana* appeared from exoskeleton of hosts insect, and covered all parts of the exterior surface of host integument, so that host body has white color, reverse pale to yellow colony, hyaline or colorless, single cell, and globular and conidia as well as insulate hypha. *M. anisopliae* causes integument which has colors of whitish green to dark green because its mycelia cover exoskeleton of hosts insect; it has green to yellow conidia, single cell and cylindrical conidia as well as insulate hypha (Driver et al. 2000; Humber 2005).

Beauveria bassiana and *M. anisopliae* have parasitic and saprophytic phases during the killing process of their host insect (Augustyniuk-Kram and Kram 2012; El-Ghany 2015). Parasitic phase starts viz, the fungal conidia attach to the host insect cuticle, and then the conidia germinate on the host cuticle (El-Ghany 2015). The fungal penetration into the insect cuticle can be performed in producing specific infection hyphae originating at appressoria of the fungus (Fernandes et al. 2007; El-Ghany 2015). Gürlek et al. (2018) reported that both species could produce germ tubes growing over the surface of the insect cuticle until the tubes contacted weakness area of cuticle where penetration could easily be achieved. After the fungus successfully penetrateds, then mycelia distributed into the hemolymph by the formation of blastospores (El-Ghany 2015). Finally, the host insect would die within four days of penetration (Gürlek et al. 2018). Saprophytic phase starts viz, the fungus grew mechanically in the dead insect body, retrieved nutrients from the insect body, and then the fungus produceds toxins (El-Ghany 2015).

The success of both fungi in conducting the process of parasitic and saprophytic phases was affected by several external factors such as moisture, pH, temperature, ultraviolet (UV) radiation, and vegetation (El-Ghany 2015). This research showed that highland soil planted with cabbage and mustard in Talang Patai, Pagaralam had more inoculum potentials of entomopathogenic fungi than other locations because cabbage and mustard were inhabited by insect pests dominated by Lepidoptera, such as *Plutella xylostella*, *Crocidolomia binotalis*, *Spodoptera litura*, and *Chrysodeixis chalcites*; while the larvae of Lepidoptera were the most suitable hosts for entomopathogic fungi (Godonou et al. 2009; Nunilahwati et al. 2012). This study also found that a lot of infected larvae of the insects pests hung above of the mustard and cabbage canopy. The host insects attacked by entomopathogenic fungi on the cabbage canopy at highlands, South Sumatra were generally dominated by *P. xylostella*, *S. litura*, and *C. chalcites.* The symptoms of sick or infected insects larvae were dry and in the stiff condition, white or greenish white in color and attached on the upper surface of cabbage leaves. The infected larvae insects which showed the symptoms of dry and stiff condition as mummy and covered with white fungal mycelia were insects which were attacked by *B. bassiana*, whereas insect body covered by fungal mycelia having greenish white or dark green color were symptoms of insects attacked by *M. anisopliae.* Symptoms of insects attacked by *B. bassiana* and *M. anisopliae* in this research matched to the symptoms reported by El-Ghany (2015) and Mora et al. (2017).

In this research, more inoculum potentials of the entomopathogenic fungi were found in the highlands and tidal lowlands than that in freshwater swamps and peatlands because it was affected by soil pH and moisture. Zhong et al.

(2010) reported that soil pH had more significant role in determining the existence of fungal propagules within soils than that of soil texture and organic matter. However, Inubushi et al. (2003) had stated that soil moisture is one of the most important controlling factors for biological reactions in the soil. Soil pH in this research was in the range of 4 to 4.5 in freshwater swamps, 4.3 to 5 in tidal lowlands, 3.60 to 4 in peatlands, and 5 to 6.7 in highlands. Kodir and Juwita (2016) stated that the pH value of soil in freshwater swamps in Indonesia are in the ranges of 4 to 4.5, pH 4.17 to 5.35 in tidal lowlands (Marlina *et al.* 2016), and 3.60 to 3.95 in peatlands (Utami *et al.* 2009), and 5 to 6 in highlands (Supriadi *et al.* 2016). The variation of pH values of soils at each location could affect the adaptation capability of entomopathogenic fungi surviving (Bugeme *et al.* 2008). The conidial viability of entomopathogenic fungi, such as *B. bassiana* and *M. anisopliae* were affected by pH of in-vitro medium for entomopathogenic fungi. Rizkie et al. (2017) reported that high acidity (pH < 4) of in-vitro medium for fungus growing significantly decrease conidial viability of *B. bassiana* and *M. anisopliae*. Therefore, inoculum potentials of the entomopathogenic fungi from peatland soil and freshwater swamp soil were lower than that from the soil in the tidal lowlands and highlands.

In addition to soil pH and moisture, soil texture also determines the existence and distribution of fungal propagules. Soil texture has low clay content, and sandy soil texture tends to have the low capability in maintaining the existence of fungal propagules (El-Ghany 2015). Water saturated soil also tend to have low capability in maintaining the existence of fungal propagules (Garrido-Jurado et al. 2011). Soils from freshwater swamps and peatlands in South Sumatra had lower clay content and in water saturated condition (Marlina et al. 2016; Kartika et al. 2018). The low existence of fungal propagules finding at freshwater swamp and peatland soils was due to both factors. Soils in freshwater swamps are water saturated for more than 6 to 7 months per year usually occurred from November to April (Herlinda et al. 2018) and soil moisture in the peatlands reach 500% (Maftu'ah and Susanti 2009).

Peatlands have soil pore saturated with water all year long resulting in the anaerobic condition of soil. Peat soil has no clay, sand, and silt content, but it has organic matter (Sudana 2005). High organic matter finally can decrease the pH of soil (Utami et al. 2009). Rizkie et al. (2017) confirmed that pH < 4 within medium in-vitro for growing fungi could decrease the ability to live of fungal propagules. In highland ecosystems, the portion of the soil texture among clay, sand, and silt fractions was found in the same composition or balance (Utomo et al. 2013; Supriadi et al. 2016). While tidal lowlands contain silt from sedimentation mixture of river water and seawater with balance clay and sand fractions (Marlina et al. 2016). Higher inoculum potentials in highlands and tidal lowlands in this research was due to balance or higher of clay texture and organic soils than that of freshwater swamps and peatlands.

The balance or higher of clay texture and organic soils are capable of maintaining the existence of fungal propagules (Garrido-Jurado et al. 2011). Zhong et al. (2010) stated that soil in ecosystems which apply composted manure or no synthetic fertilizer had higher propagules content of *B. bassiana* than that of soil in the ecosystem which applies synthetic fertilizers. Also, application of synthetic pesticides is capable of decreasing the existence of entomopathogenic fungi within the soil (Mietkiewski et al. 2010). Local farmers in freshwater swamp and peatland areas of South Sumatra usually do not apply synthetic pesticides, whereas many local farmers in tidal lowland and highland areas apply synthetic pesticides (Herlinda et al. 2018). Although no synthetic pesticides were applied in the freshwater swamp and peatland areas, fungal propagules or inoculum potentials in these areas were lower than those of tidal lowlands and highlands areas. In this study there was no evidence that having no synthetic pesticide application in freshwater swamp and peatland areas can cause high existence of fungal propagules. However, soil pH and soil texture have more effect on the existence of propagules of *B. bassiana* and *M. anisopliae* in lowland swamp and peatland ecosystems.

Higher inoculum potentials in highlands and tidal lowlands in this research were closely related to specific cultivated vegetations or crops. Most peatlands in Indonesia were utilized for forestry and conservation areas (Suriadikarta and Sutriadi 2007). Paddy generally is cultivated at freshwater swamps (Herlinda et al. 2018; Lakitan, et al. 2018a; Lakitan, et al. 2018b) as well as in tidal lowlands; however, paddy cultivation at tidal lowlands was more intensive (with two to three planting indices) than that of freshwater swamps (one planting index). Thus, diversity species of vegetation or crop plants could affect abundant and diverse species of the soil microorganisms associated with plant and crop roots (El-Ghany 2015).

This research found two species of entomopatogenic fungi from soils in South Sumatra i.e., *B. bassiana* and *M. anisopliae.* The highest percentage of the inoculum potentials and prevalence of both fungi occurred in the highland ecosystems and the lowest percentage of the inoculum potentials of the fungi was found in the peatland ecosystems. In highland ecosystems, percentage of the inoculum potentials was affected by the locations and the vegetation or the crop plants. These fungi will make an important contribution to the biological control for insect pests from lowland to highland ecosystems in Indonesia.

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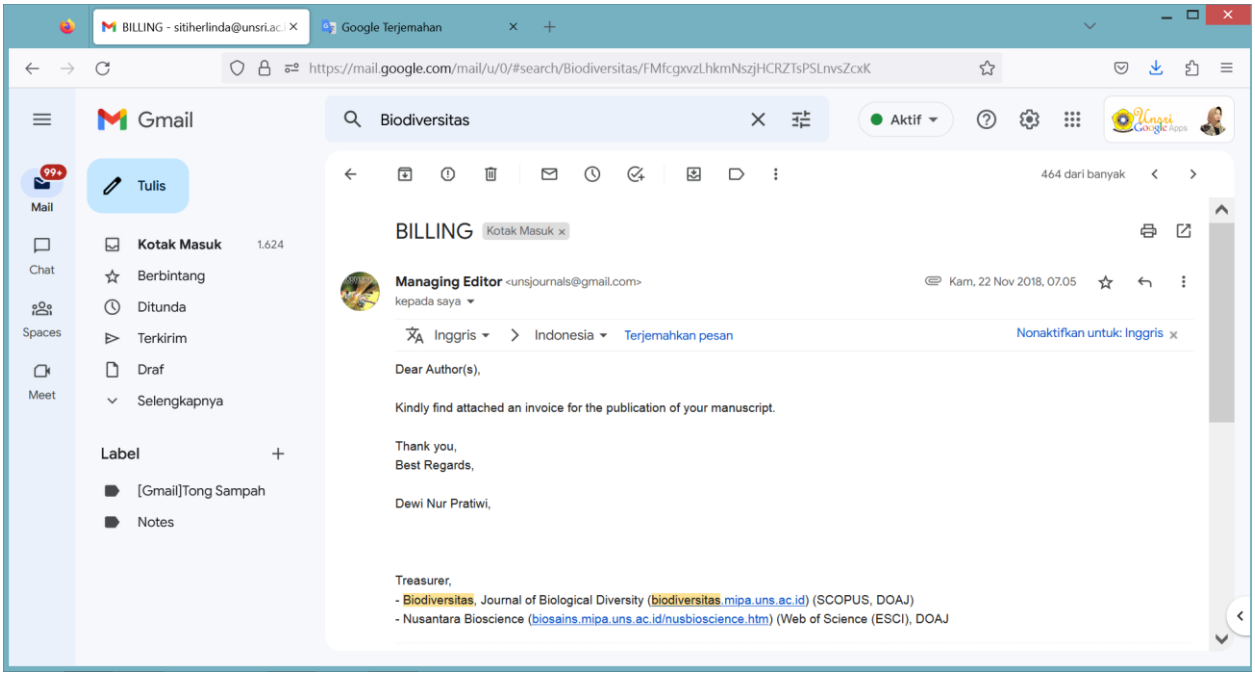

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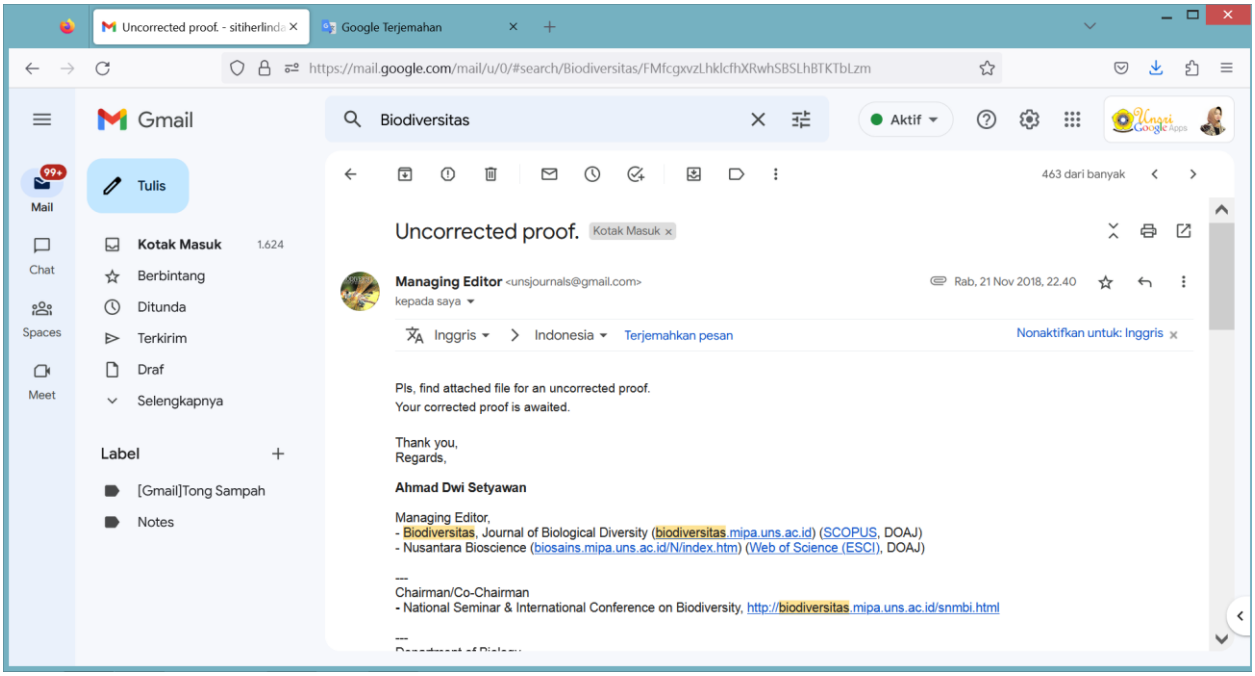
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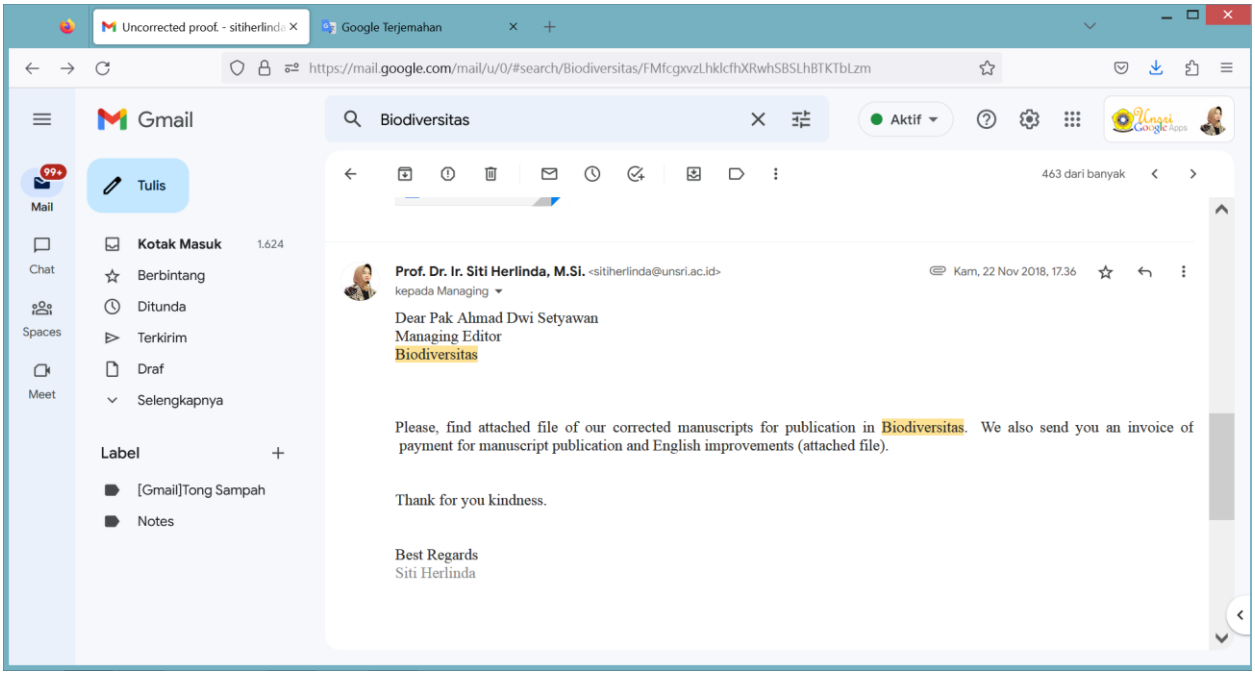
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Entomopathogenic fungi of soils of freshwater swamps, tidal lowlands, peatlands, and highlands of South Sumatra, Indonesia

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Abstract. *Safitri A, Herlinda S, Setiawan A. 2018. Entomopathogenic fungi of soils of freshwater swamps, tidal lowlands, peatlands, and highlands of South Sumatra, Indonesia. Biodiversitas 19: xxxx.* Ecosystems of lowlands and highlands in South Sumatra have specific characteristics of soils and vegetations that can affect the availability of entomopathogenic fungi. This study aimed to explore and identify species and to determine inoculum potentials of the entomopathogenic fungi from soils of freshwater swamps, tidal lowlands, peatlands, and highlands. Baiting of entomopathogenic fungi on soil samples used the larvae of *Tenebrio molitor*. The entomopathogenic fungi species found in this research were *Beauveria bassiana* and *Metarhizium anisopliae*. The number of the fungal isolates were 30 isolates consisting of nine isolates of *B. bassiana* and 21 isolates of *M. anisopliae*.The highest number of isolates was found in the highland ecosystem (11 isolates) and the lowest was found in peatland ecosystem (4 isolates). The highest percentage average of inoculum potentials of the fungi was found in the high land ecosystem (4.04%) and the lowest one was found in freshwater swamps ecosystem (2.11%). Based on the vegetation type, the soil planted with mustard in Talang Patai-Pagaralam (highland ecosystem) had the highest inoculum potentials (9.33%). These fungi will make an important contribution to the biological control for insect pests in lowland to highland ecosystems in Indonesia.

Keywords: *Beauveria bassiana*, lowlands, *Metarhizium anisopliae*, peat soils, wetlands

INTRODUCTION

Many parts of lowlands and highlands of Indonesia are used for agriculture. Lowlands consisting of freshwater swamps, tidal lowlands, and peatlands are the ecosystems having water saturated condition for the whole year (Sudana 2005). The lowland ecosystems for agricultural purposes in Indonesia are distributed in Sumatra, Kalimantan, and Papua Islands covering 11 million ha of tidal lowlands, comprising 9.2 million ha of freshwater swamps, and 14.9 million ha of peatlands, respectively (Mulyani and Sarwani 2013). While the highland ecosystems managed for agriculture with the area of 16.15 million ha are distributed all over Indonesia islands (Center for Agriculture Data and Information System, Secretariat General 2017).

Soils between lowlands and highlands in Indonesia have different characteristics, especially in the moisture, texture, and acidity (pH). The specific characteristics of lowland and highland soils at the four typologies of ecosystems consist of the freshwater swamp, tidal lowlands, peatlands, and highland closely related to specific cultivated vegetations or crops. Paddy usually is cultivated at freshwater swamps (Herlinda et al. 2018; Lakitan et al. 2018a; Lakitan et al. 2018b). There is only a small part of soils in peatlands that can be cultivated with seasonal crops and most peatlands in Indonesia are utilized for forestry and conservation areas (Suriadikarta and Sutriadi 2007). Highlands area usually planted with various types of seasonal and annual crops. Specific vegetation or crop plants could affect the soil microorganisms associated with plant and crop roots (El-Ghany 2015).

Microorganisms such as fungi are found in freshwater swamps and highlands of South Sumatra and can be used to control insect pest called entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* (Herlinda et al. 2008; 2010). The entomopathogenic fungi are proven to be an effective agent to control some insect pests (Chinniah et al. 2016), and they are not harmful toward natural enemies of insect pests (Gholamzadeh-Chitgar et al. 2017). However, there is no complete information for fungi of tidal lowlands and peatlands in that region. *B. bassiana* and *M. anisopliae* were found to kill insect pests such as *Crocidolomia pavonana* (Hasyim *et al.* 2009), *Aphis gossypii* (Herlinda 2010), *Plutella xylostella* (Loc and Chi 2007), *Lygus* spp. (Leland et al. 2005), and *Oryctes rhinoceros* (Moslim et al. 2009). Entomopathogen, such as entomopathogenic fungi can be explored in soils (El-Ghany 2015). Certain entomopathogen species have adaptation capability in certain soils (Bugeme et al. 2008). Entomopathogen already adapted in freshwater swamp soils or highland soils generally has the specific advantage, i.e., it can adapt more effectively in this soil environment (Erler and Ates 2015). Exploration of entomopathogen starting from lowland to highland ecosystems will produce the high variation of species and genetics that can be utilized in the extended area and specific location. The purposes of this study were to explore and identify species and to determine inoculum potentials of the entomopathogenic fungi of soils of freshwater swamps, tidal lowlands, peatlands, and highlands in South Sumatra.

MATERIALS AND METHODS

Study area

Soil sampling was carried out in December 2017. The study sites were selected in the agricultural center with specific typology ecosystems in some locations of South Sumatra (Figure 1 and Table 1). The sites consisted of freshwater swamps, tidal lowlands, peatlands, and highlands. Those ecosystems chosen for explorations of entomopathogenic fungi aimed to obtain the fungi adapting in lowland and highland soils. The selected freshwater swamp locations were in Gandus and Musi Subdistricts of Palembang City; Rambutan Subdistrict of Banyuasin District, and Pemulutan Subdistrict of Ogan Ilir District. The selected locations of tidal lowlands were Mulya Sari, Telang Sari, Muara Sungsang Subdistricts of Banyuasin District. The selected peatland locations were in Talang Dabok, Sepucuk, and Kedaton Subdistricts of Ogan Komering Ilir District. The selected highland locations were in Lematang, Tanjung Payang, and Pulau Pinang of Lahat District, and Rimau and Talang Patai of Pagaralam City.

Fungal isolation, purification, and identification were conducted from January to March 2018 in the Laboratory of Entomology, Department of Plant Pest and Disease, College of Agriculture, Sriwijaya University, Indonesia. Supporting data were recorded in terms of soil sampling period, village or city names, coordinate points, and vegetation types or crop plants for each exploration locations (Figure 1 and Table 1). The pH of soil samples was measured by using a method of Kartika et al. (2018). In this survey, the application of pesticides in the sampling locations was also monitored.

Procedures

Observation of inoculum potentials of entomopathogenic fungi in the soil

Inoculum potential observation of the entomopathogenic fungi in this research was conducted by exploration of fungi in soils of the freshwater swamps, tidal lowlands, peatlands, and highlands. Entomopathogenic fungi exploration was carried out by modifying the methods of Anwar et al. (2015), i.e., using newly-moulting-third instar of *Tenebrio molitor* (Yellow mealworm beetle) as insect bait (*Tenebrio* bait method) which was infested on fungal inoculum of soil samples.

The soil samples were collected from some crop fields in the sampling locations (Table 1). Soils were taken from each location at the diagonal position. Soil samples of 5000 g were obtained by digging soil at the depth of 5-15 cm in the vicinity of crop roots, and then they were put into the plastic pouch and labeled with the information of period of the sampling and crop plants.

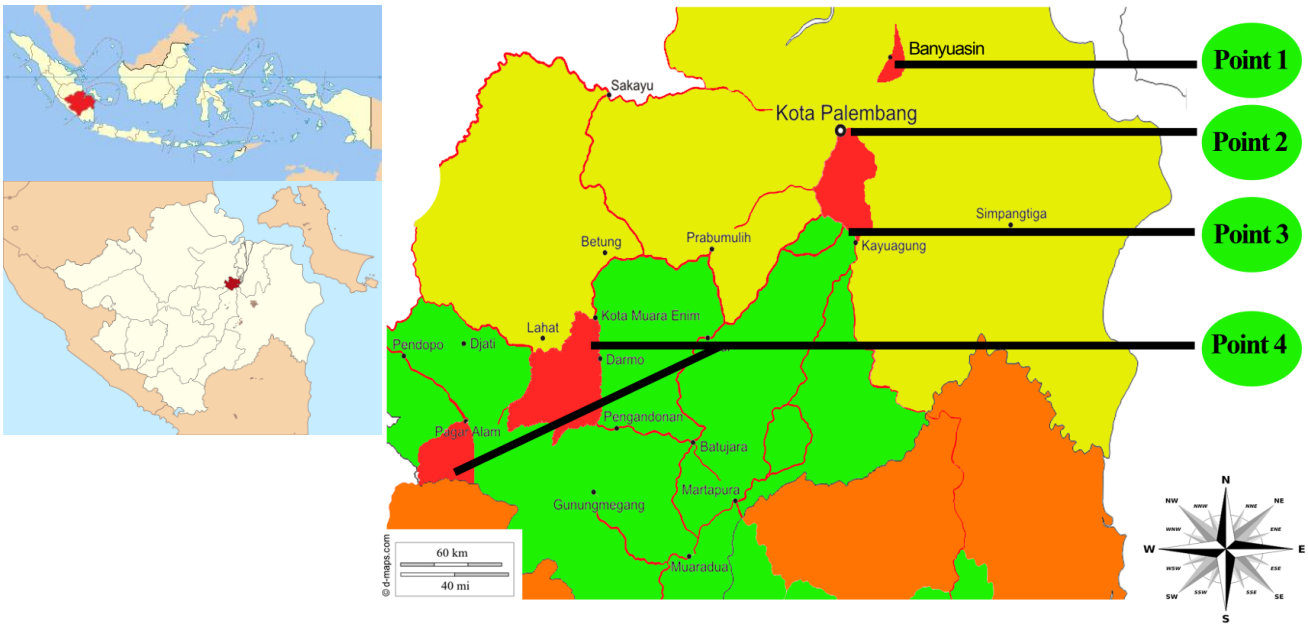


Figure 1. Locations of exploration for entomopathogenic fungi of soils in ecosystems of South Sumatra, Indonesia: point 1 = tidal lowlands (S 02°40.866’ E 104°44.298’), point 2 = freshwater swamps (S 03°02.581' E 104°51.231'), point 3= peatlands (S 03°24.840’ E 104°53.362’), and point 4 = highlands (S 03°48.063’ E 103°32.072’ and S 03°50.174’ E 103°31.293’)

Table 1. Locations of exploration for entomopathogenic fungi in South Sumatra, Indonesia

Ecosystems	Village or city	Vegetation or crop plants	Height from sea level (m)

Freshwater swamps	Rambutan, Banyuasin	Paddy	15
	Gandus, Palembang	Paddy	12
	Musi Dua, Palembang	Paddy	16.67
	Pemulutan, Ogan Ilir	Paddy	22
Tidal lowlands	Mulya Sari, Banyuasin	Corn, paddy, watermelon	16.33
	Telang Sari, Banyuasin	Corn, coconut, corn + coconut	18.33
	Muara Sungsang, Banyuasin	Coconut, banana, pineapple	15
Peatlands	Talang Dabok, Ogan Komering Ilir	Palm, rubber, pineapple	24
	Sepucuk, Ogan Komering Ilir	Oil palm, rubber, pineapple	23
	Kedaton, Ogan Komering Ilir	Oil palm	19.67
Highlands	Talang Patai, Pagaralam	Cabbage	170
	Pulau Pinang, Lahat	Rubber + coffee	161
	Tanjung Payang, Lahat	Rubber	121
	Lematang, Lahat	Paddy	121.3
	Rimau, Pagaralam	Tea	1,326

Previously, soil samples were sieved by using 5-mesh siever to separate them from crop roots. Then, samples were put into plastic tray (size of 32 cm x 25 cm x 5 cm) and yielded 1000 g of finer soil sample. The finer soil samples were subsequently moisted with sterile aquadest until they obtained the soil moisture of 80-90% using the method of Chen et al. (2014). The number of larvae used per sampling location was 150 larvae (5 plastic trays each containing 30 larvae) (Table 1), so the total larvae per ecosystem type was as follows: in the freshwater swamps it was 1,800 (150 larvae x 12 sampling locations), in tidal lowlands it was 1,350 (150 larvae x 9 sampling locations), in peatlands it was 1,350 (150 larvae x 9 sampling locations), and in highlands it was 2,250 (150 larvae x 15 sampling locations).

The larvae were sterilized with 70% alcohol, and put on soil-sample surface in a plastic tray. The body of larvae was sprinkled using soil sample of about 5 mm thick. Subsequently, the plastic trays containing soil samples were covered with black cloth and sprayed with sterile aquadest to maintain humidity of soil samples. The larvae were incubated within soil sample for seven days to provide enough time for entomopathogenic fungi to infect them. Then, the dead larvae infected by the entomopathogenic fungi were recorded daily to determine inolum potential. The inoculum potentials of entomopathogenic fungi in this research were measured according to the percentage of infected *Tenebrio* bait or hosts (Hofgaard et al. 2016). The inoculum potential (IP) was calculated using the equation below:

$$\mathrm{IP} = \frac{ib}{tb} \times 100$$

ib was the number of infected *Tenebrio* bait, and *tb* was the total number of *Tenebrio* baits.

Entomopathogen isolation

The dead *Tenebrio* baits were subsequently isolated and purified by using the methods of Herlinda (2010). Entomopathogenic fungi infecting and growing on integument of *Tenebrio* bait were isolated and grown on medium of Sabouraud Dextrose Agar (SDA, Merck). The integument surface of larvae infected by the entomopathogenic fungi was previously sterilized using the modified method of Nuraini et al. (2017) with 1% natrium hypochlorite for 3 minutes and subsequently was rinsed three times with sterile aquadest and air dried on sterile filter paper. The larvae were put into a Petri dish containing sterile humid tissue paper and then incubated in order to stimulate the growth of entomopathogenic fungi. Conidia of entomopathogenic fungi emerging from the dead larvae body were taken by using sterile ose needle and moved into a Petri dish containing SDA medium, and incubated for seven days at the constant temperature of 25 ^{o}C within the incubator.

Identification of Entomopathogen Fungi

The purified fungi were identified according to macroscopic and microscopic characteristics using the method of Guilherme et al. (2015). Fungi growing on SDA medium with the area of 1 cm^2 were taken by using ose needle and put into preparations containing SDA medium, incubated for three days and then microscopically observed. Furthermore, fungi were identified by using books of Humber (2005) and El-Ghany (2015).

Data analysis

Data on inoculum potentials based on percentage of *Tenebrio* bait which was infected by the entomopathogenic fungi among the treatments were analyzed descriptively.

RESULTS AND DISCUSSION

Insect characteristics infected by entomopathogenic fungi

The fungi obtained in this study were identified as *B. bassiana* and *M. anisopliae*. There were only 30 isolates found from these two fungal species (Table 2). These 30 isolates were isolated from 223 infected larvae of *Tenebrio* bait by the entomopathogenic fungi (Table 3-7); however a lot of larvae were failed to be isolated due to contamination mostly by aerial fungi and *Trichoderma* spp. These 30 isolates consisted of nine isolates of *B. bassiana* and 21 isolates of *M. anisopliae***.**

Tenebrio bait infected by the entomopathogenic fungi showed macroscopic and microscopic characteristics or symptoms that could be used to confirm the determination of the entomopathogenic fungal species. Sick or dead *Tenebrio* bait infected by *B. bassiana* showed symptoms as follows: insect body was dry and wrinkle, its outer integument was coated by white mycelia similar to silk, rigid, easily broken and no smell (Figure 2). The *Tenebrio* bait attacked by *M. anisopliae* showed symptoms as follows: dry and wrinkle, no smell, brittle and easily broken, but its outer integument was coated by mycelia having greenish white to dark green or dark color (Figure 3).

The pure isolate was obtained from dead *Tenebrio* bait body which was attacked by entomopathogenic fungi with colony characteristics for each species as follows; colony of *B. bassiana* had the white color similar to cotton, but gradually its color changed into yellowish white as fungi become older. Colony of *M. anisopliae* initially had white color similar to color of *B. bassiana*, but the color changed into greenish and dark green or dark as fungi become older (Figure 4). Conidia of *B. bassiana* and *M. anisopliae* with specific characteristics were obtained from each colony species of entomopathogenic fungi. Conidia of *B. bassiana* has a single cell and a round shape, whereas conidia of *M. anisopliae* also has a single cell but with a cylindrical shape (Figure 5). Mycelia of *B. bassiana* and *M. anisopliae* insulate with upright, branches, and layers of conidiophores.

Inoculum potentials of entomopathogenic fungi in the soil of South Sumatra

Inoculum potentials of entomopathogenic fungi in this research were measured according to the percentage of infected *Tenebrio* bait. The inoculum potentials of entomopathogens fungi derived from the freshwater swamp soil, tidal lowlands, peat soils, and high land each was different among the survey locations (Tables 3-6). The value of inoculum potential in soil of freshwater swamps, tidal lowlands, peatlands and high lands were in the range of 0.67-3.33%, 2.67-7.33%, 0-4%, and 1.33-9.33%, respectively.

From the freshwater swamp soils, we only could obtain five isolates of the entomopathogenic fungi isolated from 35 infected *tenebrio* baits (Table 3). A lot of the infected *Tenebrio* baits were failed to be isolated due to the contamination by other fungi. The inoculum potentials of the entomopathogenic fungi from the tidal lowlands gained were only 10 isolates (Table 4). The peat soils in sepucuk, Ogan Komering Ilir were planted with rubber had higher inoculum potentials (Table 5). The highland soils in Talang Patai, Pagaralam were planted with mustard also had higher inoculum potential (Table 6). The highest percentage of inoculum potentials of the fungi was found in the highland ecosystem and the lowest one was found in the freshwater swamp ecosystem. However, the highest number of isolates was found in highland ecosystem (11 isolates) and the lowest one found in peatland ecosystem (4 isolates) (Table 7).

A B

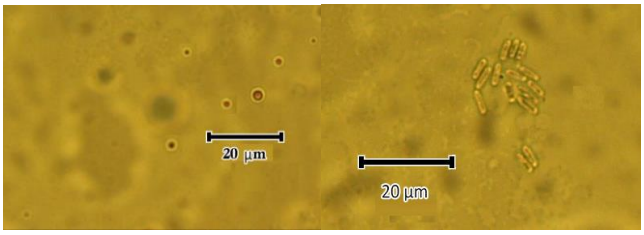
Figure 2. *Tenebrio* bait infected by *Beauveria bassiana* (A) and healthy *Tenebrio* (B)

A B

Figure 3. *Tenebrio* bait infected by *Metarhizium anisopliae* (A) and healthy *Tenebrio* (B)

A B

Figure 4. Colony of *Beauveria bassiana* (A) and *Metarhizium anisopliae* (B)



A B

Figure 5. Conidia of *Beauveria bassiana* (A) and *Metarhizium anisopliae* (B) (400x magnification)

Table 2. Species and isolates of entomopathogenic fungi found in South Sumatra, Indonesia

Ecosystems	Species of fungi	Number of isolate	Vegetation or crop plants	Village or city
Freshwater swamps	*M. anisopliae*	3	Paddy	Rambutan
Freshwater swamps	*M. anisopliae*	1	Paddy	Pemulutan
Freshwater swamps	*B. bassiana*	1	Paddy	Rambutan
Tidal lowlands	*M. anisopliae*	1	Corn + coconut	Telang Sari
Tidal lowlands	*M. anisopliae*	2	Corn	Telang Sari
Tidal lowlands	*M. anisopliae*	2	Corn	Mulya Sari
Tidal lowlands	*M. anisopliae*	3	Paddy	Mulya Sari
Tidal lowlands	*B. bassiana*	1	Corn	Telang Sari
Tidal lowlands	*B bassiana*	1	Watermelon	Mulya Sari
Peatlands	*B bassiana*	4	Oil palm	Talang Dabok
Highlands	*M. anisopliae*	1	Rubber + coffee	Pulau Pinang
Highlands	*M. anisopliae*	4	Cabbage	Talang Patai
Highlands	*M anisopliae*	4	Mustard	Talang Patai
Highlands	*B. bassiana*	1	Rubber + coffee	Pulau Pinang
Highlands	*B. bassiana*	1	Cabbage	Talang Patai

Table 3. Inoculum potentials of entomopathogenic fungi in the freshwater swamp soils of South Sumatra, Indonesia

Village or city	Vegetation or crop species	GPS (coordinat)	Inoculum potentials (%)			
			Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (Total)
Rambutan, Banyuasin	Paddy	S 03°02.581', E 104°51.231'	0	0.00 (0)	0.67 (1)	0.67 (1)
Rambutan, Banyuasin	Paddy	S 03°02.591', E 104°51.217'	2	0.67 (1)	2.00 (3)	2.67 (4)
Rambutan, Banyuasin	Paddy	S 03°02.586', E 104°51.201'	2	0.67 (1)	0.67 (1)	1.33 (2)
Gandus,Palembang	Paddy	S 03°00.401', E 104°42.380'	0	1.33 (2)	2.00 (3)	3.33 (5)
Gandus, Palembang	Paddy	S 03°00.632', E 104°42.532'	0	0.67 (1)	0.67 (1)	1.33 (2)
Gandus,Palembang	Paddy	S 03°00.632', E 104°42.801'	0	1.33 (2)	1.33 (2)	2.67 (4)
Musi Dua, Palembang	Paddy	S 03°02.120', E 104°43.021'	0	0.67 (1)	1.33 (2)	2.00 (3)
MusiDua, Palembang	Paddy	S 03°02.150', E 104°43.612'	0	1.33 (2)	2.00 (3)	3.33 (5)
Musi Dua, Palembang	Paddy	S 03°02.510', E 104°43.120'	0	1.33 (2)	1.33 (2)	2.67 (4)
Pemulutan, Ogan Ilir	Paddy	S 03°03.148', E 104°46.230'	0	0.67 (1)	0.67 (1)	1.33 (2)
Pemulutan, Ogan Ilir	Paddy	S 03°03.115', E 104°46.218'	0	0.67 (1)	0.67 (1)	1.33 (2)
Pemulutan, Ogan Ilir	Paddy	S 03°03.113', E 104°46.201'	1	1.33 (2)	1.33 (2)	2.67 (4)

Table 4. Inoculum potentials of entomopathogenic fungi in the tidal lowland soils of South Sumatra, Indonesia

Village or city	Vegetation or crop species	GPS (coordinat)	Inoculum potentials (%)			
			Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (total)
Mulya Sari, Banyuasin	Corn	S 02°40.866', E 104°44.298'	2	1.33 (2)	2.67 (4)	4.00 (6)
Mulya Sari, Banyuasin	Paddy	S 02°40.944', E 104°44.621'	3	1.33 (2)	2.00 (3)	3.33 (5)
Mulya Sari, Banyuasin	Watermelon	S 02°40.896', E 104°44.676'	1	1.33(2)	2.00 (3)	3.33 (5)
Telang Sari, Banyuasin	Corn	S 02°38.842', E 104°45.369'	2	1.33 (2)	2.67 (4)	4.00 (6)
Telang Sari, Banyuasin	Coconut	S 02°38.813', E 104°45.801'	1	0.67 (1)	1.33 (2)	2.00 (3)
Telang Sari, Banyuasin	Corn + coconut	S 02°38.875', E 104°44.495'	1	2.00 (3)	5.33 (8)	7.33 (11)
Muara Sungsang, Banyuasin	Coconut	S 02°21.736', E 104°50.635'	0	1.33 (2)	4.00 (6)	5.33 (8)
Muara Sungsang, Banyuasin	Banana	S 02°21.823', E 104°50.632'	0	0.67 (1)	2.00 (3)	2.67 (4)
Muara Sungsang	Pineapple	S 02°22.542', E 104°50.324'	0	0.67 (1)	2.00 (3)	2.67 (4)

Table 5. Inoculum potentials of entomopathogenic fungi in the peatland soils of South Sumatra, Indonesia

Village or city	Vegetation or crop plants	GPS (coordinat)	Inoculum potentials (%)			
			Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (Total)
Talang Dabok, Ogan Komering Ilir	Oil palm	S 03°23.570', E 104°51.498'	4	3.33 (5)	1.33 (2)	4.67 (7)
Talang Dabok, Ogan Komering Ilir	Rubber	S 03°25.673', E 104°53.258'	0	2.67 (4)	1.33 (2)	4.00 (6)
Talang Dabok, Ogan Komering Ilir	Pineapple	S 03°25.280', E 104°52.940'	0	2.00 (3)	0.67 (1)	2.67 (4)
Sepucuk, Ogan Komering Ilir	Oil palm	S 03°24.840', E 104°53.362'	0	2.00 (3)	1.33 (2)	3.33 (5)
Sepucuk, Ogan Komering Ilir	Rubber	S 03°23.715', E 104°52.275'	0	3.33 (5)	2.00 (3)	5.33 (8)
Sepucuk, Ogan Komering Ilir	Pineapple	S 03°23.535', E 104°51.780'	0	1.33 (2)	1.33 (2)	2.67 (4)
Kedaton, Ogan Komering Ilir	Oil palm	S 03°23.308', E 104°51.487'	0	1.33 (2)	2.00 (3)	3.33 (5)
Kedaton, Ogan Komering Ilir	Oil palm	S 03°23.277', E 104°51.398'	0	1.33 (2)	0.67 (1)	2.00 (3)
Kedaton, Ogan Komering Ilir	Oil palm	S 03°23.204', E 104°51.459'	0	1.33 (2)	0.67 (1)	2.00 (3)

Table 6. Inoculum potentials of entomopathogenic fungi in the highland soils of South Sumatra, Indonesia

Village or city	Vegetation or crop plants	GPS (coordinat)	Inoculum potentials (%)			
			Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (total)
Pulau Pinang, Lahat	Rubber + coffee (A)	S 03°48.819', E 103°32.677'	2	1.33 (2)	1.33 (2)	2.67 (4)
Pulau Pinang, Lahat	Rubber + coffee (B)	S 03°48.852', E 103°32.698'	0	1.33 (2)	2.00 (3)	3.33 (5)
Pulau Pinang, Lahat	Rubber + coffee (C)	S 03°48.883', E 103°32.688'	0	0.67 (1)	0.67 (1)	1.33 (2)
Tanjung Payang, Lahat	Rubber (A)	S 03°48.094', E 103°32.145'	0	0.67 (1)	1.33 (2)	2.00 (3)
Tanjung Payang, Lahat	Rubber (B)	S 03°48.122', E 103°32.162'	0	3.33 (5)	2.67 (4)	6.00 (9)
Tanjung Payang, Lahat	Rubber (C)	S 03°48.177', E 103°32.166'	0	2.00 (3)	2.67 (4)	4.67 (7)
Lematang, Lahat	Paddy	S 03°48.063', E 103°32.072'	0	0.67 (1)	1.33 (2)	2.00 (3)
Lematang, Lahat	Paddy	S 03°48.051', E 103°32.069'	0	3.33 (5)	4.00 (6)	7.33 (11)
Lematang, Lahat	Paddy	S 03°48.020', E 103°32.064'	0	1.33 (2)	1.33 (2)	2.67 (4)
Talang Patai, Pagaralam	Cabbage	S 03°50.180', E 103°31.325'	2	4.00 (6)	4.67 (7)	8.67 (13)
Talang Patai, Pagaralam	Cabbage	S 03°50.174', E 103°31.313'	3	0.67 (1)	1.33 (2)	2.00 (3)
Talang Patai, Pagaralam	Mustard	S 03°50.174', E 103°31.293'	4	4.67 (7)	4.67 (7)	9.33 (14)
Rimau, Pagaralam	Tea	S 04°02.161', E 103°10.484'	0	2.00 (3)	2.00 (3)	4.00 (6)
Rimau, Pagaralam	Tea	S 04°02.144', E 103°10.487'	0	1.33(2)	1.33(2)	2.67 (4)
Rimau, Pagaralam	Tea	S 04°02.136', E 103°10.485'	0	0.67 (1)	1.33(2)	2.00 (4)

Table 7. Inoculum potentials of entomopathogenic fungi in the soil of South Sumatra, Indonesia

Ecosystems	Inoculum potentials (%)			
	Number of isolate	*B. bassiana*	*M. anisoplia*	Fungi (total)
Freshwater Swamps	5	0.89 (16)	1.22 (22)	2.11 (38)
Tidal Lowlands	10	1.18 (16)	2.67 (36)	3.85 (52)
Peatlands	4	2.07 (28)	1.25 (17)	3.33 (45)
Highlands	11	1.87 (42)	2.17 (49)	4.04 (91)

Discussion

Entomopathogenic species found in this research were *B. bassiana* and *M. anisopliae*. Macroscopic and microscopic characteristics of both entomopathogenic fungi found in this research matched with the previous studies. Humber (2005) stated that mycelia of *B. bassiana* appeared from exoskeleton of hosts insect, and covered all parts of the exterior surface of host integument, so that host body has white color, reverse pale to yellow colony, hyaline or colorless, single cell, and globular and conidia as well as insulate hypha. *M. anisopliae* causes integument which has colors of whitish green to dark green because its mycelia cover exoskeleton of hosts insect; it has green to yellow conidia, single cell and cylindrical conidia as well as insulate hypha (Driver et al. 2000; Humber 2005).

Beauveria bassiana and *M. anisopliae* have parasitic and saprophytic phases during the killing process of their host insect (Augustyniuk-Kram and Kram 2012; El-Ghany 2015). Parasitic phase starts viz, the fungal conidia attach to the host insect cuticle, and then the conidia germinate on the host cuticle (El-Ghany 2015). The fungal penetration into the insect cuticle can be performed in producing specific infection hyphae originating at appressoria of the fungus (Fernandes et al. 2007; El-Ghany 2015). Gürlek et al. (2018) reported that both species could produce germ tubes growing over the surface of the insect cuticle until the tubes contacted weakness area of cuticle where penetration could easily be achieved. After the fungus successfully penetrated, then mycelia distributed into the hemolymph by the formation of blastospores (El-Ghany 2015). Finally, the host insect would die within four days of penetration (Gürlek et al. 2018). Saprophytic phase starts viz, the fungus grew mechanically in the dead insect body, retrieved nutrients from the insect body, and then the fungus produced toxins (El-Ghany 2015).

The success of both fungi in conducting the process of parasitic and saprophytic phases was affected by several external factors such as moisture, pH, temperature, ultraviolet (UV) radiation, and vegetation (El-Ghany 2015). This research showed that highland soil planted with cabbage and mustard in Talang Patai, Pagaralam had more inoculum potentials of entomopathogenic fungi than other locations because cabbage and mustard were inhabited by insect pests dominated by Lepidoptera, such as *Plutella xylostella*, *Crocidolomia binotalis*, *Spodoptera litura*, and *Chrysodeixis chalcites*; while the larvae of Lepidoptera were the most suitable hosts for entomopathogenic fungi (Godonou et al. 2009; Nunilahwati et al. 2012). This study also found that a lot of infected larvae of the insect pests hung above of the mustard and cabbage canopy. The host insects attacked by entomopathogenic fungi on the cabbage canopy at highlands, South Sumatra were generally dominated by *P. xylostella*, *S. litura,* and *C. chalcites.* The symptoms of sick or infected insect larvae were dry and in the stiff condition, white or greenish white in color and attached on the upper surface of cabbage leaves. The infected larvae which showed the symptoms of dry and stiff condition as mummy and covered with white fungal mycelia was larvae attacked by *B. bassiana*, whereas larvae body covered by fungal mycelia having greenish white or dark green color was symptoms of larvae attacked by *M. anisopliae.* Symptoms of larvae attacked by *B. bassiana* and *M. anisopliae* in this research matched to the symptoms reported by El-Ghany (2015) and Mora et al. (2017).

In this research, more inoculum potentials of the entomopathogenic fungi were found in the highlands and tidal lowlands than that in freshwater swamps and peatlands because it was affected by soil pH and moisture. Zhong et al. (2010) reported that soil pH had a more significant role in determining the existence of fungal propagules within soils than that of soil texture and organic matter. However, Inubushi et al. (2003) had stated that soil moisture is one of the most important controlling factors for biological reactions in the soil. Soil pH in this research was in the range of 4 to 4.5 in freshwater swamps, 4.3 to 5 in tidal lowlands, 3.60 to 4 in peatlands, and 5 to 6.7 in highlands. Kodir and Juwita (2016) stated that the pH value of soil in freshwater swamps in Indonesia are in the ranges of 4 to 4.5, pH 4.17 to 5.35 in tidal lowlands (Marlina *et al.* 2016), and 3.60 to 3.95 in peatlands (Utami *et al.* 2009), and 5 to 6 in highlands (Supriadi *et al.* 2016). The variation of pH values of soils at each location could affect the adaptation capability of entomopathogenic fungi surviving (Bugeme *et al*. 2008). The conidial viability of entomopathogenic fungi, such as *B. bassiana* and *M. anisopliae* were affected by pH of in-vitro medium for entomopathogenic fungi. Rizkie et al. (2017) reported that high acidity (pH < 4) of in-vitro medium for fungus growing significantly decrease the conidial viability of *B. bassiana* and *M. anisopliae*. Therefore, inoculum potentials of the entomopathogenic fungi from peatland soil and freshwater swamp soil were lower than that from the soil in the tidal lowlands and highlands.

In addition to soil pH and moisture, soil texture also determines the existence and distribution of fungal propagules. Soil texture has low clay content, and sandy soil texture tends to have the low capability in maintaining the existence of fungal propagules (El-Ghany 2015) as well as the water saturated soil (Garrido-Jurado et al. 2011). Soils from freshwater swamps and peatlands in South Sumatra had lower clay content and in water saturated condition (Marlina et al. 2016; Kartika et al. 2018). The low existence of fungal propagules finding at freshwater swamp and peatland soils was due to both factors. Soils in freshwater swamps are water saturated for more than 6 to 7 months per year usually occurred from November to April (Herlinda et al. 2018) and soil moisture in the peatlands reach 500% (Maftu'ah and Susanti 2009).

Peatlands have soil pore saturated with water all year long resulting in the anaerobic condition of the soil. Peat soil has no clay, sand, and silt content, but it has organic matter (Sudana 2005). The high organic matter finally can decrease the pH of the soil (Utami et al. 2009). Rizkie et al. (2017) confirmed that pH < 4 within medium in-vitro for growing fungi could decrease the ability to live of fungal propagules. In highland ecosystems, the portion of the soil texture among clay, sand, and silt fractions was found in the same composition or balance (Utomo et al. 2013; Supriadi et al. 2016). While tidal lowlands contain silt from sedimentation mixture of river water and seawater with balance clay and sand

fractions (Marlina et al. 2016). Higher inoculum potentials in highlands and tidal lowlands in this research was due to balance or higher of clay texture and organic soils than that of freshwater swamps and peatlands.

The balance or higher of clay texture and organic soils are capable of maintaining the existence of fungal propagules (Garrido-Jurado et al. 2011). Zhong et al. (2010) stated that soil in ecosystems which apply composted manure or no synthetic fertilizer had higher propagules content of *B. bassiana* than that of soil in the ecosystem which applies synthetic fertilizers. Also, the application of synthetic pesticides is capable of decreasing the existence of entomopathogenic fungi within the soil (Mietkiewski et al. 2010). Local farmers in freshwater swamp and peatland areas of South Sumatra usually do not apply synthetic pesticides, whereas many local farmers in tidal lowland and highland areas apply synthetic pesticides (Herlinda et al. 2018). Although no synthetic pesticides were applied in the freshwater swamp and peatland areas, fungal propagules or inoculum potentials in these areas were lower than those of tidal lowlands and highlands areas. In this study, there was no evidence that having no synthetic pesticide application in freshwater swamp and peatland areas can cause the high existence of fungal propagules. However, soil pH and soil texture have more effect on the existence of propagules of *B. bassiana* and *M. anisopliae* in lowland swamp and peatland ecosystems.

Higher inoculum potentials in highlands and tidal lowlands in this research were closely related to specific cultivated vegetations or crops. Most peatlands in Indonesia were utilized for forestry and conservation areas (Suriadikarta and Sutriadi 2007). Paddy generally is cultivated at freshwater swamps (Herlinda et al. 2018; Lakitan et al. 2018a; Lakitan et al. 2018b) as well as in tidal lowlands; however, paddy cultivation at tidal lowlands was more intensive (with two to three planting indices) than that of freshwater swamps (one planting index). Thus, diverse species of vegetation or crop plants could affect abundant and diverse species of the soil microorganisms associated with plant and crop roots (El-Ghany 2015).

This research found two species of entomopathogenic fungi from soils in South Sumatra i.e., *B. bassiana* and *M. anisopliae*. The highest percentage of the inoculum potentials and prevalence of both fungi occurred in the highland ecosystems and the lowest percentage of the inoculum potentials of the fungi was found in the peatland ecosystems. In highland ecosystems, the percentage of the inoculum potentials was affected by the locations and the vegetation or the crop plants. These fungi will make an important contribution to the biological control for insect pests from lowland to highland ecosystems in Indonesia.

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