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Occurrence of soil-inhabiting entomopathogenic fungi within a conventional and organic farm and their virulence against *Spodoptera litura*

AMINUDIN AFANDHI♥, FERY ABDUL CHOLIQ, ITO FERNANDO, YOSEP MINAR ALBERT NANDUS MARPAUNG, YOGO SETIAWAN

Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia.
Tel.: +62-341-575843; ♥emails: aaf_fp@ub.ac.id

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Abstract. *Afandhi A, Choliq FA, Fernando I, Marpaung YMAN, Setiawan Y. 2022. Occurrence of soil-inhabiting entomopathogenic fungi within a conventional and organic farm and their virulence against* Spodoptera litura. *Biodiversitas 23: 1172-1180.* Naturally occurring entomopathogenic fungi (EPF) are important components in agroecosystems as they serve as biocontrol agents of insect and mite pests. However, some cultivation practices may have deleterious effects on EPF. In this study, the occurrence of soil-inhabiting EPF was investigated between a conventional and organic farm. EPF was baited using *Tenebrio molitor* larvae, and their virulence was tested against *Spodoptera litura* larvae. The results showed a higher occurrence of EPF in the organic farm than the conventional farm, with *Aspergillus* sp., *Beauveria* sp., and *Gliocladium* sp. were exclusively found in organic soils. Among the twenty-five EPF isolates obtained, only four isolates were avirulent against *S. litura* larvae. Isolates belonging to *Beauveria*, *Metarhizium*, and *Paecilomyces* genera caused high mortality of *S. litura* larvae ranging from 40 to 65%. There was a significant positive relationship between the conidial viability of EPF and larval mortality. Since virulent isolates were found in conventional soils, efforts in preserving EPF prevalence are needed through the implementation of appropriate cultivation practices. The synthetic agrochemicals exclusion, organic fertilizers application, and crop rotation practiced in the organic farm should be integrated into any other agroecosystems as a form of conservation biological control strategies to strengthen the pest control service provided by EPF.

Keywords: Biocontrol agents, conidial viability, conservation biological control, cultivation practices, pest control service

INTRODUCTION

Since the 1960s, entomopathogenic fungi (EPF) have been viewed as excellent biocontrol agents in the regulation of phytophagous insect and mite populations in agroecosystems (Li et al. 2010; Nelly et al. 2019; Khun et al. 2020; Islam et al. 2021). The pest control efficacy of various EPF species has been reported in Hypocreales (Ascomycota), but not limited to *Beauveria* (Mascarin and Jaronski 2016; Harith-Fadzilah et al. 2021), *Metarhizium* (Aw and Hue 2017; Brunner-Mendoza et al. 2019), and *Paecilomyces* (Moreno-gavíra et al. 2020). As a result, numerous EPF species are already commercialized as mycopesticides worldwide (Goettel et al. 2010; Dara et al. 2019; Islam et al. 2021). Moreover, a plethora of research has also revealed the ability of EPF in promoting crop performance and crop resistance against abiotic and biotic stressors by colonizing rhizospheres and plant tissues (Sammaritano et al. 2018; Afandhi et al. 2019; Dara 2019; Bamisile et al. 2021). EPF also contribute to maintaining agroecosystem function and health as they partake in nutrient cycling (Ghaley et al. 2014). These multitude benefits from EPF highlight their importance for eco-friendly and sustainable agroecosystems (Sharma et al. 2021). It is important to preserve the prevalence of naturally occurring EPF in agroecosystems by understanding farming systems that may encourage or impede their proliferation (Clifton et al. 2015).

Soil is considered to be the main reservoir of EPF. These fungi inhabit soil for a significant duration of their life cycle when outside their hosts or crops are absent (Quesada-Moraga et al. 2007; Medo and Cagáň 2011). Consequently, exogenous disturbances in the like of cultivation practices may have effects on EPF. It is evident that conventional farms, which are characterized by excessive application of agrochemicals, significantly reduce the abundance and diversity of EPF in soil (Klingen et al. 2002; Tkaczuk et al. 2014). In contrast, organically farmed soil is considered more suitable habitat for EPF. Hence, organic farms may harbor greater occurrence and diversity of EPF compared to conventional farms (Clifton et al. 2015; Ramos et al. 2017).

Increased public awareness of the harmful effects of agrochemicals on the environment and human health has led to a progressive conversion from conventional to organic farms (Reganold and Wachter 2016; Mie et al. 2017). One of the integral parts of organically managed farms is the total exclusion of synthetic agrochemicals. As a result, pest management programs depend entirely on natural control, one of which is the action of natural enemies, including EPF. Therefore, the aims of this study were (i) to elucidate whether or not an organic farm has a higher occurrence of soil-inhabiting EPF than a

conventional farm; and (ii) to assess the virulence of the isolated EPF against insect pests by using larvae of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) as the target organism. Knowledge of these aspects is essential when indigenous EPF is expected to provide a pest control service in agroecosystems. The findings of this study may also assist in identifying cultivation practices that positively affect the persistence of EPF in soils and those that are not, so that could be integrated into a conservation biological control program.

MATERIALS AND METHODS

Study area and collection of soil samples

Sampling sites were located in Batu, East Java, Indonesia, a city laid in high latitude zones with a significant part of its area is intended for agricultural fields of vegetables and fruit trees. The annual rainfall, temperature, and humidity in Batu were approximately 102 mm, 22°C, and 94%, respectively, based on data maintained by the Meteorological, Climatological, and Geophysical Agency of Malang, Indonesia.

Two vegetable farms were sampled in Sumber Brantas village, Bumiaji districts (07°44'23.4"S, 112°31'05.2"E; 07°44'34.6"S, 112°31'59.0"E), with broccoli (*Brassica oleracea* var. *italica* L. [Brassicales: Brassicaceae]) as the main crop. One of the farms was certified as an organic farm, while the other was a conventional farm. Three sampling plots (approximately 20 m × 20 m) were made within each farm. After that, five sampling units were established using a diagonal pattern in each sampling plot. After removing surface litter, 500 g soil at 10-15 cm depth was collected using a garden spade from each sampling unit. The garden spade was sterilized with 85% ethanol after being used to hinder any possible cross-contamination of EPF among the sampling sites (Clifton et al. 2015; Puspitarini et al. 2021a). Soil samples were put in a plastic bag and brought to the laboratory. A total of 30 soil samples were collected as the source of EPF.

Fungal isolation

EPF was isolated by exposing the late instar larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) to the sampled soils (Sharma et al. 2018a). Before being used, soil samples were sieved to remove debris with a 2 mm mesh sieve. The soil from each sampling unit was moistened to field capacity and put into a plastic container (16 cm height; 12 cm diameter). Twenty larvae of *T. molitor* were then introduced into each container. The containers were perforated laterally for soil aeration (Ali-Shtayeh et al. 2002) and incubated at 27 ± 2°C in complete darkness for two weeks. Containers were inverted daily to allow the larvae came into maximum contact with the soil. Dead larvae (cadavers) were inspected every second day and immediately taken from containers.

The obtained cadavers were surface-sterilized in 1% v/v NaOCl (sodium hypochlorite) for three minutes, followed by three consecutive washes with sterile distilled water for one minute each (Puspitarini et al. 2021a). Each cadaver was then placed in a 9 cm diameter Petri dish lined with moistened filter paper to maintain a humid environment for stimulating the growth and sporulation of EPF (Clifton et al. 2015). The Petri dish was then tightly sealed with parafilm and left in dark condition for one to two weeks.

Fungal identification

A small portion of fungal mycelia excised from insect cadaver was inoculated into a sabouraud dextrose agar with yeast (SDAY) medium supplemented with 1% w/v chloramphenicol. All cultures were then incubated for seven days at the same environmental condition described previously. The pure fungal isolates were identified morphologically using taxonomic keys (Barnett and Hunter 1987; Domsch et al. 2007). The morphological characteristics of sporulation structures of each isolate were observed with light microscopy at 400x magnification. Moreover, all isolates were subjected to Koch's postulates to ensure their infectivity.

Conidia production and conidial viability assay

Initially, each isolate was sub-cultured by placing upside-down a 5 mm diameter disc of un-sporulated mycelium in the center of a new SDAY medium. Five culture plates were prepared for each isolate as replications. After the sub-cultures were incubated for two weeks, conidia were harvested by scraping the surface of colonies with an inoculation needle. The obtained conidia were suspended with 10 mL of sterile distilled water containing 0.02% v/v Tween 80 in a falcon tube. The tube was then centrifuged for 5 minutes at 3,000 rpm. The supernatant was removed, and the obtained pellet was mixed with 5 mL of sterile distilled water. The conidial concentration of the suspension was then determined using a hemocytometer (Puspitarini et al. 2021b).

After being left overnight, remaining conidial suspensions were used to assess the spore germination (conidial viability). An aliquot of 0.1 mL of each suspension was dropped into a microscope slide and covered with a slip. The percentage of conidial viability was then determined by counting the germinated and non-germinated spores under a light microscope. The number of observed spores for each replicate was at least 100 spores (Ali-Shtayeh et al. 2002).

Fungal virulency assay

This assay was conducted to evaluate the virulence of each isolate against second instar larvae of the common cutworm *S. litura*. The larvae were obtained from the Indonesian Sweetener and Fibre Crops Research Institute, Malang, Indonesia. The bio-assay described by Anand and Tiwary (2009) was followed with slight modifications. Summarily, the conidial suspension concentration of each isolate was standardized to 10^6 conidia mL^{-1}. Each treatment (isolate) was replicated five times, and each replication used twenty larvae. All larvae were initially surface-sterilized with 0.5% v/v NaOCl and rinsed three times with sterile distilled water. The larvae were then dried with sterile filter paper. Subsequently, the larvae were dipped into each conidial suspension for 1 minute and

placed into a plastic cup (7.4 cm height; 7.2 cm top diameter; 5.3 bottom diameter). Larvae in control groups were treated with sterile distilled water containing 0.02% v/v Tween 80. The larvae were regularly fed with surface-sterilized castor (*Ricinus communis* L. [Malpighiales: Euphorbiaceae]) leaves to avoid the infection from phylloplane EPF. The number of dead larvae was recorded daily for seven days and the cumulative mortality was expressed in percent. Larvae with a mycelial mass growing in their cuticle were assumed to have died due to fungal infection. Experiments with a control mortality rate of higher than 5% were discarded and repeated.

Field history

A standardized set of questions regarding cultivation practices applied in the studied farms was asked to the farmers. The question included the types of fertilizers and pesticides used, the implemented cropping system, and how the tillage and weeding were carried out.

Data analysis

Based on the Shapiro-Wilk normality test, data on the conidia production, conidial viability, and mortality of *S. litura* larvae had a normal distribution. The data were then subjected to analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). The data on mortality of *S. litura* were not corrected by Abbott's formula as the larval mortality in the control groups was less than 5% (WHO 2016; Puspitarini et al. 2021b). In addition, Pearson's correlation was used to determine the relationship between conidial viability and mortality of *S. litura* larvae. In all analyses, the difference among the data was considered significant for at least $P < 0.05$. All analyses were performed using R statistics (R Core Team 2020).

RESULTS AND DISCUSSION

Occurrence of entomopathogenic fungi soils

The organic farm had a relatively higher occurrence of soil-inhabiting EPF than the conventional farm. A total of 25 soil-inhabiting EPF isolates were successfully purified, of which 16 isolates were from organic soils, while 9 isolates were from conventional soils (Table 1). The fungal isolates were able to be distinguished among each other based on their colony and conidial structure characteristics (Figure 1-2). The genus *Aspergillus*, a non-hypocrealean EPF, had three isolates; the genus *Beauveria* had two isolates; the genus *Fusarium* had nine isolates; the genus *Gliocladium* had one isolate; the genus *Metarhizium* had eight isolates; and lastly, the genus *Paecilomyces* had two isolates. In this study, the genus *Aspergillus*, *Beauveria*, and *Gliocladium* were exclusively found in organic soils, while *Fusarium* and *Metarhizium* were the richest genera in both studied farms.

Table 1. Entomopathogenic fungi obtained from organic and conventional farm soils

Farming systems	Fungal genera	Fungal isolates
Organic	*Aspergillus*	*Aspergillus* sp.1
		Aspergillus sp.2
		Aspergillus sp.3
	Beauveria	*Beauveria* sp.1
		Beauveria sp.2
	Fusarium	*Fusarium* sp.1
		Fusarium sp.2
		Fusarium sp.3
		Fusarium sp.4
		Fusarium sp.5
	Gliocladium	*Gliocladium* sp.
	Metarhizium	*Metarhizium* sp.1
		Metarhizium sp.2
		Metarhizium sp.3
		Metarhizium sp.4
	Paecilomyces	*Paecilomyces* sp.1
Conventional	*Fusarium*	*Fusarium* sp.6
		Fusarium sp.7
		Fusarium sp.8
		Fusarium sp.9
	Metarhizium	*Metarhizium* sp.5
		Metarhizium sp.6
		Metarhizium sp.7
		Metarhizium sp.8
	Paecilomyces	*Paecilomyces* sp.2

Table 2. Conidia production and conidial viability of entomopathogenic fungi obtained from organic and conventional farm soils at seven days after inoculation in a SDAY medium

Farming systems	Fungal isolates	Conidia amount (10^6 conidia mL^{-1})	Conidial viability (%)
Organic	*Aspergillus* sp.1	2.45 ± 0.08 u	9.00 ± 0.08 q
	Aspergillus sp.2	11.00 ± 0.10 j	28.87 ± 0.24 j
	Aspergillus sp.3	3.75 ± 0.04 r	20.19 ± 0.28 m
	Beauveria sp.1	13.85 ± 0.23g	36.54 ± 0.29 f
	Beauveria sp.2	7.77 ± 0.04 n	29.01 ± 0.16 j
	Fusarium sp.1	8.15 ± 0.11 m	28.93 ± 0.22 j
	Fusarium sp.2	5.85 ± 0.08 p	29.10 ± 0.09 j
	Fusarium sp.3	24.65 ± 0.13 b	35.71 ± 0.12 g
	Fusarium sp.4	2.90 ± 0.12 t	11.08 ± 0.07 p
	Fusarium sp.5	12.65 ± 0.13 i	28.18 ± 0.08 k
	Gliocladium sp.	7.90±0.09 mn	20.39 ± 0.24 m
	Metarhizium sp.1	11.00 ± 0.11 j	40.30 ± 0.29 e
	Metarhizium sp.2	8.85 ± 0.08 k	39.74 ± 0.27 e
	Metarhizium sp.3	6.75 ± 0.09 o	27.01 ± 0.18 l
	Metarhizium sp.4	17.82 ± 0.14 f	52.07 ± 0.39 a
	Paecilomyces sp.1	5.30 ± 0.09 q	27.26 ± 0.12 l
Conventional	*Fusarium* sp.6	8.50 ± 0.09 l	12.25 ± 0.18 o
	Fusarium sp.7	22.31 ± 0.08 d	41.23 ± 0.20 d
	Fusarium sp.8	22.71 ± 0.05 c	31.04 ± 0.22 i
	Fusarium sp.9	1.70 ± 0.07 v	4.02 ± 0.16 r
	Metarhizium sp.5	3.60 ± 0.07 rs	31.14 ± 0.15 i
	Metarhizium sp.6	3.35 ± 0.06 s	16.77 ± 0.17 n
	Metarhizium sp.7	13.40 ± 0.06 h	34.13 ± 0.28 h
	Metarhizium sp.8	20.50 ± 0.10 e	42.46 ± 0.17 c
	Paecilomyces sp.2	43.06 ± 0.09 a	47.52 ± 0.10 b

Note: Means followed by the same letters within each column are not significantly different at $P < 0.05$ according to DMRT. Mean ± SE of five replicates.

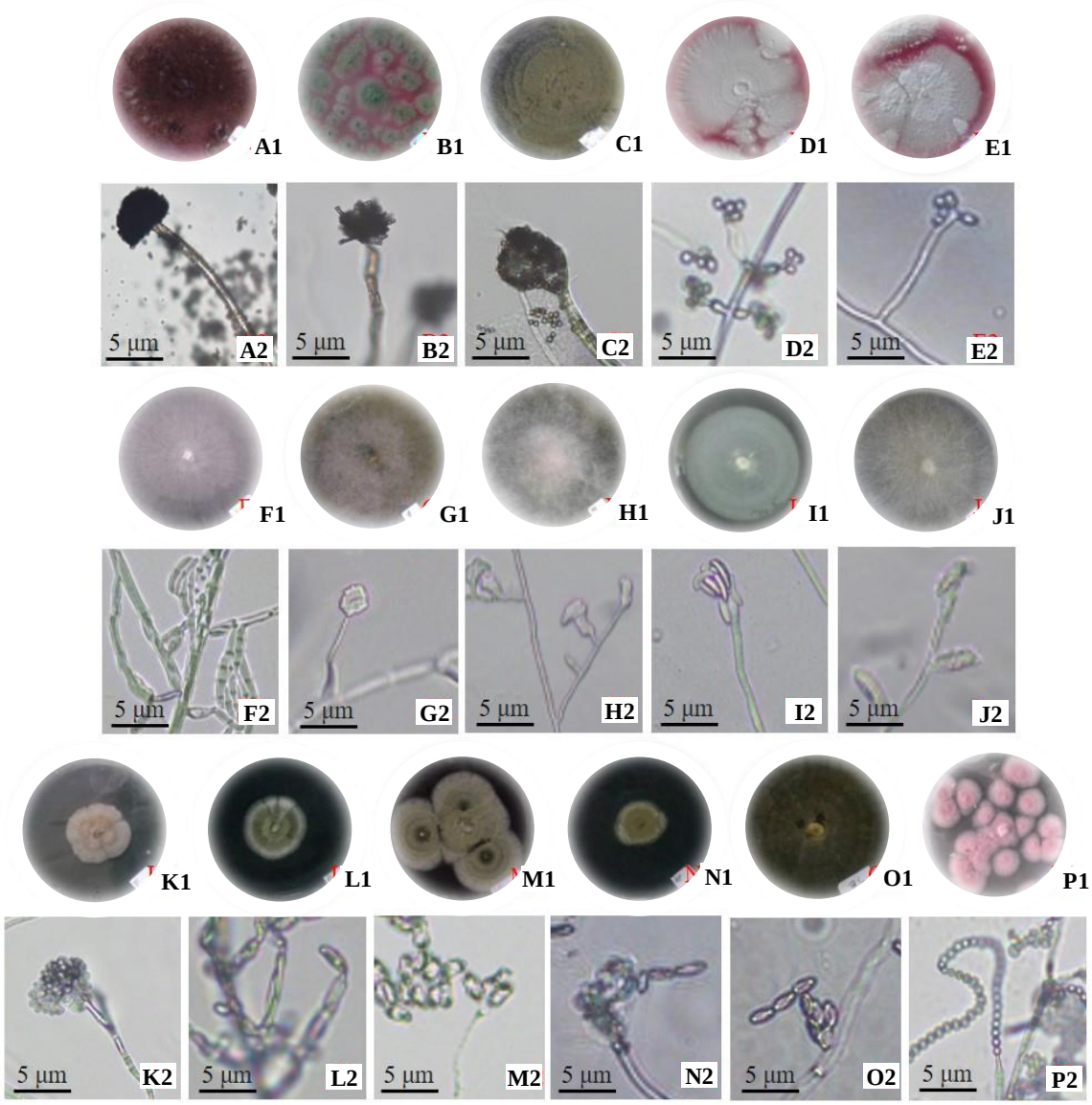

Figure 1. The morphological characteristics of entomopathogenic fungi obtained from organic farm soils. *Aspergillus* sp.1 (A); *Aspergillus* sp.2 (B); *Aspergillus* sp.3 (C); *Beauveria* sp.1 (D); *Beauveria* sp.2 (E); *Fusarium* sp.1 (F); *Fusarium* sp.2 (G); *Fusarium* sp.3 (H); *Fusarium* sp.4 (I); *Fusarium* sp.5 (J); *Gliocladium* sp.(K); *Metarhizium* sp.1 (L); *Metarhizium* sp.2 (M); *Metarhizium* sp.3 (N); *Metarhizium* sp.4 (O); and *Paecilomyces* sp.1 (P). A1 to P1: colony and A2 to P2: conidial structure

Conidia production and conidial viability of the entomopathogenic fungal isolates

The conidia production and conidial viability among the EPF isolates were significantly varied (Table 2). The lowest and highest conidia production was found in *Fusarium* sp.9 (1.70×10^6 conidia mL^{-1}) and *Paecilomyces* sp.2 (43.06×10^6 conidia mL^{-1}), respectively ($F_{24,100}$ = 7998, $P < 0.0001$). The conidial viability of all EPF isolates ranged from 4.02 to 52.07%. The lowest and highest percent germination found in *Fusarium* sp.9 and *Metarhizium* sp.4, respectively ($F_{24,100}$ = 3333, $P < 0.0001$).

The virulence of entomopathogenic fungal isolates on *Spodoptera litura* larvae

Almost all EPF isolates were virulent towards *S. litura* larvae, while *Fusarium* sp.4 and *Gliocladium* sp. were not infective (Table 3; $F_{24,100}$ = 45.68, $P < 0.0001$). *Aspergillus* sp.1 and *Fusarium* sp.9 elicited insignificant mortality compared to the control, hence they were also considered avirulent against *S. litura* larvae. The virulent isolates caused varying degrees of mortality of the larvae, ranging from 10 to 65%, with the lowest and highest value was found on *Fusarium* sp.8 and *Beauveria* sp.1, respectively. Based on Pearson's correlation analysis, we found a significant positive relationship between the conidial viability of EPF and mortality of *S. litura* larvae (r = 0.706; $P < 0.0001$) (Figure 3).

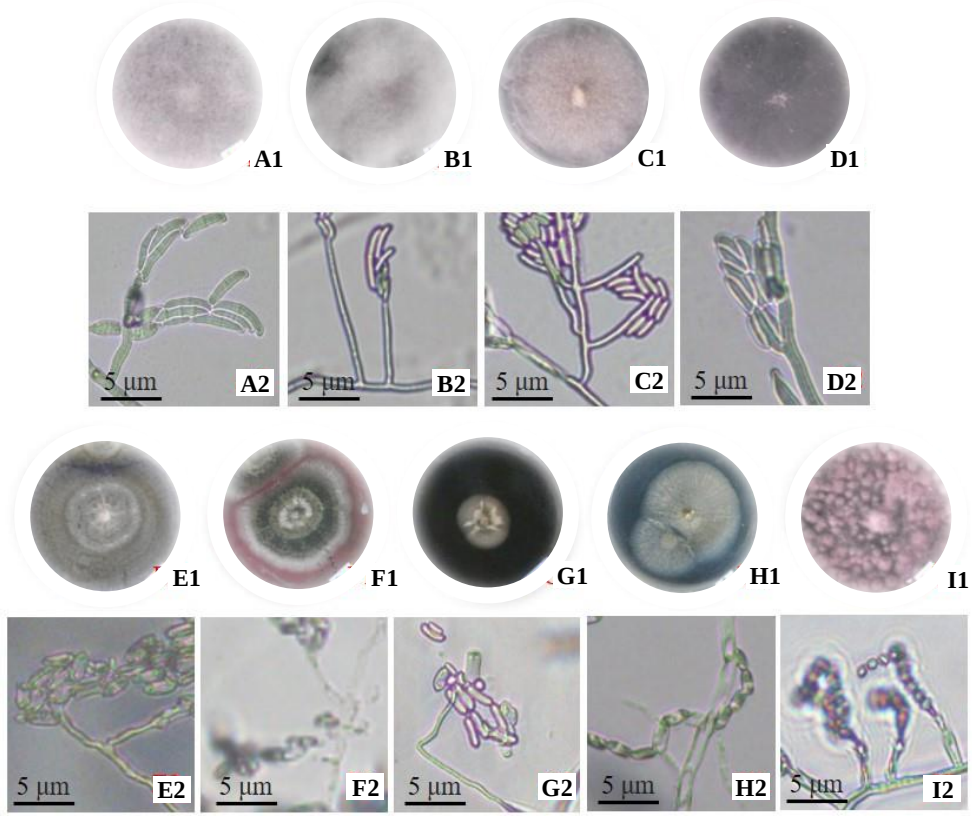

Figure 2. The morphological characteristics of entomopathogenic fungi obtained from conventional farm soils. *Fusarium* sp.6 (A); *Fusarium* sp.7 (B); *Fusarium* sp.8 (C); *Fusarium* sp.9 (D); *Metarhizium* sp.5 (E); *Metarhizium* sp.6 (F); *Metarhizium* sp.7 (G); *Metarhizium* sp.8 (H); *Paecilomyces* sp.2 (I). A1 to I1: colony and A2 to I2: conidial structure

Cultivation practices applied by the farmers

The differences in cultivation practices between the studied farms were seen in several aspects (Table 4). The studied organic farm did not apply pesticides to manage plant diseases and pests, while various pesticides were used in the studied conventional farm. For fertilization, cow manure and compost were used in the organic farm, whereas only inorganic fertilizers (nitrogen, phosphorous, and potassium) were used in the conventional farm. Crop rotation was practiced in the organic farm but not in the conventional farm. In both farms, tillage and weeding were carried out traditionally using a hoe and manually using hand, respectively.

Discussion

Our findings are in agreement with several other studies that reported a higher prevalence of soil-inhabiting EPF in organic farms than conventional farms (Klingen et al. 2002; Tkaczuk et al. 2014; Clifton et al. 2015; Ramos et al. 2017). In this study, the relatively higher occurrence of EPF in organic soils could be ascribed to differences in cultivation practices between the studied farms. The prevalence of EPF was frequently associated with soil organic matter content in soil (Bueno-Pallero et al. 2020). It was also observed that compost and cow manure application increased soil organic matter in the studied organic farm. Organic matter-rich soils are known to have high cation exchange capacities, which in turn may adsorb and retain a large amount of EPF spores (Quesada-Moraga et al. 2007; Sharma et al. 2018b). In addition, organic fertilizers could serve as a growth substrate for EPF mycelium (Klingen et al. 2002; Noble et al. 2018). Organic fertilizers also increase the abundance of soil-dwelling arthropods that are potential hosts for EPF or that act as vectors in spreading and transmitting EPF spores (Ali-Shtayeh et al. 2002; Thiele-Bruhn et al. 2012; Anslan et al. 2018; Lin et al. 2019).

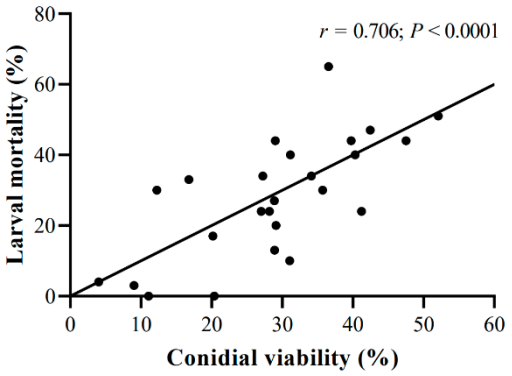

Figure 3. Pearson's correlation coefficient with conidial viability of entomopathogenic fungi in the x-axis and mortality of *Spodoptera litura* larvae in the y-axis

Table 3. Cumulative percent mortality in *Spodoptera litura* larvae at seven days after the treatment with entomopathogenic fungi obtained from organic and conventional farm soils

Farming systems	Fungal isolates	Percent mortality
Organic	*Aspergillus* sp.1	3 ± 1.22 kl
	Aspergillus sp.2	27 ± 2.55 efg
	Aspergillus sp.3	17 ± 2.55 hij
	Beauveria sp.1	65 ± 3.53 a
	Beauveria sp.2	44 ± 2.91 bc
	Fusarium sp.1	13 ± 2.55 ij
	Fusarium sp.2	20 ± 2.73 ghi
	Fusarium sp.3	30 ± 3.53 ef
	Fusarium sp.4	0 ± 0.00 l
	Fusarium sp.5	24 ± 1.87 fgh
	Gliocladium sp.	0 ± 0.00 l
	Metarhizium sp.1	40 ± 2.23 cd
	Metarhizium sp.2	44 ± 4.00 bc
	Metarhizium sp.3	24 ± 1.87 fgh
	Metarhizium sp.4	51 ± 2.91 b
	Paecilomyces sp.1	34 ± 2.44 de
Conventional	*Fusarium* sp.6	30 ± 1.58 ef
	Fusarium sp.7	24. ± 1.87 fgh
	Fusarium sp.8	10 ± 1.58 jk
	Fusarium sp.9	4 ± 1.87 kl
	Metarhizium sp.5	40 ± 3.53 cd
	Metarhizium sp.6	33 ± 2.55 de
	Metarhizium sp.7	34 ± 2.91 de
	Metarhizium sp.8	47 ± 2.55 bc
	Paecilomyces sp.2	44 ± 2.91 bc
	Control	0 ± 0.00 l

Means followed by the same letters within each column are not significantly different at $P < 0.05$ according to DMRT. Mean ± SE of five replicates.

It has been suggested that some EPF are more sensitive to exogenous disturbances than others. In this study, *Aspergillus*, *Beauveria*, and *Gliocladium* were exclusively found in organic soils. Specifically, *Beauveria* was reported to be less common in conventional farms, while it was frequently recovered in natural and semi-natural habitats as well as organic farms (Quesada-Moraga et al. 2007; Medo and Cagáň 2011; Clifton et al. 2015). The reduction in EPF occurrence and diversity in conventional soils could be due to the excessive application of fungicides (Niu et al. 2019; Litwin et al. 2020). Fungicides have a direct killing effect on EPF or may inhibit the mycelial growth, sporulation, and germination of EPF (Ali et al. 2013; Celar and Kos 2016; Sivakumar et al. 2020). The fungicides used in the studied conventional farm, namely copper oxychloride and mancozeb, have been known to significantly reduce the survival of EPF based on laboratory and field studies (Loria et al. 1983; D'Alessandro et al. 2011).

Fusarium and *Metharizium* were the richest genera found in our study. Ali-Shtayeh et al. (2002) also found a high abundance and diversity of *Fusarium* across various agricultural fields in Palestine. *Fusarium* species display diverse ecological functions, act as weak to virulent pathogens to arthropods, and have a high degree of survivability in soils (Sharma and Marques 2018; da Silva Santos et al. 2020). Regarding *Metarhizium*, a large body of knowledge has indicated its high degree of tolerance to varying levels of temperature and humidity as well as its capability to withstand the detrimental effects of fungicides and other cultivation practices (Vänninen et al. 2000; Medo and Cagáň 2011; Teja and Rahman 2016). As a result, *Metarhizium* is often become the most dominant and speciose EPF in cultivated soils (Quesada-Moraga et al. 2007; Meyling et al. 2011; Sánchez-Peña et al. 2011; Tkaczuk et al. 2014; Uzman et al. 2019).

Insecticide application might indirectly contribute to the lower occurrence of EPF in the studied conventional farm. Klingen et al. (2002) reported that excessive application of insecticides reduced the number of host arthropods for subsequent transmission. Moreover, Quesada-Moraga et al. (2007) proposed that EPF, particularly *Beauveria*, necessitates repeated infection to persist in agricultural soil. The positive relationship between insect abundance and EPF occurrence is well recognized (Pell et al. 2010; Clifton et al. 2015). The present investigation proposes that crop rotation applied in the studied organic farm could facilitate the persistence of EPF in soil. Crop rotation can increase the diversity of arthropods (Meyer et al. 2019), which some of them are maybe more susceptible to the infection of certain EPF. Moreover, by using high-throughput pyrosequencing, Ding et al. (2018) demonstrated that the establishment of crop rotation could increase the fungal diversity and richness in rhizosphere soils, which perhaps some of them were EPF.

Table 4. Cultural practices applied in the studied farms

Cultivation practices	Organic farm	Conventional farm
Crop age during the soil sampling	42 days	46 days
Pesticides	Pesticides are not applied	Pesticides are applied in a calendar manner; once or twice a week
Fungicides	-	Copper Oxychloride, Flusulfamide, Mancozeb, and Propineb
Insecticides	-	Chlorfenapyr, Cypermethrin, and Emamectin
Fertilizer	Compost and cow manure; applied 3 to 7 days before transplanting	Inorganic fertilizers, i.e, nitrogen, phosphorous, and potassium; applied at 15 days after transplanting
Tillage	Traditionally using a hoe	Traditionally using a hoe
Weeding	Manually by hand	Manually by hand
Crop rotation	Crop rotation is applied; broccoli, carrot, and sugar beet	Crop rotation is not applied

There was a large variation in the conidia production and conidial viability among the collected EPF isolates. Xavier-Santos et al. (2011) and Puspitarini et al. (2021a) suggested that those parameters are determined by the genetic backgrounds of each isolate, hence are strongly species- and strain-specific. A preliminary *in vitro* assessment on conidia production and conidial viability is carried out to search for effective naturally occurring EPF isolates as biocontrol agents. Specifically, evaluation of conidial viability is more crucial as it determines the success of EPF in penetrating the cuticle of the host arthropod. Therefore, conidial viability could serve as one of the robust predictors of virulence of EPF (Jenkins and Grzywacz 2000; Faria et al. 2015). In concordance, the result of correlation analysis indicates a significant positive relationship between the conidial viability of EPF and mortality of *S. litura* larvae.

Among the virulent EPF isolates, *Beauveria* sp.1 caused the highest mortality (65%) of *S. litura* larvae. However, it was noted that several other EPF isolates, namely *Beauveria* sp.2, *Metarhizium* sp.1, *Metarhizium* sp.2, *Metarhizium* sp.5, *Metarhizium* sp.8, and *Paecilomyces* sp.2 also caused apparent larval mortality of 40 to 51%. EPF belongs to *Beauveria*, *Metharizium*, and *Paecilomyces* genera have been widely used as biocontrol agents in agroecosystems to control myriad phytophagous insects, including *S. litura* (Nguyen et al. 2017; Ayudya et al. 2019; Yang et al. 2019). In contrast, four EPF isolates, namely *Aspergillus* sp.1, *Fusarium* sp.4, *Fusarium* sp.9, and *Gliocladium* sp., were considered avirulent against *S. litura* larvae. Nevertheless, it does not mean that those isolates are not entomopathogenic. There is a possibility that they may have a high virulency towards other insect species as they were able to kill *T. molitor* larvae on Koch's postulate. This hypothesis is supported by Butt et al. (2016), who stated that EPF species or strains had evolved different degrees of specificity and virulence. Therefore, their occurrence in agroecosystems is still of great importance.

Considering the farming system, *Beauveria* sp.1, *Beauveria* sp.2, *Metarhizium* sp.1, and *Metarhizium* sp.2 were obtained from the organic farm, whereas *Metarhizium* sp.5, *Metarhizium* sp.8, and *Paecilomyces* sp.2 were isolated from the conventional farm. It envisages that, in spite of intensive agrochemical application, conventional farms may contain EPF isolates with high potential as biocontrol agents. However, the present findings imply that the organic farm is a more appropriate farming system in preserving the occurrence and/or diversity of EPF. In addition, the removal of synthetic agrochemicals, application of organic fertilizers, and crop rotation practiced in the organic farm should be integrated into any other agroecosystems as a form of conservation biological control strategies aiming to boost the pest control service provided by naturally occurring EPF.

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