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Original Article

Entomopathogenic fungi isolated from agro-ecosystem soil in South Sulawesi, Indonesia

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Abstract

Entomopathogenic fungi have been proved controlling plant pests, and most of the entomopathogenic fungi isolated from the agroecosystem. The research was carried out to characterize the entomopathogenic fungi diversity associated with different agroecosystem on a different location in South Sulawesi, Indonesia. Fungi of rice field ecosystems were found more diverse than on dryland ecosystems, and the genus was *Fusarium* species, *Aspergillus* species, *Rhizopus* species, *Trichoderma* species, *Penicillium* species, *Rhizoctonia* species and *Metharizium* species. Moreover, *Fusarium* species and *Rhizopus* species were the most frequent genus isolated from rice field ecosystem, while on dryland ecosystems, the fungus of *Metharizium* species found more frequent than *Fusarium* species and *Aspergillus* species According to a geographical location on rice field ecosystems. The number of fungi isolated on the regency of Gowa more numerous than regency of Sidrap, Takalar, and Pinrang. Meanwhile, on the dryland ecosystems, three locations were the city of Makassar, the regency of Maros, and the regency of Polman. Among three areas, the regency of Maros founded more fungi than regency of Polman and Makassar. This study exhibits the diversity of fungi on the rice field, and dryland ecosystems were quite different.

Keywords: Entomopathogenic fungi, Agroecosystem soil, Diversity, South Sulawesi

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Introduction

Soil microorganisms services have significant influences on agricultural ecosystems, including soil formation, nutrient cycling, and biological control of pathogens and pests (Lehman et al., 2015). Soil microbial communities are closely associated with plant communities as the plant is an essential source of energy for most microorganisms through root exudates and plant waste (Schenck zu Schweinsberg-Mickan et al., 2012; Singh et al., 2016), and type of plant is one of the elements that control the activity of soil microbial (Mitchell et al., 2012; Cline and Zak, 2015).

Entomopathogenic fungi are commonly found in soil including agroecosystem soil. The fungi often isolated from soil and insects in the field (Keller and Zimmerman, 1989). One of the famous entomopathogenic genera is *Metarhizium*. They are common rhizosphere colonizers fungus in many ecosystems (Bruck, 2010). Furthermore, the entomopathogenic fungi were abundant in soil, and they were isolated from 32% of soil specimens gathered from distinct cultivated habitats across Finland (Vanninen et al., 1989). In addition, *Metarhizium anisopliae* isolated from 31% of soil samples taken from pastures across Tasmania (Rath et al., 1992). Chandler et al. (1997) isolated the fungus into 17.5% of soil specimens in the United Kingdom. Moreover, entomopathogenic fungi isolated from 14.9% of soil samples in Italy (Tarasco et al., 1997).

Biological control through the application of entomopathogenic fungi have been recommended as promising agents for managing insect pests (Gaugler, 2002). Several fungal entomopathogens have proven to reduce the infestation of several insects pests such as *Metarhizium anisopliae*, and *Beauveria bassiana* was applied against many different types of insect pests (Migiro et al., 2010; Rondot and Reineke, 2018).

Isolation and characterization of entomopathogenic fungi local species should be conducted to gain more information about them. So their plentifulness, dispersal, and their function in the local environment can be studied. Accordingly, our objective was to explore and evaluate the abundance and the diversity of entomopathogenic fungus on the rice field and dryland ecosystems in South Sulawesi, Indonesia.

Material and Methods

Soil samples were collected from different agroecosystems situated in South Sulawesi Indonesia (Figure 1) and from habitats of rice field and dryland ecosystems including cacao, cassava, banana, lemongrass, and weed vegetation (Table 1 and 2) during February until September 2017. Soil Samples were taken in the field by digging a hole at 10 - 15 cm in depth using a shovel in an area of 1000 m^2 around plant roots environment. Samples were kept in the plastic bag (25 x 25 cm) about 0.5 Kg in weight and tightly closed to prevent dryness.

Isolation of entomopathogenic fungi

Isolation of entomopathogenic fungi was carried out by collecting soil samples by "baiting" them with the larvae of darkling beetle, *Tenebrio molitor* (Tenebrionidae: Coleoptera). Ten larvae of the third instar *Tenebrio molitor* were put into a small container filled by moist soil samples. Next, The cover of the container was replaced with the smooth net as the lid. Then, the container with ten larvae was kept at room temperature in dark condition for two weeks. During the incubation process, the larvae in the container were moistened by water through the spraying method every morning, 09.00 am and afternoon, 05.00 pm.

Figure-1: Map of sampling sites in South Sulawesi, Indonesia where soil collected for isolation of fungal entomopathogen (enclosed circles).

Soil sampling

After 5-14 days, the larvae of darkling beetle was fathomed infected by fungi with an indication showing mycelia on their body surfaces were collected and maintained in the small tubes. All of the infected larvae were surface-sterilized by ethanol 70% for 3 - 5 second and in sterile water for 5 - 10 second. Then, the larvae were dried with sterile absorbent paper and put them into Petri dish contain moist tissue. Fungal isolation was made on Potato Dextrose Agar (PDA) containing chloramphenicol as antibiotics in a petri dish. After that, they are incubated at room temperature in darkness. Finally, the isolates were subcultured and identified.

Table-1: Site descriptions where soil collected for isolation of fungal entomopathogen on rice field ecosystems.

No	Location	Environmental details
1	The village of Tonronge, District of Baranti, Regency Sidrap	- Irrigated rice field - Rice phase was Generative
2	The village of Patalassang, District of Samata, Regency Gowa	- Irrigated rice field - Rice phase was Generative
3	The village of Tanah Bangka,District of Bajeng Barat, Regency Gowa	- Irrigated rice field - Rice phase was Generative
4	The village of Galesong Barat, District of Galesong, Regency Takalar	- Irrigated rice field - Rice phase was Generative
5	The village of Aressie, District of Tiroang, Regency Pinrang	- Irrigated rice field - Rice phase was Generative

Table-2: Site descriptions where soils collected for isolation of fungal entomopathogen on dryland ecosystems.

No	Location	Environmental details
1	The village of Tapango Barat, District of Tapango, Regency of Polewali Mandar (Polman)	Cacao farm, None of pesticides application in the last past seven years, and surrounded by elephant grass (*Pennisetum purpureum*).
2	The village of Purnakarya, District of Tanralili, Regency of Maros	Cacao farm, None of pesticides application.
3	The village of Samangi Balangajia, District of Simbang, Regency of Maros	Banana farm.
4	The village of Samboeja, District of Simbang, Regency of Maros	Lemongrass farm.
5	The village of Samanngi Balangajia, District of Simbang, Regency of Maros	Weeds and elephant grass vegetation.
6	The village of Samanngi Balangajia, District of Simbang, Regency of Maros	Bamboo vegetation and the area used disposal chicken manure.
7	The village of Bangkala, District of Manggala, City of Makassar	Cassava and banana farm.

Identification of entomopathogenic fungi

The fungi that emerged mainly characterized based on their morphological of macroscopic (colour, shape, and colony growth) and microscopic (conidia) according to Barnet and Hunter, 1972.

Results

The result of fungi isolation and identification through baiting method on two different areas showed that six genera of fungi were found on rice field ecosystems and three genera on dryland ecosystems (Table 3). Overall, fungi on rice field ecosystems are more diverse than on dryland ecosystems; However, on dryland ecosystems was isolated famous entomopathogenic fungus, *Metharizium* species.

Table-3: Diversity entomopathogenic fungi in soil samples related to types of ecosystems.

Ecosystem	Fungi isolates	
	No. of	Fungi Genera
Rice field	6	*Fusarium* species, *Aspergillus* species isolate 1, *Aspergillus* species isolate 2, *Rhizopus* species, *Trichoderma* species, *Penicillium* species, *Rhizoctonia* species
Dryland	3	*Metharizium* species, *Aspergillus* species isolate 1, *Fusarium* species

Six fungal genera were identified both on the rice field and dryland ecosystems (Tabel 3) namely *Fusarium* species, *Aspergillus* species, *Rhizopus* species, *Trichoderma* species, *Penicillium* species, *Metharizium* species and *Rhizoctonia* species. The fungi were identified based on morphological character including colony appearance (Figure 2).

Among the fungi that infects larvae of *Tenebrio molitor* (Figure 3) from rice field ecosystems soil, genus of *Fusarium*, *Rhizopus*, and *Aspergillus* species isolate 1 were the most often fungi that isolated from infected larvae of *Tenebrio molitor*; which is *Fusarium* species was the most prevalent isolates recovered comprise 31% of the total isolates, followed by *Rhizopus* species (27%) and *Aspergillus* species isolate 1, 23%. Then, *Trichoderma* species (8%) and *Penicillium* species, *Rhizoctonia* species, *Aspergillus* species isolate 2 with frequency 4% each on rice field land agroecosystem (Figure 4).

Meanwhile, on dryland ecosystems soil, Genus of *Metharizium* and *Aspergillus* showing the higher rate of emerged fungi. Which is *Metharizium* species was the most frequent isolate (50%), followed by *Aspergillus* species isolate 1 (41.7%) and *Fusarium* species 8.3% (Figure 5).

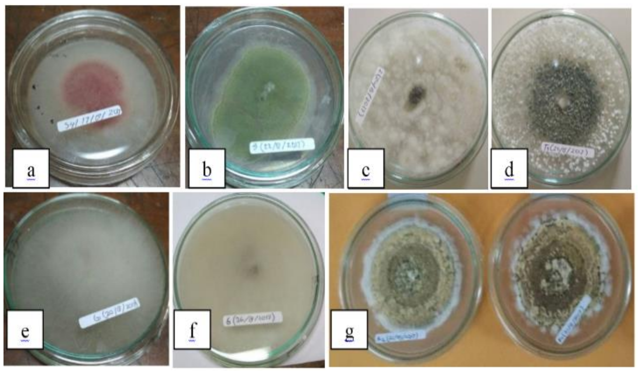

Figure-2: Colony of various fungi genera on PDA Medium; a. *Fusarium*; b. *Aspergillus*; c. *Aspergillus*; d. *Trichoderma*;e. *Penicilium*; f. *Rhizopus*; g. *Metarhizium*.

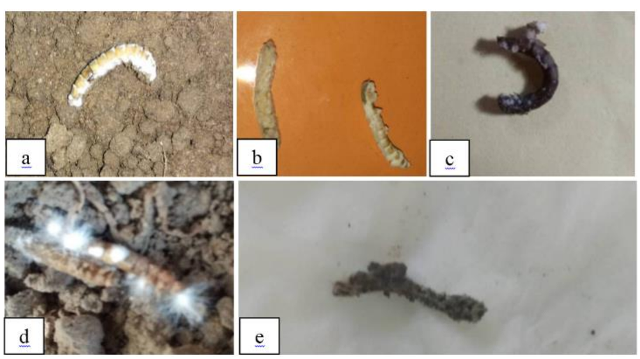

Figure-3 *Tenebrio molitor* larvae infected by various fungi; a. *Metarhizium*; b. *Aspergillus*; c. *Fusarium*; d. *Rhizopus*; e. *Trichoderma*.

According to the geographical location, the fungi isolates on rice field ecosystems soil distributed more numerous and diverse in Gowa site. Six different isolates of fungi that isolated from infected *Tenebrio molitor* larvae, which is *Aspergillus* isolates 1, and *Rhizopus* shows significant numbers, rather than other isolates such as *Fusarium, Aspergillus* isolates 2, *Trichoderma, Penicillium, Rhizoctonia*. In Takalar site, there are four different isolates found infects *Tenebrio molitor* larvae and the number of the population not significant to Pinrang and Sidrap site. The least fungal microbes found in Pinrang and Sidrap site, one and two different isolates, respectively (Table 4).

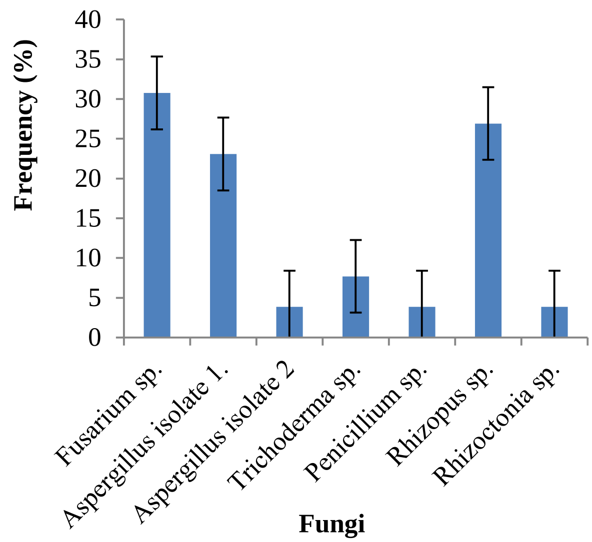

Figure-4: The frequency occurrence (%) of entomopathogenic fungi isolated from rice field ecosystems soil in South Sulawesi, Indonesia

Meanwhile, on the dryland ecosystems soil, among three different sites sample, Maros was the most common area that found three isolates of fungus namely *Fusarium*, *Aspergillus*, *Metharizium* followed by Polman and Makassar that found two isolates (Table 5). *Metharizium* isolated more frequent than two other isolates and the number not significantly different both in Maros and Polman. *Aspergillus* was isolated in Maros, Polman, and Makassar. However, the number was high in Maros and Polman while *Fusarium* was found only in Maros and Makassar with a small number.

Table-4: Distribution of entomopathogenic fungi in soil samples according to the geographical location on rice field ecosystems.

Site	*Fusarium*	*Aspergillus* Isolate 1	*Aspergillus* Isolate 2	*Trichoderma*	*Penicillium*	*Rhizopus*	*Rhizoctonia*
Sidrap	1^{ns}	1^{b}	0^{ns}	0^{ns}	0^{ns}	0^{b}	0^{ns}
Gowa	3^{ns}	5^{a}	1^{ns}	1^{ns}	1^{ns}	5^{a}	0^{ns}
Takalar	1^{ns}	0^{b}	0^{ns}	1^{ns}	0^{ns}	2^{b}	1^{ns}
Pinrang	3^{ns}	0^{b}	0^{ns}	0^{ns}	0^{ns}	0^{b}	0^{ns}

Numbers in the same column followed by the same letters are not significantly different (P<0.05, LSD test).

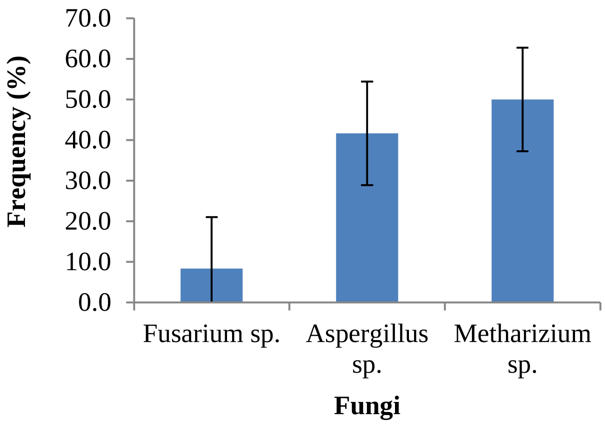

Figure-5: The frequency occurrence (%) of entomopathogenic fungi isolated from dryland ecosystems soil in South Sulawesi, Indonesia

Table-5: Distribution of entomopathogenic fungi in soil samples according to the geographical location on dryland ecosystems.

Site	*Fusarium*	*Aspergillus*	*Metharizium*
Makassar	1	1^b	0^b
Maros	1	5^a	6^a
Polman	0	4^{ab}	6^a

Numbers in the same column followed by same letters are not significantly different (P<0.05, LSD test).

Discussion

This study compared the diversity and frequency of entomopathogenic fungi on different ecosystems. Several fungi associated with the rice field ecosystems and its found more diverse than dryland ecosystems. Genus of *Fusarium*, *Aspergillus*, and *Rhizopus* dominated the emerged fungus on the rice field ecosystems. However, on dryland ecosystems founded one of the widespread fungi that use as a biological control widely for plant pests namely *Metharizium* species. Structure of soil microbial community is affected by a number of associating elements including type of plant (Maul and Drinkwater, 2010), agricultural practices (Buyer et al., 2010; Treonis et al., 2010; Maul et al., 2014), Fertilization , and soil properties (Lauber et al., 2008 & 2009; Freedman and Zak, 2015).

Geographical distributions can influence different species of fungi, and these distributions do depend on the environment such as land use change, pollution, pesticides, fertilizers, movement of biota, vegetation, and climate change (Boddy, 2016). Furthermore, the movement of microbes in rice soil is affected by a type of agro-climatic circumstances and agricultural practices including puddling (Banerjee et al., 2006). The activity of microorganisms in rice field soil also affected by transplanting, dosage and frequency of application of chemical fertilizers, biofertilizers, pesticides and duration of drying/flooding stages during crop growth (Prasanna et al., 2012). Regarding our result on soil sample sites of the rice field ecosystems, the number of fungi isolated on Gowa was more diverse than other places. Whereas sites with the least amount of fungi isolated were Sidrap and Pinrang; Sidrap and Pinrang sites are the most intensive planting areas of rice in South Sulawesi Province. Meanwhile, the type of plant and chemical input influence the composition of the microbes of the dryland Maros, Polman, and Makassar sites.

Aspergillus species and *Fusarium* species consistently isolated both rice field and dryland soil. *Aspergillus* species has been known as one of the entomopathogenic fungi around the world, it proven inhibited and to be pathogenic to insect pests of various families and few-mode of action (Tanada and Kaya, 1993; Buhroo et al., 2002; Seye et al., 2009: Suliman and Osman, 2012; Seye et al., 2014). Similarly, the genus of *Fusarium* has been reported virulent to insect pests (Tanada and Kaya, 1993; Anand and Tiwary, 2014). Also, *Aspergillus* species and *Fusarium* species reported to be associated with a number of dryland tissue plants and have endophytic properties including cacao (Rubini et al., 2005; Asman et al., 2018). Meanwhile, well known entomopathogenic fungi, *Metharizium* species isolated only from the dryland soil. One of the famous taxa is *Metarhizium anisopliae*. It has been used as biological control agents for insects pest widely (Migiro et al., 2010; Anand and Tiwary, 2014).

Our results indicate that agroecosystems can have different soil microbial communities structure both in the rice field and dryland soil. More research will be needed to specify if these entomopathogenic fungi have significant role protects plants from various insect pests.

Conclusion

This study demonstrates that a number of entomopathogenic fungi found to be associated with both rice field land and dryland ecosystem. Therefore, these native entomopathogenic fungi could be a promising source of biological control for insect pests.

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Contribution of Authors

Abdullah T: Conceived idea, designed research methodology, manuscript writing and final approval
Irwan I: Data collection
Kuswinanti T: Designed research methodology and Variable Assessment Method
Daud DI: Data interpretation and analysis
Asman A: Manuscript writing and final approval, statistical analysis and literature search
Nasruddin A: Manuscript final reading & approval and data interpretation & analysis
Agus N: Designed research methodology and Variable Assessment Method