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Potency and Diversity of Fungi on Pine Litter and Rhizosphere in Different Land-use of Universitas Brawijaya (UB) Forest

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ABSTRACT

The agroforestry system with its diversity of vegetation has the potential on the existence of antagonistic and entomopathogen fungi. This study was conducted to evaluate the diversity of antagonistic fungi and entomopathogen fungi in the rhizosphere and pine leaf litters on pine monoculture and intercropping pine - coffee. The research was started from plot determination, sampling, fungal identification, antagonist test and pathogenicity test. The results of isolation of fungi from pine leaf litter on monoculture pine and intercropping pine-coffee fields obtained 17 genus of fungi. In monoculture pine, there 9 genus of fungi were found, while other 4 were still unidentified. In pine-coffee intercropping land 13 genus were observed. Based on the potential and bility tests, *Acremonium* sp. 3 and *Penicillium* sp. 2 has the highest inhibiton capacity, while isolate *Paecilomyces* sp. 1 and *Paecilomyces* sp. 2 had the best level of pathogenicity and mortality. Temperature and humidity did not affect the diversity of fungi. The diversity of entompathogenic and antagonistic fungi was higher in the pine-coffee intercropping land use. The litter plots had higher fungal diversity than the rhizosphere.

INTRODUCTION

Agroforestry is a land management system based on natural sustainability. Initially, agroforestry was formed from natural forests that were modified in terms of land management with human intervention. This agroforestry system is a combination of woody plants and cultivated plants. The diversity of vegetation affects the biodiversity in it. One of the locations of the agroforestry system is in the Special Purpose Forest Area of Universitas Brawijaya (SPFA-UB) or better known as UB Forest. Since 1976, most of the forest area has been converted to agroforestry land. This production forest is managed in monoculture and intercropping with pine, coffee, and annual crops. This is thought to have an impact on microbial biodiversity, especially fungi.

According to Heilmann-Clausen et al. (2014), the role of fungi in ecosystems is as a provider of habitat and important processes for other organisms, as an indicator of desirable or undesirable trends in ecosystem function, as an indicator of habitat conservation value, and as a provider of strong links between human society and the environment. In this study, antagonist and entomopathogenic fungi are discussed. Fungal antagonists play a significant role in controlling plant pathogens and diseases and they have been used as Biocontrol Agents (BCAs). The report of Brito-Vega, Espinosa-Victoria, Salaya-Domínguez, & Gómez-Méndez (2013) mentioned that *Trichoderma* spp. has been widely used as antagonistic fungal agents against several pests as well as plant growth promoters. Faster metabolic rates, antimicrobial metabolites, and physiological conformation are key factors of the exhibiting antagonistic functions of these fungi. The actions of these fungi are mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites, and induction of plant defense system. *Trichoderma* spp. has also

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been used in a wide range of commercial enzyme productions, namely, cellulases, hemicellulases, proteases, and -1,3-glucanase. Meanwhile, entomopathogenic fungi are one of the biological controllers found in nature. This group can attack at various stages of insect life development, including eggs, larvae, pupae, nymphs, and imagos. Another advantage of entomopathogenic fungi in their role as biological insecticides is the ability to attack the insect pests which does not cause resistance even though they are used continuously. The distribution of entomopathogenic fungi is also diverse, covering the phyla Zygomycota, Ascomycota, Basidiomycota, and Deuteromycota. So that their existence in nature is spread, including in agroforestry areas. The effectiveness of entomopathogenic fungi in controlling pests also significantly affected several pests. This was proven in a previous study that the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* had a significant effect on the mortality of *Oryctes rhinoseros* (Sitompul, Oemry, & Pangestiningsih, 2014). Another study also proved that the infection levels of *B. bassiana* on *H. hampei* were the highest during the first five days after application, reaching nearly 30% (Bustillo et al., 1999). Both antagonistic fungi and entomopathogenic fungi can be found in the rhizosphere and litter areas.

The rhizosphere area is a root area that contains a lot of organic matter. These organic materials are used as nutrients for fungal growth and development. According to Brito-Vega, Espinosa-Victoria, Salaya-Domínguez, & Gómez-Méndez (2013), soil organic matter (SOM) is constituted of many diverse components with different states of decomposition, which vary depending on the quality of material, during mineralization (sugars, amino acids, hemicellulose, cellulose, and lignin) and humification. During the process of decomposition, organic matter is enhanced by the participation of soil organisms that ingest and transform a mixture of organic substrates and inorganic soil (micro- and macro-organisms). At the end of this process, part of the final product is absorbed by plant roots or other organisms. As well as in litter of pine leaves, microbes were also found, especially microbial decomposers. The litter contains a quite lot of C and N which are the main sources of fungal microbial nutrition. Based on these considerations, this study focuses on fungal diversity in pine litter and plant rhizosphere. Since the beneficial fungi are antagonistic and entomopathogenic fungi, the research focused on these beneficial microbes in the area of UB Forest. In addition, this research is expected to be able to assist agroforestry farmers in determining the appropriate control on monoculture and polyculture land. The control in question is the control of pests and diseases. Not only in agroforestry systems, these biological agents can also be applied to other cultivated lands. This can be useful for maintaining sustainability, stability, and increasing biodiversity.

The objective of this research was to evaluate the type of fungus, analyze the level of diversity of fungus, and find out the potential of fungus on land use of monoculture pine and intercropping pine-coffee.

MATERIALS AND METHODS

The research is conducted on August 2018 – January 2019 by collecting soil samples and pine litter from University of Brawijaya (UB) Forest, Malang Regency (Table 1). Identification and testing of fungal antagonists were carried out at the Biological Control Laboratory, Brawijaya University.

Table 1. Geography position of observation plot

Land-use	Plot	Coordinate	Elevation (m.a.s.l)
Pine Monoculture Age group 5 year	PM KU 5	07 ° 82' S 112 ° 57' E	1296
Pine Monoculture Age group 8 year	PM KU 8	07 ° 82' S 112 ° 57' E	1310
Pine-Coffee Age group 5	PK KU 5	07 ° 82' S 112 ° 57' E	1280
Pine-Coffee Age group 8	PK KU 8	07 ° 82' S 112 ° 57' E	1271

Remarks: Age group 5 = 25 years, Age group 8 = 40 years

Determination of Observation Plots

Research was carried out at pine-coffee intercropping and pine monoculture in UB Forest, that consist of 4 observation plots; 2 observation plots on pine monoculture and 2 plots on pine-coffee intercropping.

Sampling

Pine litter samples was collected randomly from pine leaves that had fallen on the topsoil. Soil samples were taken from 5 points on each subplot.

Fungi Isolation, Purification, and Identification

Isolating antagonistic fungus from pine litter is carried out by washing the samples and filtering using membrane according to Ilyas (2007). Fungal isolation from the rhizosphere is carried out by a dilution method. Isolation of fungi from rhizosphere was conducted using the insect bait method following Trizelia, Armon, & Jailani (2015).

Purification was carried out on each fungal colony based on macroscopic differences. Each fungal colony was then rown on a PDA medium.

Identification is carried out macro- and microscopically. The identification were determined based on its morphology using a reference book of Watanabe (2002).

Diversity Index (H ')

$$H' = -\sum_{i=1} \frac{[(ni)}{n} Ln \frac{(ni)]}{n} \text{.....1)}$$

Where: H '= Shannon-Wiener Index, ni = number of individuals, N = total number of individuals of all species.

Pine Leaf Fungus Antagonist Test

The antagonism test was done in a completely randomized design. It was carried out by double culture method with a ratio of 1:1 in vitro in one confrontation dish method (Johnson, 1957; Widyastuti, 2007). Colony fingers from the two isolates were measured for their length every 24 hours up to the fifth day since the two isolates were put together. On the 6th day, the observations on the inhibition zone and percentage of inhibition were carried out.

Measurements of inhibition zone are made by measuring the length of the empty zone. Percentage of inhibition was calculated by formula according to Muniroh, Nusaibah, Vadamalai, & Siddique (2019):

$$PP = \frac{r1\text{-}r2}{r1} x100\% \text{.....2)}$$

Where: PP = percentage of inhibition; r1 = radius of a pathogenic colony that grows towards the antagonist isolate; r2 = radius of the pathogenic colonies that grow near the antagonist isolate.

Pine Leaf Fungus Pathogenicity Test

Conidia density calculation was carried out bassed on Effendy, Robby, Abdullah, & Abdul (2010) by taking 1 ml of fungal suspension and dripping it on a *haecytometer*. The spore density was calculated using the formula of Gabriel & Riyatno (1989):

$$C = \frac{t}{(n.x)} x\ 10^6 \text{.....3)}$$

Where: C = spore density, t = number of spores, n = number of sample boxes, x = 0.25 correction factor for the use of small-scale sample cities on *haemocytometer.*

Calculation of conidia viability was carried out using the of Gabriel & Riyatno (1989):

$$V = \frac{g}{(g+u)}\ x\ 100\% \text{.....4)}$$

Where: V = conidia viability, g = number of germinated conidia, u = the number of conidia that did not germinated

The mortality of *T. molitor* larvae was calculated based on the formula of Gabriel & Riyatno (1989):

$$Larva\ mortality\ (\%) = \frac{A}{D} X\ 100\% \text{.....5)}$$

Where: A = the number of death insects, D = number of tested insects.

If *T. molitor* larvae were found died in the control treatment with conditions less that 20%, it was corrected using Abbott (1987) formula:

$$\frac{x-y}{x} X\ 100 \text{.....6)}$$

Where: x = percentage alive *T. molitor* larvae on control; y = percentage survived *T. molitor* larvae on treatment

Data Analysis

The diversity index can be measured by using Shannon-Wiener (H') method. The antagonist fungi inhibitory data were analyzed using analysis of variance (F test). If the results have significant differences, the analysis were proceeded using DMRT with 95% level of confidence.

RESULTS AND DISCUSSION

Type of Fungus

The type of fungus obtained from pine leaf litter on coffee pine fields was more diverse than that of monoculture pine. Based on the analysis of the Shannon-Wiener diversity index, it was shown that the pine-coffee intercropping area had higher diversity than the pine monoculture area. Differences in fungal diversity found in pine-coffee intercropping and pine monocultures were due to climatic conditions, vegetation and land use patterns. The diversity of fungal species in intercropping land was higher than in monoculture, presumably it was due to different land use patterns. This is because the coffee pine intercropping area has a closer distance among the plants and the wide coffee plant canopy makes the soil surface condition more moist. Several reports describes that the land use affects on the diversity of fungi (Frac, Hannula, Belka, & Jędryczka, 2018; Fracetto et al., 2013)and the closely related terms of soil quality and fertility, is considered as one of the most important characteristics of soil ecosystems. The integrated approach to soil health assumes that soil is a living system and soil health results from the interaction between different processes and properties, with a strong effect on the activity of soil microbiota. All soils can be described using physical, chemical, and biological properties, but adaptation to environmental changes, driven by the processes of natural selection, are unique to the latter one. This mini review focuses on fungal biodiversity and its role in the health of managed soils as well as on the current methods used in soil mycobiome identification and utilization next generation sequencing (NGS. The content of organic matter in the litter is used by microorganisms to obtain nutrients. According to Hooper et al. (2001) changes in food quality and quantity caused by the change in plant diversity will change the number, activity and diversity of microorganisms. The difference in land use patterns is thought to be a factor that causes the diversity of fungi in pine-coffee intercropping areas higher than in pine monoculture lands.

Comparison of genera of antagonistic fungi and entomopathogen fungi in the different land-use were presented in Table 2 and Table 3. The identification results (Table 2 and Table 3) of the antagonist fungi and entomopathogen fungi genus obtained from the rhizosphere and pine leaf litter in pine monoculture and pine-coffee intercropping were depicted with a Venn diagram (Fig. 1) to determine which fungi were found in the two pine fields. The antagonist fungi found in the two fields came from 4 genera, namely *Acremonium, Fusarium, Penicillium* and *Trichoderma*. The fungal genus found only in pine-coffee intercropping were *Cladosporium. Mortierella* and *Aspergilus* were found only in litter and *Scopulariosis* was only in the rhizosphere.

Table 2. Exploration result of entomopathogenic fungi and antagonistic fungi in rhizosphere

Genera of fungi	Category		Land use		Total of species
	Entomopathogen	Antagonistic	PM	PK	
Papulaspora	√		√		1
Metarhizium	√		√	√	3
Mortierella	√		√		1
Fusarium	√	√	√	√	5
Cochliobolus	√			√	1
Aspergillus	√	√	√	√	4
Penicillium	√	√	√	√	8
Acremonium		√	√	√	4
Scopulariosis		√		√	1
Unidentified 1	√		√		1
Unidentified 2	√			√	1
Unidentified 3		√	√		1
Unidentified 4		√		√	1

Remarks: PM = Pine monoculture, PK = Pine-Coffee intercropping

Table 3. Exploration result of entomopathogenic fungi and antagonistic fungi in pine litter

Genera of fungi	Category		Land-use		Total of species
	Entomopathogen	Antagonistic	PM	PK	
Aspergillus	√		√	√	4
Gliocladium	√		√		4
Geotrichum	√		√		1
Fusarium	√	√	√	√	6
Acremonium	√	√	√	√	10
Cylindrocarpon	√	√	√		1
Paecilomyces	√	√	√	√	2
Mortierella	√	√		√	2
Gongronella	√	√		√	1
Humicola	√			√	1
Penicillium		√	√	√	5
Trichoderma		√		√	2
Cladosporium		√		√	1

Remarks: PM = Pine monoculture, PK = Pine-Coffee intercropping

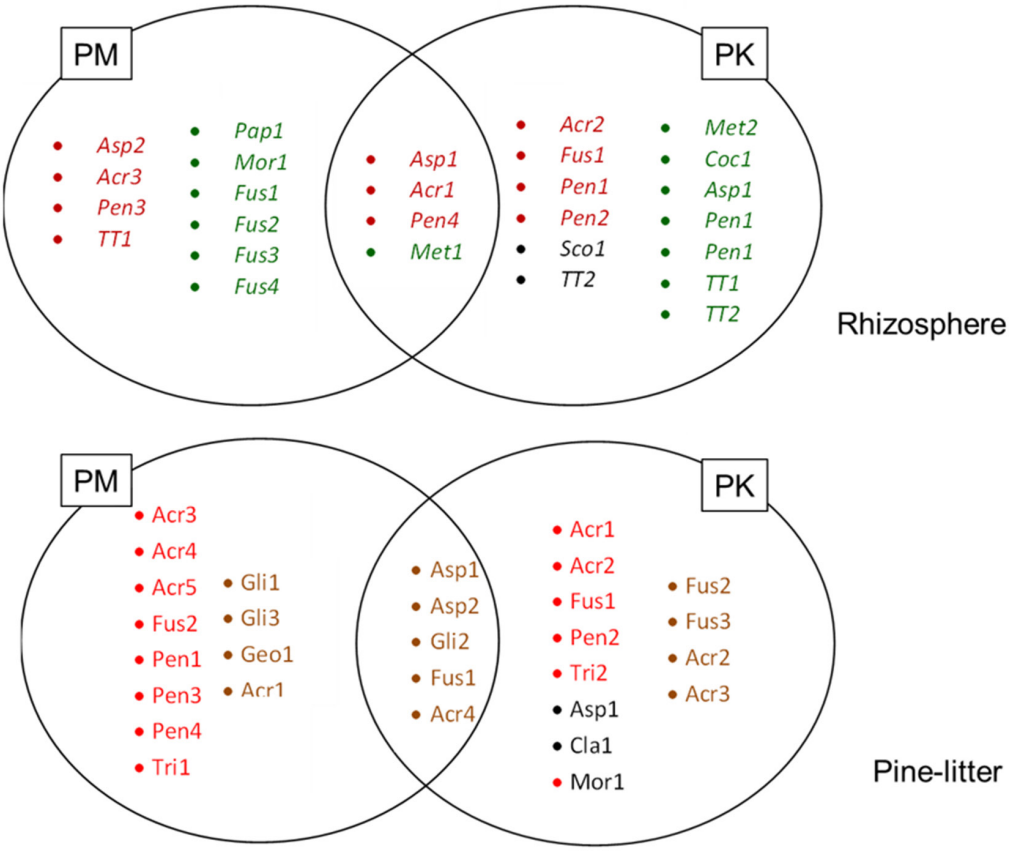

Fig.1. Diagram Venn of exploration result of entomopathogen fungi and antagonistic fungi in rhizosphere and pine-litter. Remarks: PM = Pine monoculture, PK = Pine-Coffee intercropping

Entomopathogenic genera found in two fields came from 4 genera, namely *Metarizium, Fusarium, Aspergilus,* and *Penicilium*, and those found only in pine-coffee intercropping were from the genus *Scopulariosis.* It is known that different land uses cause different fungal genera found. The genus of antagonistic fungi and entomopathogens found to be more abundant on polyculture lands, Baker & Cook (1982) stated that the use of polyculture lands will increase the amount of litter and roots and this will have a direct impact on the presence of beneficial microorganisms. In general, carbohydrate (such as sugars) are taken up by the fungal cell and converted to energy or biomolecule precursors through a variety of metabolic pathways (Mäkelä, Aguilar-Pontes, Van Rossen-Uffink, Peng, & De Vries, 2018).

The Diversity of Fungi in Pine-Coffe Intercropping and Monoculture

The diversity of fungi in pine-coffee intercropping was higher than that in pine-monoculture (Table 4). This is because the coffee-pine intercropping area has a closer distance among the plants and the wide coffee plant canopy makes the soil surface condition more humid. According to Frac, Hannula, Belka, & Jędryczka (2018) the pattern of land use is a factor that can affect the diversity of fungi and the soil health. The content of organic matter is expected to be higher. It is used by microorganisms in obtaining nutrients. According to Hooper et al. (2001), changes in food quality and quantity caused by changes in plant diversity will change the number, activity, and diversity of microorganisms.

The diversity of the fungal genus found in pine monoculture and pine-coffee intercropping is thought to be uninfluenced by microclimate conditions in both land uses as indicated by data on temperature and humidity observations that are not much different (Table 5 and Table 6). One of them is genus Fusarium. Sharma & Sharma (2021) reported that the factors that support the development of this fungus are temperature, low soil moisture and low light intensity. Low temperature will cause low soil temperature, and vice versa.

Table 4. Biodiversity Index

No	Resource of fungal		Land-use		Category		Biodiversity indeks (H')
	Rizosfer	Litter	PM	PK	Entomopathogens	Antagonistic	
1	√		√		√		1.95
2	√			√	√		2.02
3	√		√			√	1.93
4	√			√		√	2.16
5		√	√		√		2.40
6		√		√	√		2.93
7		√	√			√	1.39
8		√		√		√	1.95

Remarks: PM = Pine monoculture, PK = Pine-Coffee intercropping

Table 5. Correlation of the effect of temperature on the diversity of antagonistic fungi and entomopathogen fungi

Type	Correlation of Coefficient	Category	Description
Rhizosphere (Entomopathogenic fungi)	0.79 (P=0.21)	-	Uncorrelated
Rhizosphere (Antagonistic fungi)	0.79 (P=0.21)	-	Uncorrelated
Pine- Litter (Antagonistic fungi)	0.79 (P=0.21)	-	Uncorrelated
Pine-Litter (Entomopathogenic fungi)	0.79 (P=0.21)	-	Uncorrelated

Table 6. Correlation of the effect of humidity on the diversity of antagonistic and entomopathogenic fungi

Type	Correlation of Coefficient	Category	Description
Rhizosphere (Entomopathogenic fungi)	-0.72 (P=0.28)	-	Uncorrelated
Rhizosphere (Antagonistic fungi)	-0.72 (P=0.28)	-	Uncorrelated
Pine- Litter (Antagonistic fungi)	-0.72 (P=0.28)	-	Uncorrelated
Pine-Litter (Entomopathogenic fungi)	-0.72 (P=0.28)	-	Uncorrelated

The Effect of Different Land Use to Species Richness and Abundance

The differentiation of land-use was not influenced of individual and species fungi abundance. Based on the results obtained in the Tukey test on the individual abundance of pine monoculture and pine-coffee intercropping in UB Forest, the probability value shows that it is not significantly different (Table 7). Based on these results, it can be concluded that the abundance of vegetation species in the two land uses is not significantly different. This phenomenon is thought to be caused by cultural practice employed by coffee farmers in pine-coffee intercropping. Weeding and shrubs were carried out and during field observations, pine monoculture had a denser vegetation density than pine-coffee intercropping. It is possible that the abundance of vegetation species in the two land uses is not significantly different thus contribute to the insignificant effect on the diversity and potential of antagonistic fungi in land use, especially on various types of land use in the UB Forest.

Based on the results of the Tukey test (Table 8) on the abundance of fungal species in the land use of pine-coffee intercropping and pine monoculture, there was no difference. The diversity of fungal species in pine-coffee intercropping and pine monocultures was thought to be unaffected by vegetation conditions. There was no difference in the vegetation conditions in the two land uses. This is supported by the results of the Tukey test on the abundance of individual vegetation. The types of vegetation in the two land uses are not much different, so there is no significant difference in the diversity of fungi. In addition, plant diversity contributes the composition, abundance, and biological activity (Reese, Lulow, David, & Wright, 2018).

Potential and Abillity of Exploration Result of Fungi

Based on exploration results, the highest number of antagonist fungal genera was found in the litter plot. They were slected and choosen to be tested for fungal isolates on the ability of inhibition. Testing of the growth inhibition of 16 isolates from exploration results in UB Forest with the pathogen *F. moniliforme* was observed for 7 days. Analysis of variance showed that the fungus which has the potential as an antagonist agent had a significant inhibitory effects againts the pathogen under in vitro testing on sugarcane after 3, 5 and 7 days observation (Table 9).

The control mechanism with biological agents on plant pathogenic fungi is generally divided into three types, i.e. competition for growth and nutrition, antibiosis, and parasitism (Baker & Cook, 1982). The success of antagonistic agents in inhibiting pathogens in this study is the competition and the suppression process carried out by isolates of the fungus *Penicillium* sp. 2 and *Acremonium* sp. 3 against the pathogen *F. moniliforme*. In addition, the antagonistic fungus can be categorized as potential antagonits when showing supression capacity more than 60% (Wicaksono Jati et al., 2022).

Table 7. Effect of vegetation diversity on individual and species fungi

Vegetation abundace	Rhizosphere		Pine litter	
	Antagonistic fungi	Entomopathogenic fungi	Antagonistic fungi	Entomopathogenic fungi
Individual	0.937	0.937	0.937	0.937
Species	0.186	0.186	0.186	0.186

Table 8. Effect of vegetation diversity on individual and species fungi

Fungi abundace	Rhizosphere		Pine litter	
	Antagonistic fungi	Entomopathogenic fungi	Antagonistic fungi	Entomopathogenic fungi
Individual	0.377	0.571	1	Not defined
Species	0.777	0.763	0.4709	0.318

When the percentage of supression was less than 30%, the antagonistic fungus is categorized to have a minimal inhibitory effect. The inhibiting mechanism of the two fungi isolates of *Penicillium* sp. 2 and *Acremonium* sp. 3 were competition with different abilities. There were several evidence that some fungi of the genus Penicillium inhibit the growth of pathogens only through a mechanism of competition and antibiosis (Harman, Khadka, Doni, & Uphoff, 2021; Köhl, Kolnaar, & Ravensberg, 2019; Liang et al., 2021; Win, Bo, Malec, & Fu, 2021).

Table 9. Inhibition of antagonistic fungi against pathogen

Isolate code	Isolate	Inhibition percentage (%)	
P3-PKCla	*Cladosporium* sp.	16.32	a
P3-PKMor	*Mortierella* sp.	24.31	a
P3-PKTri2	*Trichoderma* sp. 2	34.37	ab
P2-PKAcr1	*Acremonium* sp. 1	45	ab
P3-PKFus1	*Fusarium* sp. 1	13.35	ab
P3-PKAsp	*Aspergillus* sp.	35.12	bc
P3-PKAcr3	*Acremonium* sp. 3	66.56	cd
P3-PKPen2	*Penicillium* sp. 2	72.38	d

Remarks: Numbers accompanied by the same letter in the same column show no significant difference based on Duncan's test with an error rate of 5%

Table 10. Mortality percentage of *T. molitor*

Isolate Code	Fungi species	Mortality rate (%)	
Control	-	10	a
PB1	*Aspergillus* sp. 1	36.67	cd
PC1	*Aspergillus* sp.1	36.67	cd
PB2	*Aspergillus* sp. 2	30.00	b
PC2	*Aspergillus* sp.2	30.00	b
PA1	*Fusarium* sp. 1	33.33	bc
PD1	*Fusarium* sp. 1	40.00	cd
PC3	*Fusarium* sp. 2	33.33	bc
PD2	*Fusarium* sp. 3	26.67	ab
PA2	*Acremonium* sp. 1	26.67	ab
PD3	*Acremonium* sp. 2	26.67	ab
PC4	*Acremonium* sp. 3	30.00	b
PC5	*Acremonium* sp. 4	26.67	ab
PA3	*Acremonium* sp.4	50.00	de
PB3	*Gliocladium* sp. 1	23.33	ab
PB4	*Gliocladium* sp. 2	26.67	ab
PC6	*Gliocladium* sp.2	53.33	de
PA4	*Gliocladium* sp. 3	33.33	bc
PA5	*Cylindrocarpon* sp.	26.67	ab
PA6	*Paecilomyces* sp. 1	63.33	e
PD4	*Paecilomyces* sp. 2	60.00	e
PD5	*Mortierella* sp.	40.00	cd
PB5	*Geotrichum* sp.	23.33	ab
PD6	*Gongronella* sp.	33.33	bc
PD7	*Humicola* sp.	26.67	ab

Remarks: PA = Plot A; PB = Plot B; PC = Plot C; PD = Plot D; Each treatment invested 10 *T. molitor* larvae. Numbers followed by the same letter in the same column were not significantly different according to DMRT ($\alpha \leq 5\%$)

The best growth of fungal colonies were *Penicillium* sp. 2 and *Acremonium* sp. 3 as viewed on the 3rd days after incubation (DAI). The development of colony was observed on the 3rd, 5th and 7th DAI. The antagonistic mechanism of *Penicillium* sp. 2 and *Acremonium* sp. 3 was competition and had relatively the same inhibitory rate. Based on observations for 7 DAI, the area of *F. moniliforme* was reduced by an average of 1-3 mm per day.

The results of the exploration of entomopathogenic fungi in the rhizosphere and litter area showed that the fungi found in the litter area had a higher diversity index than the root area. Thus, the isolates of entomopathogenic fungi found in the litter area were selected to be tested for pathogenicity. The results of the application of pine leaf litter fungus suspension caused changes in the behavior and morphology of *Tenebrio molitor*. The changes in the behavior of *T. molitor* are characterized by weakened larvae movements. Morphological changes of *T. molitor* are characterized by a change in color. The overgrown mycelia might result in the dead of larvae.The increase in mortality of *T. molitor* larvae occurred on the second to tenth day. According to Li, Liu, Lewis, & Tarasco (2016) insect death due to entomopathogenic fungi occurs 2 to 14 days after infection, but can also occur in less than 24 hours. The rate of insect mortality depends on insect susceptibility, density and viability of fungal conidia. Entomopathogenic fungi can work effectively depending on insect age, developmental stage, insect cuticle surface and spore density (Ortiz-Urquiza & Keyhani, 2013; Sharma & Sharma, 2021).

Observations of fungal pathogenicity against *T. molitor* showed different values. The results of observations at 10 days after planting (DAP) showed that the highest mortality of *T. molitor* was obtained in the treatment of *Paecilomyces* sp.1 with a percentage of 63.33%, followed by *Paecilomyces* sp. 2 (60%), *Gliocladium* sp. 4 (53.33%) and *Acremonium* sp. 4 (50%). In the control treatment, the mortality of *T. molitor* larvae was 10% (Table 10). The fungal genus Paecilomyces had the highest level of pathogenicity compared to other fungal genera (63.33%). The genus Paecilomyces is an entomopathogenic fungus that can infect insect pests. The highest spore concentration of *Paecilomyces* sp., were found to cause percent mortality of 51% (Rishi, Pandey, & Kumar, 2016). The fungal genera Cylindrocarpon, Aspergillus, Fusarium, Acremonium, Gliocladium, Geotrichum, Mortierella, Gongronella, and Humicola are opportunistic fungi that are capable to infect weak or injured larvae. Sun & Liu (2008), stated that the group of opportunistic pathogens are fungi other than insect pathogens which have the characteristics of fast-growing fungi and can infect weak insects. Mortality in controls was suspected to be physical susceptibility of *T. molitor* larvae which had thin and soft cuticles after molting.

Factors Affecting Mortality

Larval susceptibility is a factor causing mortality. The *T. molitor* larvae used in the pathogenicity test were those that had just procceded molting stage. Newly molted larvae have a thin and soft cuticle, thus make them susceptible to infection. These condition was predictably to be the factor in the occurrence of mortality in the control and treatment of antagonistic fungus. The thin cuticle layer have made the fungus easly penetrate into the larva's body. Li, Liu, Lewis, & Tarasco (2016) stated that in general insects can be infected by entomopathogenic fungal conidia through contact with the cuticle or through gaps between body segments. According to Shahid, Rao, Bakshs, & Husnain (2012), conidia of entomopathogenic fungi generally infect insects. When the conidia attach to a suitable host, they germinate, initiating the recognition reaction and enzyme activation. During penetration, the fungus produces enzymes to be able to degrade the cuticle. A number of cuticle-degrading enzymes are produced during penetration, called proteases, lipases, and chitinases (Smith & Grula, 1981). In addition to larval susceptibility, host compatibility also affects insect mortality.

Another factor that causes mortality of *T. molitor* larvae is the density and viability of the fungus. Fungal isolates from pine leaf litter had different conidia density and viability. While Li, Liu, Lewis, & Tarasco (2016) state that the number of fungal conidia is related to the level of concentration. The higher of concentration, the higher of mortality. Viability (germination) of fungal conidia is one of the factors that determine the success of fungi in infecting insect pests. The higher the conidia concentration, the more conidia have direct contact with the insect body, so that the penetration and infection of fungal conidia that successfully germinate will be higher (Huang et al., 2021).

CONCLUSION

The diversity of entompathogenic and antagonistic fungi was higher in the pine-coffee intercropping land use. The litter plots had higher fungal diversity than the rhizosphere. The isolate *Acremonium* sp. 3 and *Penicillium* sp. 2 has the highest inhibition. *Paecilomyces* sp. 1 and *Paecilomyces* sp. 2 has the best level of pathogenicity and mortality. The factors of temperature and humidity did not affect the diversity of fungi. For the further research, it is recommended to analyze the role of soil temperature and soil moisture on diversity of fungi.

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