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ENTOMOPATHOGENIC FUNGI FROM COCOA RHIZOSPHERE IN INDONESIA

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ABSTRACT

Cocoa (*Theobroma cocoa* L.) is a native Asian plant, and is generally grown in large quantities as a result of production in Pacitan. The study was conducted on 2 types of land, namely land recommended by the Department of Agriculture and land managed by traditional from farmers. In 2 fields to identify the diversity of entomopathogenic fungi and clarify the chemical content in the soil. Research from June to November 2019. Seven variables were also investigated such as acidity, nutrient, Relationship Diversity Index of the Organic Matter and Soil Fertility Genus, Distribution, Quantity, and Composition of the Genus of Soil Fungi. The results showed the highest Shannon diversity index of cultivated land according to tradtional, the index of diversity of cultivated land according to traditional was higher than conventional. Genus diversity in cultivated land according to traditional is higher (7 genera). Rather than land conventional (6 genera). Land on farmed cocoa land according to traditional is more fertile than recommended cocoa land. Rhizosphere fungi diversity level becomes bioindicator of soil fertility.

Keywords: biodiversity, soil fungi, soil fertility, cocoa, pacitan.

INTRODUCTION

Pekarangan is one form of complex agroforestry which has a very diverse structure and composition. The function of the *pekarangan* is as a food source, wood producer, trade commodity, spices, medicines, raw materials and ornamental plants [1]. Biodiversity is variation between organisms that exist in different ecosystems, including diversity within a species and between species, and comparative diversity between ecosystems. Soil biodiversity reflects the diversity of living organisms in the soil. Soil biodiversity is drastically reduced when forests are converted to agricultural land, and when agricultural land use is intensified. These organisms interact with each other and with plants and small animals to form biological activities. They include bacteria, fungi, protozoa, insects, worms and other invertebrates. Their numbers, types and populations are very abundant. For example, the soil that was extracted was then identified as containing billions of bacteria, fungi, arachnids and worms. Microorganisms in the soil have an important role in maintaining soil fertility because microorganisms have a role that is as a decomposer. The main function of this decomposer is to weather residues: immobilizing nutrients in their biomass, producing new organic compounds as a source of nutrition and energy for other organisms [2]. Collaborative functions of soil microorganisms will produce nutrients that can be used by plants.

Soil microorganisms as biological components play a role to support soil health. Soil fungi are important microorganisms in the process of pest control which can reduce agricultural production. Soil fungi can infect insects and cause death, so soil fungi can be used as bioinsecticides or biological agents. Biological control using pathogenic fungi for insect pests has the potential to be developed because it has a positive effect on the environment because it does not attack natural enemies [3]. The presence of PBK (fruit borer - *Conopomorpha cramella* S.) and BBK (fruit rot - Phytophtora palmivora) diseases in cocoa can be reduced by biodiversity in ZWCC gardens. Cocoa (*Theobroma cocoa* L.) is one of the regional commodities that has an important role in the national economy, especially as a source of income and foreign exchange for the country [4]. In addition to cultivating cocoa in a sustainable and healthy manner, it is important to design agroforestry systems that resemble the structure of local native forests, promote high biodiversity, and stratify production systems [5]. The number of microorganisms in the soil sample from the cocoa area is higher than in the cultivated cocoa area according to traditional [6]. Brown area (*Theobroma cocoa* L.). In the cocoa region (*Theobroma cocoa* L.). Sulawesi, shows "there is a relationship" between soil diversity and cocoa pest attacks [7]. In Pacitan, Cocoa is one of the plantation commodities developed by 820 families, in addition to other food crops [8]. The area of developed cocoa plantation is 6089 ha [9].

On our preliminary studies in the last 2019, there are some cocoa ecosystem such as *pekarangan* ecosystem in Pacitan. Based on productivity and soil fertility are difference diantara banyak ecosystem. The purpose of this study was to analyze soil fertility with indicators of soil microorganisms in the yard of cocoa land with a cultivation system according to traditional and

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conventional in Pacitan Regency. Knowing the diversity of the genus of soil fungi in the cocoa yard with cultivation systems according to traditional and conventional of the yard ecosystem. Determine management model conventional for typology of the cocoa area in the Pacitan District yard (sustainable cocoa cultivation in the community cocoa yard, Pacitan Regency, East Java).

MATERIALS AND METHODS

Materials

The materials used include soil samples, sterile distilled water, 70% alcohol, SDAY media, plastic wrap, tissue and NaOCl. The tools used are Petridish, test tubes, measuring cups, autoclaves, enlemeyers, orbital shakers, bunsen, L sticks and scales.

Methods

Soil sampling was carried out in Pekarangan Cocoa ecosystem at Sanggrahan Village, Kebonagung District, Pacitan Regency. Soil mold isolation was carried out at the Laboratory of Biological Control, Faculty of Agriculture, University of Brawijaya, and soil analysis was carried out at the Soil Laboratory, Agribusiness and Horticulture Development Unit, Bedali, Lawang. The study was conducted from 1 June to 7 December 2019.

A. Research Implementation

a) Soil sampling

Soil samples were taken near the roots of the cocoa plant using a shovel. Soil samples were taken at 5 points with a diagonal system [10]. Rhizosphere part soil was taken with a depth of 30 cm [11]. From 5 soil sample points, each soil was mixed into one. Soil samples are inserted into a 25x30 cm plastic clip that has been removed and labeled and then taken to the laboratory to be isolated and identified macroscopically and microscopically. Sample locations are distinguished based on differences in cultivation systems namely cultivation according to traditional and conventional.

b) Isolation of soil fungi

Performed by the multilevel dilution method. Every 10g of sample was dissolved with sterile water so that 100 ml of soil suspension was obtained by using an orbital shaker for 20 minutes at a speed of 150 rpm. The suspension is then diluted immediately in series by mixing 1 ml of ground suspension with 9 ml of sterile aquades in a test tube so that a 10-1 dilution is obtained. The 10-1 dilution suspension is diluted by mixing 1 ml of a 10-1 solution with 9 ml of sterile aquades in a test tube so that a 10-2 dilution is obtained. Dilution continues until the dilution level is 10-5. For dilutions of 10-3 to 10-5, 1 ml is taken and then it is cultured in a Petri dish containing SDAY (Sabouraud dextrose agar) with solid yeast extract. Breeding results were observed seven days after capturing (HSP) assuming all microbes had grown. Work is done carefully and repeated until it gets pure culture. The final stage is to identify soil fungis.

c) Identification of soil fungus

Purified and prepared soil fungi isolates were macroscopically and microscopically observed and identified with guidance on fungal identification namely [12], [13] and according to research journals. Macroscopic observations were made by observing the morphological appearance of fungal colonies which included the color of the colony, the shape of the colony, and the pattern of colony distribution in a petri dish (concentric and non-concentric). Microscopic characterization is based on the reference books [12], [13].

B. Data Analysis

Data analysis was carried out qualitatively and quantitatively. Quantitative analysis uses the following formula:

Shannon Wienner Diversity Index [14] is:

$$H' = -\sum_{t=1}^{s}\left(\frac{ni}{N}\right) ln \left(\frac{ni}{N}\right)$$

H' = Shannon Wienner Diversity
S = Number od Species
ni = Number of individuals of type i
N = Total number of individuals

a) Evenness index

Evenness index is used to measure community balance. This is based on a measure of the similarity of the number of individuals between species in a community [15]. The calculation of uniformity (E) is as follows:

$$E = \frac{H'}{\ln s}$$

E = Uniformity index
H' = Shannon Wienner Diversity
s = Number genus / species

Qualitative analysis is done to determine the dominance of a species in the ecosystem. The formula based on the Shipmson Dominance Index is used, the following:

$$\sum_{i=1}^{s}\left(\frac{\mathrm{Ni}}{N}\right)^2$$

C = Domination Index
Ni = Number of individuals of type i
N = Total number of individuals

C. Soil fertility analysis

Soil fertility analysis includes: (1) soil organic matter is carried out to determine the content of organic matter in the cocoa community area of Pacitan district; (2)

Soil pH analysis is carried out to determine the pH of the soil; and (3) soil macro / micro element content.

RESULT AND DISCUSSIONS

Soil Acidity (pH)

Soil pH has something to do with the land's physiographic position where on lower land physiography, the soil's pH will be higher (closer to base) than the land above it. This is caused by the process of washing and run-off (nutrient elements) (from fertilizer) on the land to the land under the water [16]. The pH on the conventional cocoa farm (R2) is lower than the cultivated cocoa farm according to traditional due to differences in physiographic position. The physiographic position of the recommended cocoa field is higher than that of traditional cultivated cocoa land. Soil fungi will be able to grow optimally in soils with a pH in the range of 5-6 [12]. R2 has the lowest acidity and the lowest pH (4.84) which causes the fungus to not live so that the value of the H' is low. Apart from its physiographic position, the application of bokashi fertilizer on recommended land results in acidic soil pH. This is caused by the bokashi being applied to decompose to produce CO2. If CO2 reacts with soil pH it has to do with the physiographic position of the land where on lower land physiography the soil pH will be higher (closer to the base) than the land above it. This is caused by the bokashi being applied to decompose to produce CO2, when CO2 reacts with water that forms H2CO3 and will break down into $HCO3^-$ $+H^+$ which causes the soil to become acidic [16]. Based on Table-1 shows that differences in cocoa cultivation systems affect soil pH, soil organic matter, nutrients and H'.

Table-1. pH percentage of organic material, nutrients and shannon-winner diversity rhizosfer fungi (H') on cocoa at *Pekarangan* ecosystem.

Type of cultivation at *Pekarangan* ecosystem	pH	Organic Matter (%)	Nutrient			H'
			N (%)	$P_2 0_5$ (ppm)	K (me)	
Conventional (*)	5.48	3.83	0.10	11	0.70	1.46
Conventional (**)	4.84	3.72	0.10	10	0.90	0.86
Traditional 1 (*)	5.48	5.34	0.10	13	0.70	1.35
Traditional 2 (**)	5.73	5.07	0.16	8	0.30	1.76

Data source: Soil Laboratory in UPT for Food Crops and Horticulture Development, Lawang-Malang Indonesia, November 2019
Note: * = pest free area
** = area attacked by pests

Nutrient (N,P,K)

Essential plant nutrients include N, P, K. The availability of nutrients is influenced by the cultivation system. The effect of applying bokashi fertilizer on the conventional system has an effect on the availability of element K, where on the recommended land the K content is higher than the cultivated land according to traditional. Bokashi application on land causes K values in very high criteria [16]. The highest availability of phosphorus (P) on land in cultivated land according to traditional *. The content of N elements in all rhizosphere of cocoa R1, R2, and P1 is classified as very low, whereas P2 is classified as low. This indicates that the biochemical processes in the soil are low [17].

Relations Diversity Index Genus Fungi Soil Organic Matter and Soil Fertility

The index of diversity of soil fungus genus is an indicator of soil fertility. This is because the presence and persistence of soil fungi are closely related to the soil disturbance. Fertile land always has a high H' value. This is because fertile soils are always able to be ideal growing media for various microorganisms, including soil fungi. Soil with high H' content will occur the decomposition process, nutrient cycling and decomposition of other organic and inorganic compounds. Based on Table-1, all of the studied areas have different H' values, but none are classified in the high category (> 3), so that none of the observed areas have high fertility. However, in cultivated cocoa land according to traditional has a higher H' value compared to conventional cocoa land (Figure-1). This condition is related to the accumulation of organic material which is ideal for the life of biodiversity of soil microorganisms. Organic matter has a positive influence, which means more organic matter in the soil, so the level of soil fertility will also increase. This is because organic matter is a source of energy for soil microorganisms to carry out the process of decomposition, nutrient cycling and decomposition of organic and inorganic compounds, so that it becomes a factor to improve soil fertility [16]. Higher fungus diversity in the cultivation cocoa rhizosphere according to traditional also indicates that cultivated cocoa land according to traditional is more fertile than recommended cocoa soil cultivation.

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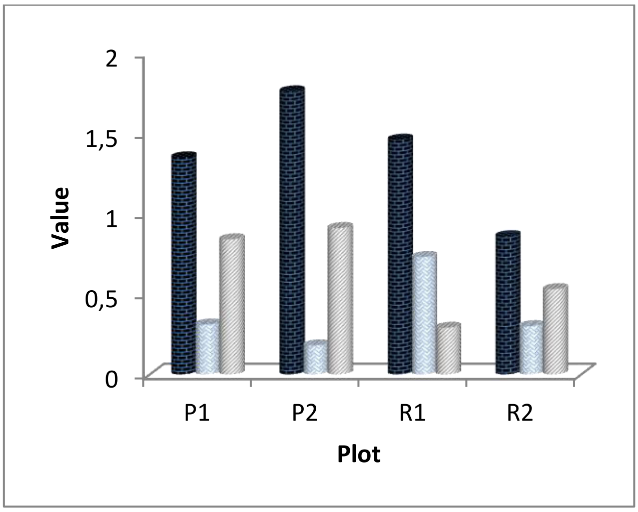

Figure-1. Value of diversity index, dominance index and evenness index of fungi in the rhizosphere of cultivated cocoa according to traditional and conventional recommendations.

Soil fungi as bio-indicators in soil fertility play an important role in the overhaul of soil organic matter which is crucial for nutrient availability in fertilizing the soil [18]. Organic matter acts as a provider of nutrients such as N, P and S for plants, a source of energy for soil microorganisms, and as a buffer against changes in pH [19]. The more diverse soil fungus, the decomposition process of organic matter into micro and macro nutrients will be faster so that soil fertility will increase and cocoa production is high. The relationship between soil factors, especially between pH and organic matter with fungal diversity index was analyzed by Pearson Correlation (Table-2).

Table-2 shows the correlation between pH and BO yielding 0.670. This figure shows the strong correlation between pH and BO because the value is above 0.5. While the "*" sign indicates that the higher the H', the higher the pH will be and vice versa. For the correlation between pH and H', yielding 0.981. This figure means that the two variables have a very strong correlation because it is above 0.5. As for the correlation between BO and H', the figure is 0.579. This figure means that both variables have a strong correlation because they are close to 0.5. A significant relationship was shown from 0.981 (pH with H'; 0.670 (Ph with BO); 0.579 (BO with H').

Table-2. Person Correlation Coefficients between pH, Organic Matter and H'.

Correlations			
	pH	**Organic Matter**	**H'**
pH	1	0.670	0.981*
		0.330	0.019
	4	4	4
BO	0.670	1	0.579
	0.330		0.421
	4	4	4
H'	0.981*	0.579	1
	0.019	0.421	
	4	4	4
*. Correlation is significant at the 0.05 level (2-tailed)			

Distribution, Quantity, and Composition of the Genus of Soil Fungi in Cocoa Rhizosphere Traditional and Conventional

The distribution of soil fungus illustrates the existence of the fungus genus in each cocoa plantation system. The distribution, number and composition of the genus of soil fungi in the rhizosphere of traditional and conventional different (Table-3). In the recommended cocoa rhizosphere, 6 genus fungis were found with a total of 192 individuals, whereas in the non-intensive cocoa rhizosphere, 7 genus fungis were found with a total of 77 individuals. In intensive cocoa rhizosphere fungi of the genus *Mucor* were not found. This relates to environmental factors (Table-4). In the recommended cocoa rhizosphere, 6 genus fungis were found with a total of 192 individuals, whereas in the non-intensive cocoa rhizosphere, 7 genus fungis were found with a total of 77 individuals. In intensive cocoa rhizosphere fungi of the genus *Mucor* were not found. This relates to environmental factors (Table-4).

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Table-3. Distribution, amount and composition of fungi genus in cocoa rhizosphere conventional and traditional.

Genus	Conventional		Traditional	
	R1	R2	P1	P2
Aspergillus	14	30	5	3
Verticillium	78	10	5	6
Metarhizium	31	-	2	1
Beauveria	3	19	11	13
Fusarium	23	44	-	12
Paecilomyces	19	1	1	8
Mucor	-	-	-	10
Amount	168	24	24	53
Total	192		77	

Table-4. Results of edafic factor measurements in conventional and traditional system.

Parameter	Conventional		Traditional	
	R1	R2	P1	P2
Temperature (^{o}C)	26±29	26±30	14±25	16±27
Humidity (%)	20±45	20±80	40±80	20±70

The large number of fungi genus in the soil can be a bio-indicator of the fertile soil [20]. The number of genera on cultivated cocoa land according to traditional is more than conventional of cultivated cocoa land, this means that the land on cultivated cocoa land according to traditional is more fertile than the land on conventional cultivation land.

CONCLUSIONS

The conclusions of this research, as follow:

a) The entomopathogenic fungus genus of the cocoa rhizosphere is *Aspergillus, Verticillium, Metarhizium, Beauveria, Fusarium, Paecilomyces, Mucor.*
b) The cocoa yard with traditional has a higher index of soil fungi diversity than conventional at *pekarangan* ecosystem.
c) The development of cocoa in *pekarangan* ecosystem by the concept of BAP (Biodiversity Agriculture Practice), which is the concept of agriculture based on biodiversity conservation policies.
d) Diversity of soil fungi can be used as a bio-indicator of soil fertility. The higher the diversity index, the higher the fertility of the soil.

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