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Biodiversity of Arbuscular Mycorrhizal from Rhizosfer Soil Contaminated Petroleum Hydrocarbon in Bojonegoro, East Java

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ABSTRACT

Arbuscular mycorrhiza is one of fungi which symbiosis with plant roots. Arbuscular mycorrhiza is influenced by environmental factors, one of them is hydrocarbon. Bojonegoro is one district as producer petroleum hydrocarbon in Indonesia. This research aim to analyse biodiversity of arbuscular mycorryzha in the rhizosfer soils contaminated petroleum hydrocarbon in Bojonegoro. Sampling was carried out using simple randomized method. It was used descriptive method by Microsoft Excel 2007, whereas correlation between relative abundance relative and frequency index was analyzed by SPSS 22.0. Results of this research was found and identified 13 type of plants in there with 32 type of arbuscular mycorrhiza and 979 spores density. Five genus were find are Glomus, Gigaspora, Acaulospora, Enthrospora, dan Scutellospora. Diversity index of mycorrhiza is 2,82 (medium) with eveness 0.81 (high), and index of dominance 0.086. This indicates that diversity mycorrhizal arbuscular within the community stable, good, and without dominance species.All that condition indicate in the worst condition(TPH 5600 mg/kg), arbuscular mycorrhiza canadaptive and tolerance with this condition and still can do symbiosis with plants.
KEYWORDS: arbuscular mycorrhyza, Bojonegoro, petroleum hydrocarbon

INTRODUCTION

Arbuscular mycorrhiza is one type of fungi that associate with plant roots. These symbiosis with roots more than 80% of plant species [1]. In this symbiosis, arbuscular mycorrhiza obtain carbon source of photosynthesis in plants about 20% [2]. While mycorrhizal able to expand the field of absorption of water and nutrients, especially phosphorus from the soils [3-5] through a smaller diameter hyphae (2-15 m) [6] and longer (5-50 m) [7] compared to the plant roots.

Besides that, mycorrhizal arbuscular can release glomalin exudate. Glomalin is hydrophobic glycoprotein secreted mycorrhizal arbuscular as a protector of hyphae when absorb water and minerals [8].The concentration of high glomalin favorable to the ground for forming the aggregated soils, accumulate carbon, and reduce erosion [9,10]. Depositions of glomalin contributes around 5 to 10 percent organic soil carbon (SOC) [11] and 5 to 13 percent N soils [12].

In their stage development, arbuscular mycorrhiza is influenced by environmental factors. They are temperature, pH, moisture content, nutrients, and toxic compounds such as heavy metals and hydrocarbons. One type of hydrocarbons are toxic for arbuscular mycorrhiza is petroleum hydrocarbons. The impacts of various abiotic stresses on biodiversity, abundance and development of AMF to morphological, biochemical and molecular mechanisms implemented by AMF for survive in the presence of these stresses was reviewed by [13].

Bojonegoro district is one of the oil producer in Indonesia. This area has estimated reserves of about 445 million barrels of oil. The high oil content in Bojonegoro which as its main component hydrocarbons may have a negative impact on the diversity of mycorrhizal. This is due to Polycyclic Aromatic Hydrocarbon (PAH) in petroleum hydrocarbons that are mutagenic and carcinogenic for soil organisms [14]. The toxicological properties of the compound is due to the lipophilic nature which may affect the structure of cell membranes and low water solubility [15].

Beside that, the characteristics of hydrophobic and non-polar nature of petroleum that can promote accumulation and the presence of these contaminants in the soil [16]. Petroleum hydrocarbons that enter the soil environment can cause damage to the soil physical and chemical structure because the compound is able to bind to soil particles and organic matter contained in the soil, causing the formation micropollutant hard ground [17].

According to research by Desalme et al. [18], PAH can effect to arbuscular mycorrhyza especially in the top part of soils. But there are mycorrhizal spores on contaminated land and colonization of the roots shows that there is a mechanism was performed by mycorrhiza and plants to adapt with environments contaminated petroleum hydrocarbons. Diversity of arbuscular mycorrhyza in the extreme area have recently been reported.

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Research by Aguilera et al. [19]shows that there is an effect of wheat cultivar on AM fungal diversity and an important level of AM fungal specificity in Al tolerant wheat cultivars grown in Andosols with high Al^{3+} levels.

Information about isolates mycorrhizal arbuscular and their diversity in the soil contaminated petroleum hydrocarbon can play an important role in the process of bioremediation and revegetation polluted contaminated soils. For that reason, this study will do analyzed about diversity of arbuscular mycorrhiza isolates and the host, especially in the soils contaminated petroleum hydrocarbon.

MATERIALS AND METHODS

Study Analysis

This research conducted in July 2016 until December 2016 in the microbiology laboratory, Airlangga University, Surabaya. Samples taken from polluted soil petroleum hydrocarbon in the Bojonegoro district, East Java, Indonesia with broad plot 100 m^2 (10x10 m). The sample was conducted using simple random sampling technique [20].

Sampling soil samples collected from the surface polluted soil petroleum hydrocarbons by 20 cm deep in rhizosfer from the different types of plants using soil corer. Soils drained and stored to process of analysis done. Rhizosfer used to extract spores and know characteristic of soil. One group of soils collected from the point of sampling as many as 100 grams for analysis spores. While to analyze physic-chemical several soils of each different vegetation mixed and than are packed using plastic packaging. Sample soils drained and stored on temperature 4°C for analysis [21].

Procedures

Isolation of mycorrhizal arbuscularspores using wet sieving method and than decanting and sucrose centrifugation [22].Soil samples had been obtained from rhizosfer in the each of plant vegetation soil contaminated petroleum hydrocarbon. Soil was taken 100 gr and moistened with water 500 ml. And than it is mixed and settled for 10 minutes until particles were settled.

The next, it was filtered with sieve by sized 300, 180, 78, 63 and 38 µm. Filtrate of the last sieve moved into a tube centrifuge and added solution sucrose 60% then was done centrifuge for 5 minutes in 2000 rpm. Supernatant formed cast in the last filter (38 µm) and flushed with water to clean spores from sucrose. Then spores on the filter was moved in a petri dish for observed with a stereo microscope (magnitude 84X).

Observation morphological characteristics mycorrhizal arbuscular based on manual by using invam website [23] (http:// invam.caf.wvu.edu / myc _ info / taxonomy). Identification done by using a microscope compound with magnification 400x [24]. Identification mycorrhizal done based on spores morphology characters covering a form of spores, spores colour, number of cell walls, and ornamentation [22].

Staining mycorrhizal roots and type colonization mycorrhizal arbuscular roots taken, washed clean, and cut into pieces ±1 cm. The pieces roots and stored in the FAA before staining process. Then it was soaked in KOH and heated by autoclave for ±15-20 minutes with the temperature 121°C and pressure 1 atm. After that, it was washed by water. The next, roots is bleached with hydrogen peroxide alkaline to colored whitish and washed with water again. The next step, roots submerged into HCl1% for ±1 minute and the last in acid fuchsin for staining. And then pieces of roots included in a bottle fial that contains washing solution and heated for ±15 minutes with temperature 121°C.After that, roots arrange on the object glasses to observed type of mycorrhizal arbuscular colonization with compound microscope (magnitude 400X).

Calculation infection done by means of counting the number of hyphae, arbuscular, vesicles and spores that are coloured. Percent roots infected calculated based on the formula:

Colonisation = JAT/JSP

JAT = the number of roots infected, JSP = the number of the entire chunk of the root of which observed. Levels percent infection are 1 = 0-5%, 2 = 6-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-100% [21,25].

Data Analysis

Identification of plants based on APG III [29]. The other date which were analyzed are spores density and colonization, relative abundance, frequency index, richness, eveness, and index of dominance using descriptive method by Microsoft excel 2007. Corelation between relative abundance and frequency index was analyzed by SPSS 22.0.

Soil was analyzed physical and chemically. Temperature and humidity soil use tester were measured. pH was measured using pH meter. Analysis soil types, soil nutrients were measured in the soil laboratory, Brawijaya University, Malang.

RESULTS

Soils in the sampling area containing organic matter of 1,68% and C-organic 0,97%. Results show that pH values is 6.7 and temperature 32°C. Total Petroleum Hydrocarbon (TPH) contents in soils are 2,500 mg/kg which was caused due to oil drilling are traditionally. Texture of soils is loam dusty. They are sand 2%, dust 73% and clay 25%.

In this study, we were found 13 plants from 10 families, they are Passifloraceae, Poaceae, Salicaceae, Caprifoliaceae, Asteraceae, Zingiberaceae, Apocynaceae, Tiliaceae, Lamiaceae, and Euphorbiaceae. Every plants have found different spores density (table 1).

Lonicera japonica, Muntingia calabura, Andropogon acciculatus, and *Imperata cylindrica* are four plants with the highest spore density (more than 100 spores/100 gram rhizosphere). Beside that, *Imperata cylindrica* has highest spore richness than other plants (6 type spores) (table 1).

A total of 979 spores of arbuscular mycorrhiza were wet sieved from 13 rhizosphere soil samples collected, with 32 arbuscular mycorrhiza species were identified (table 2 and figure 1). Genus of arbuscular mycorrhizal spores with the highest relative abundance is come from genus *Glomus* (60.8%), followed *Acaulospora* (17.8%), *Gigaspora* (14.5%), *Enthrospora* (4.49%), and *Scutellospora* (2.45 %).

Table 1. Identification of plants and colonization stage

Code	Host Plants	SR	C	SD
H1	*Passiflora foetida*	3	4	8
H2	*Imperata cylindrica*	6	5	130
H3	*Flacourtica indica*	5	5	64
H4	*Lonicera japonica*	3	5	204
H5	*Bidens spilosa*	3	3	88
H6	*Hedychium coronarium*	3	4	37
H7	*Catharanthus roseus*	5	5	47
H8	*Muntingia calabura*	4	5	185
H9	*Tectona grandis*	4	5	74
H10	*Poa annua*	2	5	11
H11	*Manihot utilisima*	2	5	27
H12	*Vibirnum* sp.	1	5	5
H13	*Andropogon aciculatus*	4	5	163

SR=spores richness; C= colonisation; SD=spores density

Glomus also have the highest frequency index. We were found 15 type from this genus (84,62%). This value was followed from genus *Gigaspora, Acaulospora, Enthrospora, and Scutellospora* (table 2).

Table 2. Relative abundance and frequency index of arbuscular mycorrhiza

AM fungal class and order	Family	AMF	RA	FI
Glomeromycetes				
Glomerales	Glomaceae	***Glomus***	**60.78**	**84.62**
		Glomus multicaule	1.74	7.69
		Glomus clarum	2.25	7.69
		Glomus fasciculatum	1.33	7.69
		Glomus geosporum	0.10	7.69
		Glomus mosseae	6.13	15.38
		Glomus etunicatum	11.34	7.69
		Glomus sp.1	1.53	15.38
		Glomus sp.2	0.72	23.08
		Glomus sp.3	0.10	7.69
		Glomus sp.4	4.49	15.38
		Glomus sp.5	0.51	7.69

		Glomus sp.6	21.55	30.77
		Glomus sp.7	2.25	7.69
		Glomus sp.8	2.96	7.69
		Glomus sp.9	3.78	7.69
Gigasporales	Gigaporaceae	***Gigaspora***	**14.50**	**53.85**
		Gigaspora pellucida	3.98	15.38
		Gigaspora margarita	4.09	7.69
		Gigaspora calospora	5.72	7.69
		Gigaspora sp.1	0.20	23.08
		Gigaspora sp.2	0.10	15.38
		Gigaspora sp.3	0.41	7.69
Glomerales	Acaulosporaceae	***Acaulospora***	**17.77**	**38.46**
		Acaulospora scrobiculata	0.72	15.38
		Acaulospora delicate	6.03	7.69
		Acaulospora sp.1	0.20	7.69
		Acaulospora sp.2	0.10	7.69
		Acaulospora sp.3	1.94	7.69
		Acaulospora sp.4	2.04	7.69
		Acaulospora sp.5	6.74	7.69
Glomerales	Acaulosporaceae	***Enthrospora***	**4.49**	**15.38**
		Enthrospora infrequens	0.10	7.69
		Enthrospora sp.1	4.29	7.69
		Enthrospora sp.2	0.10	7.69
Gigasporales	Gigaporaceae	***Scutellospora***	**2.45**	**7.69**
		Scutellospora sp.1	2.45	7.69
		Sum	**100**	

RA=relative abundance ; IF= frequency index

DISCUSSION

The spores density of arbuscular mycorrhiza (table 1) were found on the sampling area in 13 type vegetation is 21 spores/grams (low density based on invam). Diversity of arbuscular mycorrhiza influenced by several factors, among of them is environmental factor (plants and soils).

Among the variabilities, soil moisture, organic compounds, and soil texture can effect to spores density and richness. Moisture soils is as much as 1,5 indicate that the level of the water that can be stored by soils. This is related to soil texture namely clay dusty that tends to able to withstand more water. This condition will affect spore mycorrhiza because humid conditions will trigger the development of fungal pathogen that can affect the quality and quantity of mycorrhiza spores.

Organic compound is like Total Petroleum Hydrocarbon (TPH) is one of the factors that also contributed to the low density of the spores. According to review by Lenoir et al. [13] indicating that the presence of crude oil in the soil can affect to arbuscular mycorrhiza colonization in the roots. Beside that, one compound of crude oil, PAH (Polyaromatic Hydrocarbon) also inhibit spore germination hypal elongation, and sporulation. Research by Desalme et al. [18] also shown that phenanthrene is come from atmosphere can decrease infectivity of arbuscular mycorrhiza in the surface area in soils.

Aside from the environment, spores density also were affected by type and stages of plants. In this study, we were found 13 plants from 10 families. Every plants have found different spores density. Spores was resulted by mycelium external when do symbiosis with plants. Large symbiotic also was determined by the system plant roots so this affect density of mycorrhiza spores indirectly.

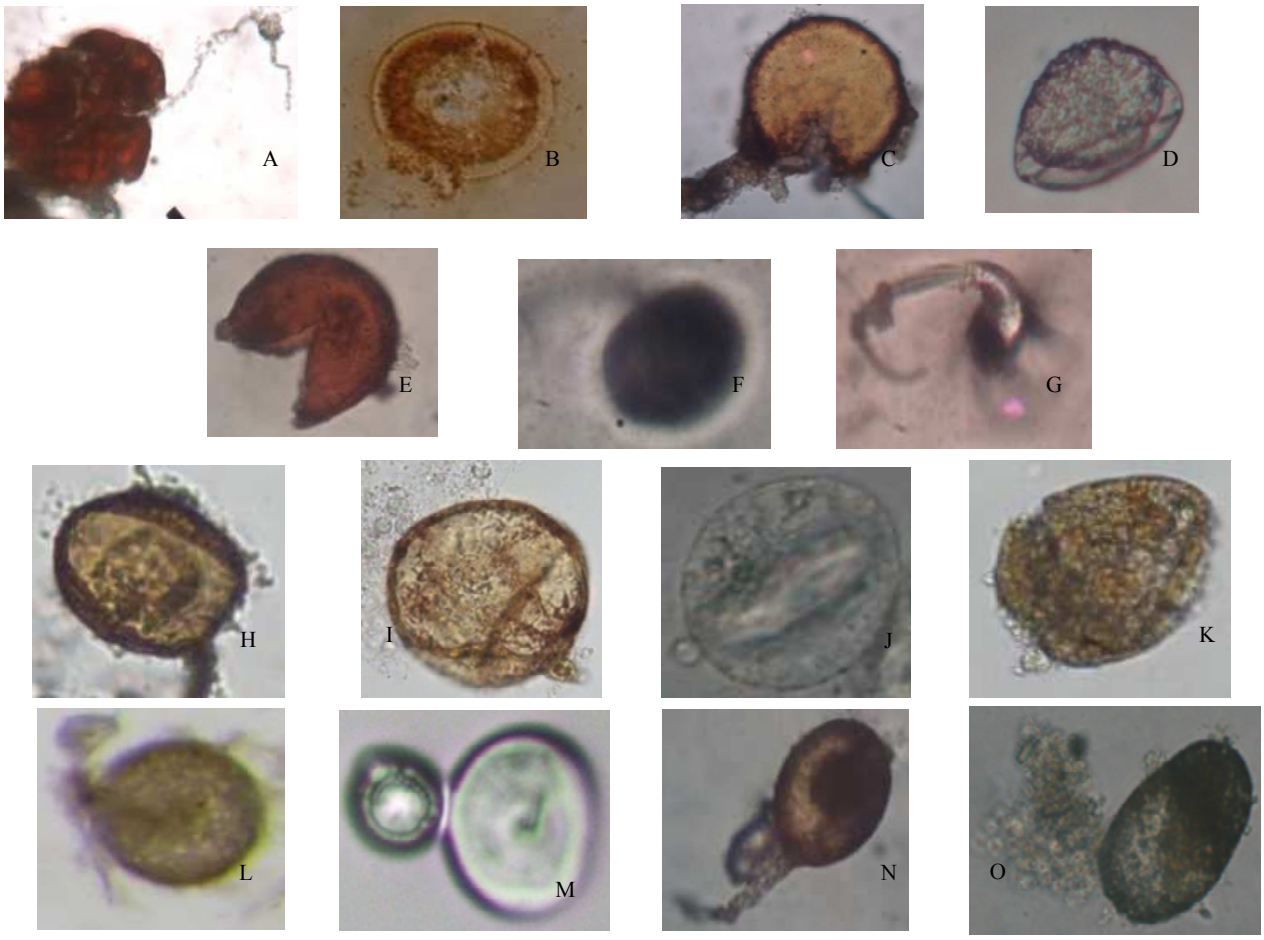

Figure 1. Various types of arbuscular mycorrhizal spores, A. *Glomus multicaule*, B. *Glomus clarum*, C. *Glomus fasciculatum*(51,24µm), D. *Glomus geosporum*, E. *Glomus* sp.9, F. *Glomus mosseae* spore, G. *Glomus mosseae* hyphae, H. *Gigaspora calospora*, I. *Gigaspora margarita*, J. *Gigaspora* sp.2, K. *Gigaspora* sp.3, L. *Acaulospora scrobiculata*, M. Developing spore of *Acaulospora delicate*, N. *Acaulospora* sp.4, O. *Enthrospora infrequens* (10x40X)

Some grass plants that are found in the area of sampling have high levels of colonization and spore density is quite high compared to other plants. Beside that, the highest richness index is *Imperata cylindrical*. If analyzed, it showed that mycorrhiza act directly or indirectly on plant growth and development. The higher of spores density and the rate of colonization showed that mycorrhizal symbiosis better able to plant, so that transfer of nutrients by mycorrhiza to the plant will also be getting better. This is what can lead to an increase in plant growth and development, especially in plants grasses that can multiply vegetatively naturally.

All of the results agree with Pagano et al. [21] which was shown that the diversity of arbuscular mycorrhiza related to heterogeneity of habitats and the soil properties (moisture).

Type the colonization of the plant is arum. Arum type is a type of colonization which mycorrhizal hyphae proliferate in the cortex grows longitudinally between plant cells. When intracellular hyphae of mycorrhiza entered into the plant cell, the cells begin to form arbuscular [27].

In the present study, *Glomus* has the highest frequency index than other genus in which it relates to the ability of the species to spread and sporulation. Beside that, *Glomus* also has highest relative abundance than other. There are positive correlation between the relative abundance and frequency index.

But, even though frequency index of *Gigaspora* higher than *Acaulospora*, value of relative abundance is smaller. This is happen because *Gigaspora* and *Scutellospora* require a longer time to form spores and mature [28]. In addition, a group of Gigasporaceae has a characteristic forming long mycelium and produce fewer spores than the group Acaulosporaceae and Glomaceae [29,30].

Mycorrhizal arbuscular having diversity index 2,82 (medium category), evenness level 0.81 (high category) and Simpson dominance index of 0.086. This indicates that diversity mycorrhizal arbuscular within the community stable and good. Index dominance Simpson shows an avoid one who having a meaning that

community more scattered and is not dominated by one or more species. The petroleum hydrocarbon in the soils is not too influence the diversity of mycorrhizal arbuscular, although the condition were slightly affecting the amount of the density of spores.

It is known very well that arbuscular mycorrhiza can improve the establishment of plants, especially to grow in the unfavorable environment [31]. Based on Gao et al. [16]mycorrhizal colonization may also alter the soil microbial (1) activity and (2) community. The former is supported by several observations on the enhanced activities of enzymes mainly generated by microbial exudation in mycorrhizal soils.

The effective microbiota in the mycorrhizal association was suggested to be responsible for the reductions in polyaromatic hydrocarbon concentrations in the soils with arbuscular mycorrhiza. This is a reason why arbuscular mycorrhiza community still stable and good in the hydrocarbon contaminated soils.

Conclusion

Results of this research was find and identified 13 type of plants in there with 32 type of arbuscular mycorrhiza spores and total of the density spores are 979 spores. Five genus were found are *Glomus, Gigaspora, Acaulospora, Enthrospora*, and *Scutellospora*. Diversity index of mycorrhiza is 2,82 (medium) with eveness 0.81 (high), and index of dominance 0.086. All that condition indicate in the unfavorable condition **(**TPH 5600 mg/kg), arbuscular mycorrhiza still can do symbiosis with plants and that diversity mycorrhizal arbuscular within the community stable and good.

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