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Spores Diversity of Arbuscular Mycorrhizal Fungi and Their Use for Land Reclamation in Coal Mining Used Land

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ABSTRACT

This research was to obtain spores of Arbuscular Mycorrhizal Fungi [AMF] from rizhosphere of corn, gaharu [Triangle wood] and cocoa plants which had been able to grow in coal mining used land that had been heavily destructed. Analysis of the soil in research location showed that the soil did not have good chemical character and fertility marked by low pH and low content of N, P, K and Ca. Observation on soil from the rhizospheres of corn, gaharu [Triangle wood], and cocoa growing in coal mining used land showed that there were nine species of AMF spores identified, namely *A. spinosa, A. scrobiculata,A. tuberculata, G. claroideum, G. etunicatum, G. fistulosum, G. luteum, G. versiforme,* and *G. Sp.* After being identified, the FMA spores were multiplied in greenhouse as a main source for manufacturing FMA natural fertilizer which would be applied in greenhouse and field.

***KEYWORDS**: Arbuscular Mycorrhizal Fungi, Coal Mined Land, Rhizosphere*

INTRODUCTION

Most of land used as coal mining is let fallow after being exploited mainly in an opened mining land. If the land is not rehabilitated it would cause the land to be critical and the ecosystem is destructed. To improve the ecosystem quickly it needs a series of appropriate reclamations in the degraded land. This activity was aimed at improving the unstable land condition, reducing erosion, and in long term improving micro climate in revegetation areas. Using appropriate pre-reclamation methods, one of them implementing Arbuscular Mycorrhizal Fungi [AMF], the growth of revegetation in mining used land could increase highly. AMF is an old symbiont that has been known for about 600 million-1billion years and much older than the age of monocotyle and dicotyle plants [200 million years], or other symbionts [1].

The application of AMF in agriculture is in the form of inoculants with active ingredient of living thing which functions to facilitate the availability of nutrients for plants in soil [2]. AMF with external hyphae in soil produces phosphatase enzyme which functions to release Phosphor [P] fixed by Al and Fe in acid soil like Ultisol, therefore it is available for plants [3-5].

There have been many reports found about AMF and so many efforts have been done in producing AMF. This is caused by the role of AMF that facilitates to increase yield of plants. AMF could increase yield of food crops like corn, estate crops like cocoa, and forestry crops like gaharu especially in critical or marginal land. However, there has been no research done in coal mining used land [6-8].

Besides agroclimate suitability which is the comparative superiority, this research is also important to increase the society income in coal mining used land in SawahLunto. Information obtained from the head of county more than 50 % of residents migrates to other areas like Bataman di Pekanbaru, some of them become riders of public vehicles, contruction workers and hired farmers, and the women become house maids.

The objectives of research to obtain indigenous AMF diversity from rhizosphere of several plants growing around coal mining land in Sawahlunto. This research aim too for multiplying dominant indigenous AMF spores in laboratory and greenhouse to obtain FMA inoculants as a source of AMF natural fertilizer that could be used directly in field.

MATERIALS AND METHODS

Study Area

This research carried coal mined land in Nagari Sikalang, Sawahlunto city, West Sumatra, Indonesia. This location was chosen by the researchers to determine the diversity of spesies of spore in the former land of coal mines. So that the results of this study can be seen how the FMA effect on soil fertility that is fomer coal mine.

Figure-1:Study site in Nagari Sikalang, Sawahlunto city, West Sumatra, Indonesia

PROCEDURE

Research has been conducted on four points at the coal mined land SawahLunto city that is north, south, east and west. Then take soil samples rhizosfir dominant crop in that location approximately 2 kg for observation AMF spores. Extraction and identification of the type and number of Mycorrhizal Fungi spores carried by the casting technique strain [9] and followed by centrifugation techniques [10].

AMF identification is done by morphological observation of spores. Spores placed on PVLG [Polyvinyl-Lacto-Glycerol] and PVLG + Melzer's Reagent [v/v 1:1] using a glass preparations [glass slides]. Subsequently observed using a microscope with a magnification of 40 x and labeled as unidentified. AMF spores were identified by observing the types and spore morphology based publication [11] as well as a variety of sources such as [12, 13].

Followed by counting spores directly by transferring 1 ml spore suspension on watch glass with four replications. Then all the spores were observed and counted the number of each species. Spores of each species were isolated and taken menggunakan pasteur pipette. Spores were collected as many as 30 spores per species for one treatment used in subsequent experiments.

Formulation and multiplication FMA inoculum in greenhouse

A single and compound spore cultures which produced good spores were subcultured to multiply the spores formed. The subculture was done using open pot culture system. The complete steps were as follow: First, single and compound spore cultures in test tubes were taken out carefully to avoid the FMA hyphae damage. Then all culture media containing spores, cut of hyphae, and part of root colonized by fungi were placed in culture pots.

After the development of cultures was good then they were dried and were not watered in order to trigger formation of more spores. After a week the plants were harvested by cutting 2-3 cm above media surface. This material was used as FMA inoculums for plants in fields.

RESULTS AND DISCUSSION

Results of analysis on contents of nutrients and chemical characters of soil showed that the ferlity of the soil was low [Table 1 and Table 2]. Almost all nutrients found and pH were low, and C-organic content was very low. On the other side, Al-dd content which inhibited development of plant growth had been measured.

Table-1: Results of soil analysis

No	Rhizosphere	Nutrients			
		N [%]	P [ppm]	K [me/100g]	Ca [me/100g]
1.	**Corn**	0.20R	6.3R	0.26R	0.03SR
2.	**Gaharu**	0.18R	4.4R	0.25R	1.12 SR
3.	**Cocoa**	0.21S	17.5S	0.264R	1.05 SR

Table-2:Results of analysis of some soil chemical characters

No	Rhizosphere	Chemical characters		
		pH	**C-Organic [%]**	**Al-dd [me/100g**
1.	Corn	5.81 [am]	1.25 [R]	Tu
2.	Gaharu	5.8 [am]	1.71 [R]	0.66
3.	Cocoa	5.76 [am]	1.38 [R]	0.02
Note: am : slightly acid, S : medium, R : low, SR : very low, tu : unmeasured				

Table 1 showed soil nitrogen content ranged from low to medium. P-available in soil ranged from very low to very high. Exchange calsium was in criteria of very low. Exchange calsium ranged from low to medium. All soil samples had pH which was slightly acid, C-organic ranged from very low to medium. Data in Table 1 and Table 2 indicated that soil in research location did not have good chemical characters and fertility due to mining.

Nine species of FMA spores were found, i.e. *A. spinosa, A. scrobiculata, A. tuberculata, G. claroideum, G. etunicatum, G. fistulosum, G. luteum, G. versiforme, and Gi. Sp.* Species and number of spores in three plant rhizospheres were different [Table 3]. Seven species of spore were found in corn rhizosphere , eight species in gaharurhizosphere, and six species in cocoa rhizosphere. The difference in spore number in several samples was assumed to be caused by low plant resistance due to soil pollution caused by mining activities occurred in Sawahlunto which caused low soil fertility. Land reclamation done by AMF could have direct and indirect effects to soil. Directly, it gives the effects to soil chemicals, increasing plant absorption of water and nutrients and protecting roots fro pathogens. Indirectly, it affects more soil physical characters, improving soil structure and decomposition of parent material and increasing nutrient dilution for plants.

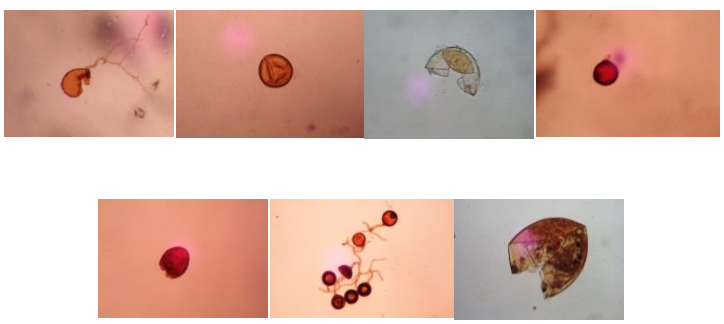
Table-3: Number of spores found in rhizospheres of three plants in coal mining used land

No	Plant Rhizosphere	Spore species	Total number
1	Corn	*Acaulospora. spinosa*	42
		Acaulospora scrobiculata	48
		Acaulospora tuberculata	20
		Glomus cloroideum	27
		Glomus etunicatum	92
		Glomus fistulosum	4
		Glomus luteum	
2	Gaharu	*Acaulospra. spinosa*	10
		Acaulospora tuberculata	25
		Glomus claroideum	50
		Glomus etunicatum	169
		Glomus fistulosum	64
		Glomus luteum	114
		Glomus versiform	61
		Gigaspora,sp	2

3	Cocoa	*Acaulospora tuberculata*	24
		Glomus claroideum	23
		Glomus etunicatum	92
		Glomus luteum	89
		Glomus versiform	13
		Gigaspora,sp	7

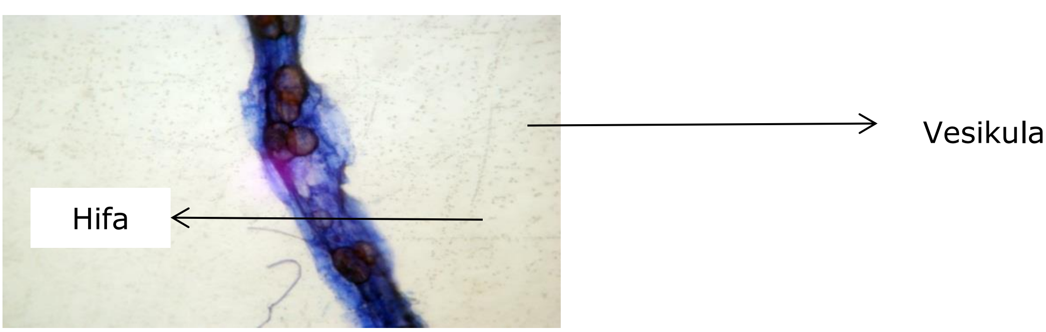
Soil physical properties could be enhanced by improving soil structure through netting external hyphae of FMA. Secretions of polisaccaride, organic acid and lender produced are able to bind soil particles to become micro agregates. Improvement of soil chemical properties can also be done by AMF through its role in increasing plant capacity in absorbing nutrients and water, mainly in marginal soil which is poor of nutrients like mining used land. In a process of filtering, single or double spore culture and mass production, spore of AMF was identified [Figure 1].

Figure-1: Spores of AMF found in coal mining used land in SawahLunto [a] G.Claroideum SC 186A.spinosa WV 861 AA.scrobiculata BR984A.tuberculata VZ 103 E and [b]G.etunicatum NE 108AGlomusLuteum SA 112Gi. Sp



Infection of AMF on rhizosphere of plant was shown in figure 2.

Figure-2:Infection of AMF on plant rhizosphere with vesicle and internal hyphae



[7], reported that application of AMF inoculums could increase percentage of root infection caused by increasing spore number. The high percentage of AMF was not determined by low and high dosages of inoculums because it depended on its compatibility with its hosts. AMF could be given in low dosage but percentage of infection could be higher than giving higher dosage.

Even though in general AMF can associate with roots of various plants, but the effectiveness is also determined by the compatibility between species of plants and the ecosystem origin of inokulum. The compatibility between AMF and host plants correlates with root system and environment condition which trigger the plants to excrete exudates which stimulate growth and development of AMF in plant roots. Generally plants with fine root system are less responsive toward growth and development of AMF. Plants try to associate with AMF in order to widen root exploitation zone to absorb nutrients, water, and other compounds. Therefore the application of AMF on plants having relatively big and less root systems would be very effective, mainly in poor environment condition [dry, low pH, not enough available nutrition]. If plants grow bad condition like drough and nutrient poor soil, thus root with mycorriza stimulate development of AMF.

CONCLUSION

Nine species of AMF spore were found in coal mining used land in SawahLunto, i.e.; *A. spinosa, A. scrobiculata,A. tuberculata, G. claroideum, G. etunicatum, G. fistulosum, G. luteum, G. versiforme, Gi. Sp.* The dominant number of spores were G. *etunicatum, G. luteum,* dan *A. tuberculata.* The three spores could be used as a source of biological fertilizer that could be applied together with other organic fertilizers mainly in critical lands like coal mining used lands.

The soil in coal mining used lands in SawahLunto had poor chemical characters and fertility. Mining has caused environmental damage, one of them is damage on soil chemical characters and nutrients. To improve environmental damage in short time, it is necessary to apply appropriate reclamation series in degraded lands. Appropriate method of pre reclamation is implementing mycorrhiza.

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