Utilizing Potential Soil Microorganisms, Humic Acid, Grasses and Legumes Forages in Marginal and Degraded Lands in Indonesia

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ABSTRACT

Marginal and degraded lands in Indonesia are considerably extensive and include many kinds of soil, for instance acid soil and post mining soil. The efforts which can be conducted are the use of biological fertilizers, soil conditioners, grasses and legumes forages. Biological fertilizers such as arbuscular mycorrhizal fungi (AMF), phosphate solvent microorganisms (MPP), and nitrogen fixing microorganisms (MPN). The soil conditioners such as humic acid. The forages such as, *Pueraria phaseoloides*, *Centrosema pubescens*, *Panicum maximum*, and *Setaria splendida.* The objectives of this research were to obtain new formulation of biological fertilizers which constitute a consortium of AMF and MPP, MPN, humic acid which could increase its ability to supply nutrients and help to increase forage plant survival in less favourable environment; to get grasses and legumes tolerances in marginal and degraded lands. There were 6 formulas of biological fertlizers which were tested in this research, namely (a) AMF with addition of MPP isolates 1, 2 and 3; (b) AMF with addition of Azospirilum isolates 1, 2 and 3; (c) AMF with addition of Rhizobium isolates 1, 2 and 3; (d) AMF with addition of humic acid; (e) AMF with addition of MPP, Azospirilum or Rhizobium; (f) AMF with addition of MPP, Azospirillum or Rhizobium and humic acid. Legumes and grasses were used *Centrosema pubescens*, *Pueraria phaseoloides*, *Panicum maximum*, and *Setaria splendida.* The results showed that the four test plant species responded differently to the latosol and post gold mining. In general, the four kinds of plants were not supported by single bio-fertilizer, but require a consortium of several types microorganisms and the results will be better when it was combined with humic acid. Growth response to the four types of plants in soil latosol was better compared to gold post-mining soil. At planting media from gold mine tailings many plants died, especially in the control treatment which was a treatment without the addition of bio-fertilizers.

Key words: soil potential microorganism, humic acid, legumes, grasses forages

INTRODUCTION

Marginal and degraded land in Indonesia was a lot, such as acid lands and post-mining lands. The existence of such lands in Indonesia is very high that covers 30% or 0.51 million km^2 of land area in Indonesia spread over the area of West Java, Sumatra, Kalimantan, Sulawesi and Irian Jaya. The main problems in acid soil are (1) decrease the solubility of P and Mo. (2) decrease the concentration of macro elements such as N, Mg, Ca and K. (3) increase concentrations of Al, Mn and Fe which can cause poisoning (4) inhibits root growth and water absorption, causing nutrient deficiencies, drought stress and increase nutrient leaching (Maschner, 1995). Post-mining land in addition to problems with acid conditions, as well as problems that may result is a heavy metal contamination.

The problems of acid soil can be overcome with the use of bio-fertilizers. Bio-fertilizers are the arbuscular mycorrhizal fungi (AMF), microorganism solvent phosphate (MPP) and Nitrogen Fixing Microorganisms (MPN). Arbuscular mycorrhizal fungi can help plants to supply and absorp of elements of low P availability in acid soil and have ability to adapt to acid soil (Koslowsky and Boerner, 1989). Phosphate solvent microorganisms are soil microorganisms that can improve the provision of P in acid soil by producing organic acids so that the solubility of Al can be reduced because it is bound by organic acids (Illmer *et al*., 1995). Malate, citrate and oxalate are organic acids that have high affinity to metal having such as Al^{3+} and Fe^{3+} (Jones and Brassington, 1998; Karti, 2003). Nitrogen fixing bacteria like *Azospirillum* and *Rhizobium* are bacteria that can cause changes in root morphology, such as an increased number of hair root, root extension, and root surface area. Influence on plant morphology may be caused by the production of compounds that support plant growth produced by Azospirillum. The speed of absorption of N, P, K and the accumulation of dry weight was higher in corn, sorghum, wheat and setaria inoculated with *Azospirillum* (Okon and Kapulnik, 1986).

Humic acid is an organic material that is not degraded by microorganisms, have the ability to help provide nutrients for carboxyl and phenolic groups. Both these groups can adsorb soil cations and anions such as Al and Fe in acid soil, heavy metals and fertilizers that do not adsorb easily will be washed. Special nature and potential of other humic acid is its ability to interact with metal ions, oxide, hydroxide, and organic minerals, including toxic pollutants by forming an association.

The objective of this research was to obtain new formulation of biological fertilizers which constitute a consortium between AMF with MPP, MPN, and humic acid which could increase its ability for supplying nutrients and help to increase forage plant survival in less favorable environment. To get grasses and legumes tolerances in marginal and degraded lands.

MATERIALS AND METHODS

This research consists of 3 stages, namely: (1) potential microbial multiplication (AMF, MPP and MPN), (2) formulation of new biological fertilizers which is a consortium of microbiology with humic acid, (3) new formulations test on marginal and gold post-mining land at laboratory scale.

Stage 1: Preparation for augmentation material: a potential microbial multiplication (AMF, MPP and MPN). AMF propagated through the open pot culture with the media and the host zeolite grown sorghum. AMF propagation procedures follow the standards from Laboratory of Forests and Environment Biotechnology, which includes the inoculation technique, nutrition, maintenance and monitoring for 4 months. Propagation from MPP and MPN was performed using liquid media and with the help of shaker to obtain the desired population, and formulated in liquid carriers. The isolates have been selected in the laboratory of Forest and Environment Biotechnology. Humic acid in the form of liquid was added 1 ml for each polybag.

Stage 2: formulation of new biological fertilizer which is a consortium of microbiology with humic acid. There were 6 formulas of biological fertlizers which were tested in this research, namely (1) Control (without microorganisms), (2) AMF (M); (3) AMF with addition of Rhizobium or Azospirilum isolates 1, 2 and 3 (MR/A); (4) AMF with addition of humic acid (MH); (5) AMF with addition of MPP, Rhizobium or Azospirilum (MPR/A); (6) AMF with addition of MPP, Rhizobium or Azospirillum, and humic acid (MPR/AH).

Stage 3: New formulations test on marginal and gold post-mining land at laboratory scale. All the formulations obtained in the nursery were tested with Legum cover crop (LCC) and grasses with the condition that the marginal growth media (acid soil/latosol soil) and gold post-mining soil. Testing with the LCC and grasses should be done to determine the production of legumes and grasses. Containers were a 3-kg capacity pot soil. Tests conducted at nursery for 3 months. Legumes and grasses used *Pueraria phaseoloides*, *Centrosema pubescens*, *Panicum maximum*, and *Setaria splendida*

RESULTS AND DISCUSSION

AMF, MPP and MPN and propagule density testing methods using most probable number (MPN) and the results are presented in Table 1. MPN test can be performed to know the quality of the isolate to be used for further testing.

Table 1. Most probable number of four type inoculans

No	Inoculans	MPN
1.	AMF	95.68 active propagule/30 g inoculan
2.	MPP	1.7×10^{10} (per g inoculan)
3.	MPN (*Azospirillum*)	1.6×10^{9} (per g inoculan)
4.	MPN (*Rhizobium*)	5.3×10^{6} (per g inoculan)

Latosol land taken from Dramaga Bogor district showed acid pH, low organic C content. The content of N, P, K, Ca, Mg was also low. Cation exchange capacity (CEC) is very low. The results of the analysis of Pongkor gold mine tailings, Bogor district generally had alkaline pH, organic C and nutrient elements such as N, P, K, Mg were very low with P and K levels of high potential. High levels of Ca and Pb could affect the availability of P. Very low value of CEC may affect the exchange of positively charged nutrients, namely micro-nutrient elements (Fe, Cu, Zn and Mn) which are essential minerals for plants.

Treatment M gave the best response for shoot dry weight for *Pueraria phaseoloides*, and *Setaria splendida* or *Panicum maximum* and the best response was MR and MA treatments. *Centrocema pubescens* did not give response (Table 2). Treatment M gave the best response to the root dry weight in *Pueraria phaseoloides* and *Panicum maximum*, whereas treatment MP for *Centrosema pubescens*. *Setaria splendida* did not give response (Table 3).

Table 2. The influence of treatments on shoot dry weight for *Pueraria phaseoloides, Centrosema pubescens, Setaria splendida* and *Panicum maximum* at latosol soil

Treatment	Shoot dry weight *Pueraria phaseoloides* (g/pot)	Shoot dry weight *Centrosema pubescens* (*g*/pot)	Shoot dry weight *Setaria splendida* (g/pot)	Shoot dry weight *Panicum maximum* (g/pot)
Control	7.412 [ab]	2.99	10.3 [b]	8.10 [ab]
M	8.016 [a]	3.08	11.0 [ab]	9.28 [a]
MR/MA	7.698 [ab]	2.78	12.2 [a]	9.60 [a]
MH	6.908 [ab]	3.02	10.7 [b]	6.56 [bc]
MP	7.102 [ab]	2.01	11.4 [ab]	7.50 [abc]
MPR/A	6.440 [b]	2.38	11.5 [ab]	5.48 [c]
MPRH	7.040 [ab]	3.00	11.4 [ab]	8.30 [ab]

Note: 1. M = Mycofer, MR/MA = Mycofer + Rhizobium/Azospirillum, MH = Mycofer + humic acid, MP = Mycofer + MPP, MPR/A = Mycofer + MPP + Rhizobium/ Azospirillum, MPR/AH = Mycofer + MPP + Rhizobium/ Azospirillum + humic acid;
2. Different superscript at the same column means significantly difference (P <0.05).

Table 3. The influence of treatments on root dry weight for *Pueraria phaseoloides*, *Centrosema pubescens, Setaria splendida* and *Panicum maximum* at latosol soil

Treatment	Root Dry weight *Pueraria phaseoloides* (g/pot)	Root Dry weight *Centrosema pubescens* (g/pot)	Root Dry weight *Setaria splendida* (g/pot)	Root Dry weight *Panicum maximum* (g/pot)
Control	2.660[bc]	1.12[ab]	13.94	5.08[ab]
M	3.93 [a]	1.14[ab]	12.30	6.62[a]
MR/MA	3.484[ab]	0.83[bc]	16.1	4.90[ab]
MH	2.276[c]	0.82[bc]	12.98	2.78[b]
MP	2.83 [bc]	1.20[a]	13.90	4.70[ab]
MPR/A	2.454[bc]	0.54[c]	15.32	3.80[ab]
MPRH	2.186[c]	0.71[c]	18.1	5.24[ab]

Note: 1. M = Mycofer, MR/MA = Mycofer + Rhizobium/Azospirillum, MH = Mycofer + humic acid, MP = Mycofer + MPP, MPR/A = Mycofer + MPP + Rhizobium/ Azospirillum, MPR/AH = Mycofer + MPP + Rhizobium/ Azospirillum + humic acid;
2. Different superscript at the same column means significantly difference (P <0.05).

Table 4. The influence of treatments on dry weight for *Pueraria phaseoloides*, *Centrosema pubescens, Setaria splendida* and *Panicum maximum* at Gold Mining soil

Treatment	Shoot Dry weight *Pueraria phaseoloides* (g/pot)	Shoot Dry weight *Centrosema pubescens* (g/pot)	Shoot Dry weight *Setaria splendida* (g/pot)	Shoot Dry weight *Panicum maximum* (g/pot)
Control	0.240[b]	0.00	1.00	0.22[b]
M	0.82 [ab]	0.77	1.05	0.42[b]
MR/A	1.710[a]	0.74	1.32	0.48[b]
MH	0.476[ab]	0.84	1.08	0.70[ab]
MP	1.224[ab]	0.68	0.90	0.33[b]
MPR/A	0.874[ab]	1.18	0.88	1.04[a]
MPR/AH	0.506[ab]	0.81	1.22	1.04[a]

Note: 1. M = Mycofer, MR/MA = Mycofer + Rhizobium/Azospirillum, MH = Mycofer + humic acid, MP = Mycofer + MPP, MPR/A = Mycofer + MPP + Rhizobium/ Azospirillum, MPR/AH = Mycofer + MPP + Rhizobium/ Azospirillum + humic acid;
2. Different superscript at the same column means significantly difference (P <0.05).

Latosol land constraints for crop growth are acid pH, low organic C, P, N, K, Ca, Mg, but very high level in P and K. The addition of biological fertilizers such as nitrogen fixing microorganisms was expected to help provide the nitrogen through N2 fixation, so that one of the constraints on the availability of land latosol N elements can be resolved. Phosphate solvent microorganisms (MPP) will help P solubilization. Through the release of organic acids by microorganisms, it can dissolve P bound by inorganic minerals such as Al, Fe or Mn. The existence of arbuscular fungi arbuscular (AMF), available nutrient elements can be quickly absorbed through the external and internal hifa so that plants will always be provided the necessary nutrients for growth. Ensuring the availability of nutrients to the plants because of the addition of biological fertilizer will help overcome the obstacles on the latosol soil. MR treatment and MPAH or MPA gave the best response to the shoot dry weight of *Pueraria phaseoloides* and *Panicum maximum* on a gold mine tailings, but did not gave response on *Setaria splendida* and *Centrocema pubescens* shoot dry weight (Table 4).

MP and MH treatmens on gold post mining stimulated the best response to dry weight of roots in *Pueraria phaseoloides* and *Setaria splendida*, but it did not gave response on *Centrosema pubescens* and *Panicum maximum* root dry weight (Table 5). *Setaria splendida* grew better than other plants, because the grass can release organic acids like malate, oxalate and citrate on the soil, root and shoot (Karti, 2003).

In general the gold mine tailings needs all types of biological fertilizer for growth. Constraints for plant growth in gold mine tailings are alkaline pH, with very high Pb, and low organic C content and macro nutrients like N, P, K, Mg. However, P and K levels were high potential. Ca levels are also high, which may affect the availability of P. Very low value of CEC may affect the exchange of positively charged nutrients, namely micro-nutrient elements such as Fe, Cu, Zn and Mn which are essential minerals for plants. The addition of biological fertilizers such as nitrogen fixing and phosphate solubilizing microorganisms will help provide nutrients N and P. AMF will assist the absorption of nutrients that are available to the plants continuously, so the plants can grow better. Humic acid which is a soil conditioner will help in providing nutrients simultaneously, because the humic acid can help to adsorb the nutrients and then be easily removed through a process of exchange. Humic acids can help to bind the heavy metal such as PB which is generally high in the gold post-mining land, so the availability decreases and eventually the plants can grow normally.

Availability of nutrients will be continuously improved the process of photosynthesis. Increased photosynthesis process wll lead to an increase of shoot dry weight and root dry weight. In the post-mining lands, plant growth seems to be lower than in the latosol soil, so the addition of biological fertilizers with different types of microorganisms is required.

Table 5. The influence of treatments on root dry weight for *Pueraria phaseoloides, Centrosema pubescens, Setaria splendida* and *Panicum maximum* at Gold Mining soil

Treatment	Root Dry weight *Pueraria phaseoloides* (g/pot)	Root Dry weight *Centrosema pubescens* (g/pot)	Root Dry weight *Setaria splendida* (g/pot)	Root Dry weight *Panicum maximum* (g/pot)
Control	0.184[b]	0.00	2.44[b]	2.06
M	0.224[b]	0.33	2.80[b]	1.74
MR/MA	0.558[ab]	0.30	3.90[ab]	1.46
MH	0.158[b]	0.38	5.08[a]	1.88
MP	0.716[a]	0.23	2.98[b]	1.13
MPR/A	0.350[ab]	0.43	3.16[ab]	2.22
MPR/AH	0.224[b]	0.23	3.78[ab]	2.24

Note: 1. M = Mycofer, MR/MA = Mycofer + Rhizobium/Azospirillum, MH = Mycofer + humic acid, MP = Mycofer + MPP, MPR/A = Mycofer + MPP + Rhizobium/ Azospirillum, MPR/AH = Mycofer + MPP + Rhizobium/ Azospirillum + humic acid;
2. Different superscript at the same column means significantly difference (P <0.05).

CONCLUSIONS

The four test plants species responded differently to the latosol and post gold mining soil. In general, the four plants were not stimulated by single bio-fertilizers, but they required a consortium of several types microorganisms and the result will be better when it is combined with the humic acid on gold post mining soil. Growth responses of the four types of plants in latosol soil were better when compared to gold post-mining. At planting media from gold mine tailings many plants die, especially in the control treatment which was treatment without the addition of bio-fertilizers. *Setaria splendida* grew better than other plants.

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