

Original Research Article

doi: <https://doi.org/10.20546/ijcrbp.2019.608.001>

Indigenous endomycorrhiza of iron and manganese contaminated soils in Indonesia

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Article Info

Date of Acceptance:
07 July 2019

Date of Publication:
06 August 2019

Keywords

Acaulospora
Fungus
Gigaspora
Heavy metal
Phytorhizoremediation

ABSTRACT

Concentrations of iron and manganese that exceed critical limits in soil and plant provide opportunities for microorganisms to become resistant. The aim of this study was to identify endomycorrhizal fungi from areas contaminated with iron and manganese for use as biostarter for phytoremediation programs. This research was conducted in two phases at April 2019, namely taking rhizosphere from *Chromolaena odorata*, *Melastoma affine* dan *Spathoglottis plicata* in Sorowako, Indonesia; and the second phase is to isolate and identify endomycorrhizal fungal spores in the Microbiology Laboratory, Center for Environmental and Forestry Research and Development in Makassar, Indonesia. The results showed that the genus *Acaulospora* was more dominant in areas that were contaminated with iron and manganese, and were able to adapt and survive compared to other genera.

Introduction

Endomycorrhizal a form of mutualism symbiotic association between root endodermis of higher plants with fungal hyphae, which have reached 80% of plant species. Some researchers have shown the positive role of endomycorrhizal on plant growth in stressful environments of salinity (Hashem et al., 2018; Hashem et al., 2019), nutrients (Chanda et al., 2014; Bowles et al., 2018) drought (Abdelmoneim et al., 2013; He et al., 2019), pathogens (Whipps, 2011; Adamec and Andrejiova, 2018) and heavy metals (Miransari, 2011; Atakan et al., 2018).

Research on the use of endomycorrhizal to reduce heavy metal contamination is a phenomenon that has received the attention of phytoremediation researchers. Arisusanti and Purwani (2013) infect *Glomus fasciculatum* on the roots of *Dahlia sp.* to reduce the metal, lead (Pb). *Rhizophagus clarus* and *R. irregularis* can provide protective effects on vines on land contaminated with copper (Cu) (Ambrosini et al., 2015). *Glomus versiforme* and *Rhizophagus intraradices* can reduce the absorption of cadmium (Cd) on *Lonicera japonica* (Jiang et al., 2016). Alam et al. (2019) and Singh et al. (2015) using endomycorrhizal species obtained from International of (Vesicular)

Arbuscular Mycorrhizal Fungi (INVAM), West Virginia University (WV), USA, inoculated on *Lens culinaris* to reduce arsenic (As), but the endomycorrhizal used are not indigenous endomycorrhizal from areas that contaminated with heavy metals.

The chemical elements iron (Fe) and manganese (Mn), respectively, have specific gravity of 7.86 g.cm⁻³ and 7.21 g.cm⁻³, so that classified as heavy metals (Engge and Hendrajaya, 2016; Dangeand Dhoble, 2017). Fe and Mn including as micro-essential heavy metals that can cause metabolic disorders for soil microorganisms if are in high concentrations (Singh and Prasad, 2015; Chu, 2017). Some research results to show that heavy metal pollution give out detrimental effect on the life cycle of soil microorganisms (Xie et al., 2016; Jin et al., 2018) and causes changes in the soil microorganism community structure (Gurave et al., 2015; Wood et al., 2016, Park et al., 2016), but ultimately causes soil microorganisms to become tolerant and resistant (Gurave et al, 2015; Harman and Uphoff, 2019). However, the level of tolerance and resistance between groups of fungus is largely determined by the type of fungus and environment (Ramage et al., 2012; Domka et al., 2019).

Researchers have found several strains of endomycorrhizal fungus that are tolerant of heavy metal stress, namely, *Gigaspora albida* and *Glomus clarum* tolerant of zinc (Zn), cadmium (Cd), lead (Pb) and copper (Cu) (Soares and Siqueira, 2008), *Glomus intraradiaces* and *Glomus mosseae* tolerant of Pb and Cd (Adewole et al., 2010; Aram and Golchin, 2013), *Acaulospora morrowiae* tolerant of Cu (Ambrosini et al., 2015), *Acaulospora mellea* tolerant to nickel (Ni) and chromium (Cr) (Spruyt et al., 2014) and *Scutellospora pellucida* tolerant of Zn (Filho et al., 2002; Bano and Ashfaq, 2013).

Endomycorrhizal tolerant and resistant to heavy metals with high concentrations can be a biotechnology agent for the success of post-mining land phytoremediation programs in Sorowako, Indonesia. So that exploring the indigenous Endomycorrhizal fungi spores from areas contaminated with Fe and Mn is the objective of this study, which also provides an opportunity for others researcher to inoculate on endemic plant of location.

Materials and methods

The study conducted in two phases at April 2019. The first phase, rhizosphere collection of *Chromolaena odorata*, *Melastoma affine* and *Spathoglottis plicata* at coordinated of 2°31'57.6 "S and 121°22'50.7" E, Sorowako, Indonesia. The rhizosphere collection method uses a diagonal system (Subramani, 2009; Astiko et al, 2016).

The second phase, endomycorrhizal fungus spores isolated from rhizosphere using the wet sieving technique (Chanda et al., 2014; Shamini and Amutha, 2014) and the sucrose gradient centrifugation method (Walker et al., 1982; Charoenpakdee et al., 2010) at Microbiology Laboratory, Research and Development Center for Environment and Forestry, Makassar, Indonesia. Spore morphology identified using a manual book from the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungus (INVAM, 2019).

Concentration Fe and Mn in soil was measured while in laboratory of chemistry, Polytechnic of Ujung Pandang, Makassar, using X-Ray Florence Spectrophotometer/Bruker/S2 Ranger, concentration Fe and Mn in soil can be seen in Fig. 1.

Results and discussion

Concentrations of Fe and Mn in nickel post-mine area of Sorowako have exceeded critical limit for the environment and plants (Fig. 1), some researchers suggest that critical limit for Fe in the soil were 100,000 ppm (Mengel and Kirdy, 1987) and in plants were 1,000 ppm (Ministry of State for Population and Environment of the Republic of Indonesia and Dalhousie University Canada, 1992), while for Mn, the critical limit in the soil were 1,500 ppm (Liphadzi and Kirkham, 2005) and in plants was 400 ppm (Jones, 1979).

Fe concentration and high Mn is likely caused by mining activities, especially when returning overburden, so that it can have a toxic effect on the activity of soil microorganisms to develop and reproduce, but according to Ivshina et al (2013) that strategy to adaptation and tolerance also owned by each organism in unfavorable environment.

Endomycorrhizal also have defense strategy to heavy metal stress, the strategy is likely to involve one of the mechanisms, including: (i) new expression of fungus gene (Cicatelli et al., 2010; Ferrol et al., 2016), (ii) metal of quarantined and deposited at extracellular (Koochi, 2014; Chen et al., 2018), (iii) produce of metal binding protein (Singh, 2012; Hryniewicz et al., 2012), (iv) reduce of metal absorption (Karimi et al., 2011; Emamverdian et al., 2015), (v) increase efflux (Hildebrandt et al., 2007; Tiwari and Lata, 2018), (vi) formation of complexes outside the cell (Violante et al., 2010; Singh et al., 2016), (vii) release of organic acids (Adeleke et al., 2017; Mishra et al., 2017), and (viii) ligand synthesis such as polyphosphates and metallothionein (Ferrol et al., 2016; Zhan et al., 2019).

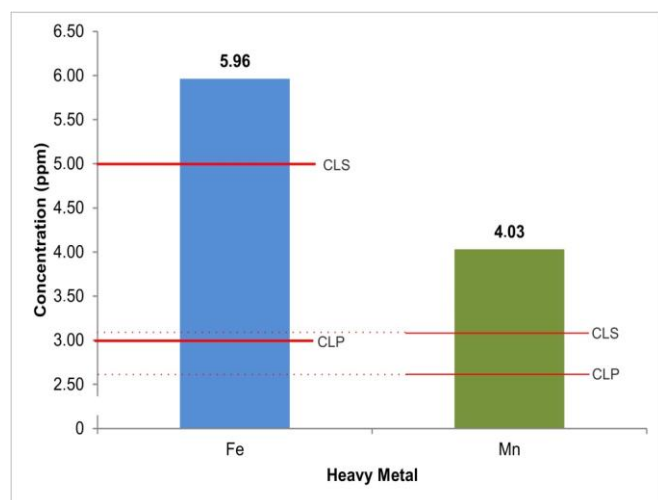


Fig. 1: Concentrations of Fe and Mn in nickel post-mine area of Sorowako, Indonesia (CLS, Critical limit in soil; CLP, Critical limit in plant). Data is displayed in logarithmic form.

Indigenous endomycorrhiza spores identified in various plant rhizosphere, obtained spores from two genera that are able to adapt in areas that have high concentrations of Fe and Mn, namely *Acaulospora sp.* and *Gigaspora sp.*; *Acaulospora sp.* was the genus mycorrhiza which belongs to the family Acaulosporaceae. This genus has several characteristics including having 2-3 spore walls, spores formed on the side of the neck of the sporiferous saccule, globose to elliptical, hyaline, yellow, or yellowish red, spore diameter between 74-289 μm (INVAM, 2019), however, the diameter of the spores found by Akib et al. (2018) on nickel-contaminated land ranged from 60 - 80 μm .

Gigaspora sp. was the mycorrhizal genus that belongs to the Gigasporaceae family. This genus has characteristics, among others, spores are produced singly in the soil, not having a layer of inner spore walls, have bulbous suspensors, globose or sub-globose shaped, creamy to yellow in color, 206-358 μm in diameter (INVAM, 2019). The research results of Akib et al. (2018) in the nickel post-mine area found spores of *Gigaspora sp.* with diameter of 203 - 235 μm .

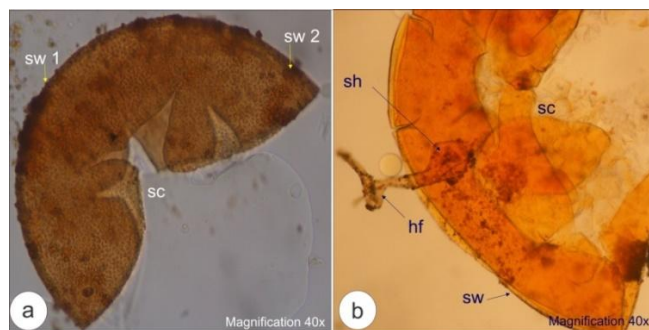


Fig. 2: Spores morphology of *Acaulospora sp.* (a) and *Gigaspora sp.* (b) which isolated from area contaminated with Fe and Mn (Note: sw, cell wall; sc, saccule; sh, substanding hyphae; hf, hyphae).

The presence of indigenous endomycorrhizal spores in environment contaminated with Fe and Mn, allegedly do one of strategic defense, as has explained by researchers. However, according to Herrera et al. (2018), the ability of adaptation and resistance of indigenous endomycorrhizal to be tolerant, it is possible follow the intracellular metal binding mechanism through ligand synthesis (metallothionein, polyphosphate), and / or accumulate metals to in vacuoles.

Calculation of spore's indigenous endomycorrhizal number per 1000 mg of rhizosphere contaminated with Fe and Mn were found in different amounts, and dominated by the genus *Acaulospora sp.* (Table 1). This is probably due to *Acaulospora sp.* having more than one cell wall, so it has a stronger defense against heavy metal stress than *Gigaspora sp.* According to Upadhyaya et al. (2010) and Koochi (2014) that endomycorrhizae can store heavy metals in hyphal cell walls, specifically stored in crystalloids in the mycelium, this explanation reinforced by Chaves et al. (2002) and Chen et al. (2018) who show that in roots of plants that are contaminated with heavy metals, it is seen

that the endomycorrhizal extra radical mycelium is able to absorb and accumulate heavy metals outside the mushy wall of hyphal wall zone, in cell wall, and in cytoplasm of fungus hyphae, while

the research results of Tuheteru et al. (2017) show that utilization *Acaulospora tuberculata* can increase Mn, Fe, Cr and Ni uptake of *Nauclea orientalis* L.

Table 1. Number of indigenous endomycorrhizal spores per 1000 mg of rhizosphere sample.

Family	Rhizosphere of pioneer plant	Number of spores	
		GS	AC
Asteraceae	<i>Chromolaena odorata</i>	0	24
Melastomataceae	<i>Melastoma affine</i>	1	1
Nephrolepidaceae	<i>Nephrolepis exaltata</i>	0	13

Note: GS, *Gigaspora* sp.; AC, *Acaulospora* sp.

Several studies have reported that indigenous endomycorrhiza effectively and efficiently infect endodermis of plant roots in areas that are contaminated with heavy metals (Vaishaly et al., 2015; Shakeel and Yaseen, 2015; Kumar and Saxena, 2019). According to Zadehbagheri et al. (2014) that indigenous endomycorrhizal isolates that develop and reproduce naturally in areas contaminated with heavy metals are more tolerant than isolates originating from non-polluted areas. Thus, filtering tolerant indigenous endomycorrhizal isolates needs to be done to ensure the effectiveness of symbiosis between endomycorrhiza and endodermis of plant roots. Therefore, it is very important to infect endodermis of endemic plant roots with indigenous endomycorrhizal isolates in future studies.

Conclusion

Indigenous endomycorrhizal of genus *Acaulospora* sp. and *Gigaspora* sp. that are able to adapt in areas contaminated with Fe and Mn have been found in Sorowako and can be used as sources of inoculum in phytoremediation programs combined with endemic plants.

Acknowledgement

Authors sincerely thank Ministry of Research, Technology and Higher Education of the Republic of Indonesia for providing support through a grant of basic research competition in 2019 and special thanks to Makole Nuha, Mr. H. Andi Baso, AM Opu To Lamattulia, my brother Mr. Syamsul Amri Akib, Mr. Komaruddin and friends, for all the help during our activities in Sorowako.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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How to cite this article:

Akib, M. A., Nuddin, A., Prayudyaningsih, R., Syatrawati, 2019. Indigenous endomycorrhiza of iron and manganese contaminated soils in Indonesia. Int. J. Curr. Res. Biosci. Plant Biol. 6(8), 1-7.

doi: <https://doi.org/10.20546/ijcrbp.2019.608.001>