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Research Article

Arbuscular mycorrhizal fungal inoculation improves *Nauclea orientalis* L. growth and phosphorus uptake in gold mine tailing soil media

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Abstract: Gold mine tailing soil media are characterized by low soil fertility and heavy metals toxicity. As an effort to improve the condition of gold mine tailing soil media, a revegetation experiment using Arbuscular Mycorrhizal Fungi (AMF) and soil media from gold mine tailing was conducted in the greenhouse. The objectives were to assess initial growth, P uptake and Pb reduction in *Nauclea orientalis* L. plants inoculated with indigenous AMF grown on gold mine tailing soil media. Three AMF fungi were used in this study, i.e. *Glomus aggregatum*, *Glomus* sp. and *Acaulospora delicata.* The experiment was conducted in Completely Randomized Design, having four treatments, i.e. control, *G. aggregatum*, *Glomus* sp. and *A. delicata*. The experiment was carried out for 3 months in a greenhouse scale. The results showed that local AMF inoculation significantly increased the height and stem diameter of lonkida by 181-213% and 284-443%, respectively, compared to control. The highest measurements of leaf's length and width of lonkida seedlings were obtained from *Glomus* sp. and *A. delicata* treatments. *Glomus* sp. and *A. delicata* each significantly increased P levels in roots and shoots. Inoculation with *G. aggregatum* reduced Pb in the root and shoots parts by 74-86% and 72-76%, respectively, compared to controls. Local AMFs are potential to be developed as biological fertilizers to support revegetation in degraded lands, such as in gold mine tailing areas.

Keywords: *Acaulospora delicata, Glomus sp, Nauclea orientalis, reforestation, tropical*

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Introduction

Mining is among factors causing deforestation, which results in the loss of topsoil and vegetation, biodiversity decrease, extreme land conditions decline, such as low soil fertility (Sheoran et al., 2010) and heavy metals toxicity. Low soil fertility is characterized by low content of essential nutrients (N, P, K and Zn) in tailing soil (Orłowska et al., 2011). While heavy metals toxicity is characterized by a high level of Pb content (Xiao et al., 2017), which is toxic to plants and has a systemic impact on human health (Lar et al., 2013; Taiwo and Awomeso, 2017). Reforestation of damaged forest ecosystems needs to be done to restore ecosystem function and forest productivity and to reduce the negative impact of deforestation. However, several issues need to be solved, such as slow natural regeneration and soil restoration. These issues are common to happen in the post-mining area, and there are very limited experiences to handle such issues in the tropics (Román-Da˜nobeytia et al., 2015). Native tree species and utilization of Arbuscular Mycorrhizal Fungi can be used to restore damaged forest ecosystems (Wang, 2017).

Arbuscular Mycorrhizal Fungi (AMF) is a mycorrhizal fungi-forming agent of phylum Glomeremycota (Smith and Read, 2018). Various international publications reported that AMF enhances growth and improves seedling quality of plants in a greenhouse scale through improved nutrient uptake and water and increased plant resistance to abiotic stresses such as salinity (Al-Khaliel, 2010), drought (Qiao et al., 2011; Zhu et al., 2012), waterlogging (Tuheteru et al., 2015; Tuheteru and Wu, 2017) and heavy metals (Wang et al., 2005; Husna et al., 2016; 2019). AMF is also potential to be developed as bioremediation agents (Simon et al., 2006; Tuheteru et al., 2017) and in forest reclamation and restoration programs (Arshi et al., 2017; Maiti, 2013). In gold tailing ecosystem and mercury-contaminated soil media, AMF is beneficial for the vitality, growth and uptake of plant nutrients, as well as enhances the rebuilding of vegetation (Orłowska et al., 2011; Madejon et al., 2012; Fiqri et al., 2016; Husna et al., 2019; 2020).

AMF form symbiosis with 97 families of land plants such as family Rubiaceae (Smith and Read, 2018). One tree species from the Rubiaceae family that is symbiotic with AMF is *Nauclea orientalis* L. (Tuheteru et al., 2015). *N. orientalis* is a multipurpose tropical tree species for wood production (Dayan et al., 2007; Muslich et al., 2013), wetland rehabilitation (Marghescu, 2001), agroforestry (Amihan-Vega and Mendoza, 2005), medicinal plants (Lim et al., 2013) and phytoremediation (Tuheteru et al., 2016). AMF inoculation promotes the growth of lonkida seedlings in mineral degraded soil media (Tuheteru et al., 2015), serpentine soil (Tuheteru et al., 2017), and mercury-contaminated soil (Ekamawanti et al., 2014).

Lonkida is potential as phytoremediation plant in Fe, Mn, Zn, Ni, Cr (Tuheteru et al., 2016; 2017) and Hg contaminated soil (Ekamawanti et al., 2014). Information about the effect of indigenous AMF to lonkida growth in gold mine tailings condition is still limited. This research aimed to assess initial growth, P uptake and Pb reduction in *Nauclea orientalis* L. plants inoculated with indigenous AMF grown on gold mine tailing soil media.

Materials and Methods

Time and location

This study was conducted for seven months (January-July 2017) in the greenhouse of Forestry Department, Faculty of Forestry and Environmental Science, Universitas Halu Oleo, Kendari. Chemical analysis of the media and absorption of nutrients and heavy metals were conducted at the Soil and Plant Laboratory of SEAMEO BIOTROP, Bogor, Indonesia.

Media and substrate

The gold tailing soil media were obtained from the disposal site of PT. Panca Logam Makmur, Bombana District, Southeast Sulawesi, Indonesia. Characteristics of physical and chemical properties of gold tailings soil media were analyzed at the Soil and Plant Laboratory of SEAMEO BIOTROP, Bogor, Indonesia. The analysis results showed that the soil media had pH (H_2O) of 5.7; C-organic content 0.20%; total N 0.09%; C/N ratio 2; P_2O_5 of 15.7 mg/kg; Ca, Mg, K, Na contents of 1.27, 1.23, 0.15 and 0.23 cmol/kg, respectively; CEC of 3.23 cmol/kg; base saturation of 87.71%; Al^{3+} and H^+ of 0.12 meq/100 g and 0.65 meq/100 g, respectively. The media consisted of 78.6% sand, 10.9% silt and 10.5% clay. The total metal contents of the media were 1,546 mg/kg of Mn, 3.23% of Fe, 14 mg/kg of Cr, 60 mg/kg (HNO_3-$HClO_4$)-AAS of Pb. The total Hg was 0.61 mg//kg (Cool Vapor-AAS). The gold tailings soil medium was mixed with vermicompost (mixing ratio of 8:2). The characteristics of sterile vermicompost were pH of 5.7, C-organic content of 1.77%; total N of 0.17%; P_2O_5 339 mg/kg and total K_2O 5081 mg/kg.

Seed germination

Seeds of lonkida were collected from the parent plants in the Konawe District of the Southeast Sulawesi Province (located at 3˚58'59.70"S and 122˚02'48.94" E). Germination of lonkida seeds does not require seed treatment. Seeds were sown to the mica tubs (20 x 20 x 5 cm), containing sterile sand media and cultured for 60 days (20 January - 20 March 2017).

Inoculation preparation and AMF inoculation

AMF inoculums used were *Glomus* sp., *Glomus aggregatum* and *Acaulospora delicate* which were isolated from the rhizosphere of *Pericopsis mooniana*. The AMF inoculums contained spores and AMF-colonized roots that were propagated for 3 months in culture pot containing zeolite media and host *Pueraria javanica* in the greenhouse condition of the Department of Forestry, Universitas Halu Oleo (located at 6°38'07.35"S and 106°49'31.72"E), Kendari, Southeast Sulawesi, Indonesia. Five grams of AMF inoculums were inoculated in roots of 60- day- old lonkida seedling in a 10 x 15 cm polybags containing gold tailing soil media and vermicompost mixture (mixture ratio of 8:2). Non-inoculated seedlings were used as controls.

Experimental design

The experimental design used was Completely Randomized Design (CRD) consisted of control, *Glomus* sp., *G. aggregatum* and *A. delicata* treatments. Each treatment consisted of 9 replications. Seedlings were grown in the greenhouse of the Department of Forestry, Universitas Halu Oleo from 02 April until 04 July 2017.

Data collection and analysis of plants

Plant height measurement (cm) was done using a ruler, starting from the base of the stem to the highest growing point on the trunk line. Plant diameter measurement (mm), was carried out using a calliper, on the stem at the height of 1 cm above the soil medium. Leaf area was measured at the length and width of the leaf. The dry weight of the seedlings, including roots and shoots (leaves and stems), were obtained by oven-drying the seedlings at a temperature of 70°C for 2 x 24 hours and then weighed. Both sections of roots and shoots were measured separately. Analysis of concentration and content of P and Pb at the roots and shoots were conducted using HNO_3-$HClO_4$. Uptake of P and Pb were obtained and analyzed, which results were then compared with the theoretical contents of the dry weight of plants. Transport factor (TF) of Pb was calculated from the ratio of C_{aerial}/C_{root}; where C_{aerial} is the concentration of metal in the shoot (stems & leaves) and C_{root} is the metal concentration in the root. Increased and reduced metal absorptions were calculated with the formula: [metal absorption of mycorrhizal plant – metal absorption of non-mycorrhizal plant/metal absorption of non-mycorrhizal plant] x 100% (Wang et al., 2005).

Observation of AMF colonization and Mycorrhizal Dependency (MD)

The root colonization was determined using technique following Brundrett et al. (1996) and was calculated using the formula: [Σ length with mycorrhizae/Σ total observed field of view] x 100%. Mycorrhizal dependency (MD) analysis was carried out according to Habte and Manjunath (1991) using the formula: MD (%) = (dry weight of mycorrhizal plant - the dry weight of non-mycorrhizal plant)/dry weight of mycorrhizal plant x 100%.

Data analysis

Data were analyzed using one way ANOVA. Comparisons of means were done using LSD Test at the 5% probability level where the F-Values were significant. All statistical analyses were conducted using SAS 9.1.3 portable statistical software.

Results and Discussion

Colonization of AMF and Mycorrhizal Dependency (MD)

Results of root staining showed that the roots of lonkida seedlings were colonized by AMF, with *A. delicata* having the highest colonization average (89%), followed by *G. aggregatum* (86%) and *Glomus* sp. (77%) (Table 1). There no significant differences in AMF colonization among *N. orientalis* inoculated with the three fungi (Table 1). MD values for *G. aggregatum*, *Glomus* sp. and *A. delicata* were 48, 43 and 37%, respectively.

Table 1. AMF colonization and Mycorrhizal Dependency (MD) of *N. orientalis* L. at 12 weeks grown in gold tailing soil media.

Treatment	AMF Colonization (%)	MD (%)
Control	0 a	-
Glomus sp.	77 b	43
G. aggregatum	86 b	48
A. delicata	89 b	37

Growth of seedlings

AMF colonization increased the height and diameter growth and leaf length of *N. orientalis* at 12 weeks after transplantation (Table 2, $p<0.05$). The inoculation of *Glomus* sp., *G. aggregatum* and *A. delicata* increased stem diameter and leaf length (Table 2). There were no significant differences in shoot height, stem diameter and leaf length among *N. orientalis* inoculated with the three fungi. Leaf widths of *N. orientalis* inoculated with *Glomus* sp. were higher compared to *N. orientalis* inoculated with *G. aggregatum* and *A. delicate* (Table 2, $p<0.05$).

Table 2. Effect of AMF inoculation on growth of *N. orientalis* L. at 12 weeks grown in gold tailing soil media.

Treatment	Height (cm)[a]	Diameter (mm)[b]	Leaf length (cm)[c]	Leaf width (cm)[d]
Control	4.93±0.895 c	1.88±0.583 b	1.67±0.396 b	1.00±0.175 c
Glomus sp.	7.83±0.636 a	2.91±0.240 a	8.82±0.407 a	3.57±0.058 a
G. aggregatum	8.73±0.233 ab	2.71±0.234 a	7.46±0.905 a	2.79±0.203 b
A. delicata	10.07±0.061 a	2.76±0.242 a	7.38±0.682 a	2.70±0.238 b

Note: Average values followed by unequal letters in the same column differs significantly at the LSD test level (p <0.05). [a,b,c,d] Mean ± SE.

Plant dry weight

AMF colonization significantly increased shoots and total dry weight of lonkida seedlings that were higher than control (Table 3, p<0.05). The inoculation of *Glomus* sp., *G. aggregatum* and *A. delicate* increased shoots and total dry weight. There were no significant differences in shoots and total dry weight among *N. orientalis* inoculated with the three fungi. AM colonization by *Glomus* sp and *G. aggregatum* increased roots dry weight of *N. orientalis* L. by contrast, while *A. delicata* did not increase roots dry weight of *N. orientalis.*

P content and uptake of plant

P concentration was higher in roots of *N. orientalis* seedlings inoculated with *Glomus* sp. (Table 4, p<0.001). Inoculation with *A. delicata* significantly increased P concentration in shoots of *N. orientalis* seedlings (Table 4, p<0.001) compared to other treatments. *Glomus* sp. and *Glomus aggregatum* significantly increased P content in roots of *N. orientalis* compared to control and *A. delicata*. AMF inoculation also increased P content in shoots seedlings compared to control (Table 4, p<0.001).

Table 3. Effect of AMF inoculation on dry weight of *N. orientalis* L at 12 weeks grown in gold tailing soil media.

Treatment	Dry Weight (g)		
	Root[a]	Shoot[b]	Total[c]
Control	0.09±0.007 c	0.246±0.028 b	0.33±0.032 b
Glomus sp.	0.19±0.039 a	0.466±0.027 a	0.66±0.035 a
G. aggregatum	0.18±0.007 ab	0.443±0.058 a	0.62±0.059 a
Acaulospora delicata	0.12±0.015 bc	0.420±0.066 a	0.54±0.081 a

Note: Average values followed by unequal letters in the same column differs significantly at the LSD test level (p <0.05). [a,b,c] Mean ± SE.

Table 4. Effect of AMF inoculation on P uptake by *N. orientalis* plants after 12 weeks grown in gold tailing soil media.

Treatments	P content (mg/g)[a]		P uptake (mg/plant)[b]	
	Root	Shoot	Root	Shoot
Control	1.27±0.016 bc	1.11±0.029 bc	0.11±0.010 b	0.27±0.025 b
Glomus sp.	1.52±0.038 a	1.09±0.007 c	0.29±0.052 a	0.52±0.033 a
G. aggregatum	1.33±0.020 b	1.16±0.006 b	0.24±0.007 a	0.52±0.069 a
A. delicata	1.21±0.035 c	1.26±0.008 a	0.11±0.014 b	0.53±0.079 a

Note: Average values followed by unequal letters in the same column differs significantly at the LSD test level (p <0.05). [a,b] Mean ± SE.

Pb content, uptake and transport factor

The highest Pb content and uptake occurred in roots and shoots of *N. orientalis* without AMF (control) (Table 5, p<0.001). There were differences on Pb uptake in shoots of *N. orientalis* seedlings between AMF treatments and control. Transport factor (TF) of Pb was less than 1 for all treatments (Table 5). AMF inoculation reduced Pb concentration at both the roots and the shoots (Table 5).

Table 5. Effect of AMF inoculation on Pb levels, uptake and transport factor in *N. orientalis* plants at 12 weeks grown in gold tailing soil media.

Treatments	Pb content (mg/g)[a]		TF	Pb uptake (mg/plant)[b]		Reduction of Pb by mycorrhiza (%)	
	Root	Shoot		Root	Shoot	Root	Shoot
Control	0.102±4.27 a	34.3±5.94 a	0.34	8.80±0.47 a	8.73±2.27 a		
Glomus sp.	0.025±1.63 bc	9.43±1.13 b	0.37	4.83±0.66 b	4.40±0.61 b	-75	-73
G. aggregatum	0.014±0.95 c	8.17±0.74 b	0.56	2.60±0.26 c	3.63±0.62 b	-86	-76
A. delicata	0.027±5.20 b	9.73±0.45 b	0.36	3.13±0.39 c	4.07±0.58 b	-74	-72

Note: Average values followed by unequal letters in the same column differs significantly at the LSD test level (p <0.05). [a,b] Mean ± SE

AMF was able to form a symbiosis with *N. orientalis* seedlings after 12 weeks grown in gold tailing soil media. In this study, local AMF such as *Glomus* sp and *G. aggregatum* improved growth and P uptake of 3-month-old *N. orientalis* L. seedlings in gold tailing soil media. AM fungi also reduced Pb content in plant tissue. Lonkida plant is highly dependent on the symbiosis with local AMF.

Results of this study indicated that AM fungi enhanced P concentration and uptake in the roots and shoots of plants. The previous study reported that the AMF also improved the P concentration in plants in various environmental conditions (Wang et al., 2005; Husna et al., 2016; Guo et al., 2014; Chen et al., 2015; Wulandari et al., 2016). P is an important macronutrient needed for plant growth, as development and defence mechanisms against environmental stress. The results of this study indicated that there was an improvement of P concentration in *N. orientalis* plants due to AM fungi inoculums followed by increased growth and dry weight at 12-week-old lonkida plant. In addition to P absorption, AM fungi also increased water absorption capacity in sand-dominated textured soils (Mickan et al., 2016) and improved tailings structure by binding soil particles and soil aggregation formation (Rillig and Mummey, 2006).

Data on plant growth and MD values showed that 12-week-old lonkida grown on gold tailing media required AMF inoculums indicated by a high degree of dependency on AMF. The high value of MD indicated that the growth of lonkida plant was highly dependent on the symbiosis with local AMF on sub-optimal conditions. Lonkida dependence on AMF also found in post-nickel mining or lateritic soil media conditions (Tuheteru et al., 2017). Increasing growth and dry weight of the plant by the AM fungi in this study was in line with some previous studies. Madejon et al. (2012) reported that the inoculation of AMF of *Glomus* sp*.,* *Scuttelospora aurigloba* dan *Acalouspora lervis* might increase biomass and nutrient absorption of *Eucalyptus cladacalyx* plant on arsenical gold-scale alkaline mine tailings. In addition, inoculation of *Glomus intraradices* species was reported to improve plant biomass and local AMF survival on *Dodonaea viscose* plant species*; Andropogon eucomus* and *Imperata cylindrica* in tailings of Hg-contaminated gold mines in a greenhouse scale (Madejon et al., 2012). Results of this study and several literature reviews indicated that AM fungi were needed by plants living in environmental stress condition. It is important to inoculate plants in a nursery scale or greenhouse condition with AM fungi to support the success in field planting. According to Schneider et al. (2016), AMF colonization played an important role in the development of vegetation on Pb contaminated soils.

In addition, the study results showed that good lonkida growth was also supported by AM fungi inoculation which detoxified lead (Pb). Local AMF inoculation was proven able to reduce Pb in plant tissue (Table 4). Some studies showed Pb reduction due to AMF inoculation in plants *Thlaspi praecox* (Vogel-Mikùs et al., 2006) and *Brassica chinensis* (Wu et al., 2016). The reduction of Pb by AMF inoculation was presumably made through production and secretion mechanisms of glomalin by AMF hyphae (González-Chávez et al., 2004, Chern et al., 2007; Vodnik et al., 2008; Malekzadeh et al., 2016), or in other AMF structures such as vesicles, hyphae (Joner and Leyval, 1997; Kaldorf et al., 1999; Chen et al., 2005) and spores (Salazar et al., 2018). In addition to the Pb detoxification mechanism, the concentration improvement of P nutrient elements in the AMF symbiotic and plants was also suspected to be other Pb detoxifying mechanisms through the provision of indirect metabolite energy such as ATP for metal compartments in vacuoles through the production of metallothionin and phytochelatin (Rabie, 2005). The results of this study indicated that local AMF isolated from rhizosphere of legume such previous studies on serpentine soil media, AMF *Glomus* sp. reduced the uptake of Fe and Ni at the root part (Tuheteru et al., 2017).

In this study, Pb transport factor of less than 1 (TF<1) indicated that lonkida seedlings limited Pb uptake in the root section. The accumulation of Pb as *P. mooniana* had the potential to be developed as a pharmaceutical agent of Pb on media or Pb polluted land. In is more in the root tissue, which is also found in most plants, of which about 90% of Pb is accumulated in the root tissue (Chen et al., 2015; Yang et al., 2015). Some plant species, such as *Pisum sativum* (Malecka et al. 2008), Zea mays (Gupta et al., 2009), *Lathyrus sativus* (Brunet et al., 2009), *Vigna unguiculata* (Kopittke et al., 2011), cultivar *Eucalyptus urophylla, Tectona grandis* (Peng *et al*., 2012), *Acacia mangium* and *Eucalyptus camaldulensis* (Yongpisanphop et al., 2017) accumulate Pb in the root tissue. Based on these data, lonkida is categorized as a phytoremedian of Pb with the exclusion category of rhizofiltration mechanism (TF<1). According to Dhir (2013), rhizofiltration is a way of removing contaminants by plant roots through adsorption or absorption followed by storage of metals within the roots. Based on this study results, lonkida was assumed to detoxify Pb and then accumulate Pb in its cell walls, intercellular spaces and vacuoles (Phang et al., 2010) as well as inducing antioxidants (Pourrut et al., 2011). The results of this study also strengthen the results of previous research where lonkida accumulated Hg, Fe, Mn, Cr and Ni more in the root tissue (Ekamawanti et al., 2014; Tuheteru et al., 2016; 2017).

Conclusion

Inoculation with indigenous AMF improved plant growth and P uptake of *Nauclea orientalis* at 12 weeks in soil media as well as reduce Pb content of the plant. The potential of local AMFs is developed as biofertilizers to support ecosystem revegetation and restoration programs. Field-Scale research is needed to test the effectiveness of local AMFs at the greenhouse scale.

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