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Research Article
Growth and Nutrient Status of Kayu Kuku [*Pericopsis mooniana* (Thw.) Thw] with Mycorrhiza in Soil Media of Nickel Post Mining Site

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Abstract
Background: Arbuscular Mycorrhizal Fungi are categorized as phytoremediant and increase the tolerance of plants under condition of heavy metal pollution. Effectiveness of AMF is determined very much by species of AMF, plant species and environmental condition. Therefore, testing the effect of local AMF on growth and absorption of nutrients and metal by *Pericopsis mooniana* planting stocks in growing media which are heavily polluted by heavy metal, need to be conducted. **Methodology:** There were testing of 6 inoculums of local AMF which were isolated from rhizosphere of *P. mooniana*, namely AMF from district of Kolaka (Lamedai Nature Reserve, Tanggetada Natural Forest, Bali Jaya Village Plantation Forest and PT. Vale Indonesia Tbk) and from Kendari town (Campus environment of Halu Oleo University and Office of Southeast Sulawesi Governor). Besides the 6 local AMF, there were treatment without AMF (control) and treatment with mycofer as comparison. **Results:** Results showed that local AMF were effective in increasing growth and biomass of plants; absorption of C, N, P and K in three parts of the plants; Ca in stems and leaves and of Mg in leaf tissues; increasing formation of plant's root nodules and were able to reduce Ni content in tissues of kayu kuku planting stocks. Effects of local AMF from Lamedai nature reserve and AMF from PT. Vale Indonesia were greater as compared with those of mycofer IPB. Content of Ni in kayu kuku plant tissue was found more in roots as shown by the value of TF<1. **Conclusion:** Based on this study, kayu kuku is categorized as excluder species (TF<1) and moderate species toward Ni (>50 mg Ni/kg of plant dry weight) and possessed very high dependence on AMF (MIE >75%). Local AMF are potential to be developed as biological fertilizer to improve planting stocks for rehabilitation of degraded land.

Key words: Phytoremediation, phosphorus, local arbuscular mycorrhizal fungi, *Pericopsis mooniana*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nickel deposits are generally found in serpentine soils (laterite) with ultramafic rock materials and are distributed in Sulawesi region[1]. Serpentine soils have characteristics of low content in particular nutrients (P, N, K and Ca) and rich in Fe, Mg and metals of Ni, Cr and Co. Mining activities of nickel deposits are conducted by scraping the topsoil and overburden. Such mining activities have environmental impacts in the form of vegetation and landscape change and occurrence of heavy metal toxicity. Heavy metal is defined as metal elements with mass weight of >5.0 g cm^{-3}. Nickel constitutes the dominant metal in ultramafic rocks, with concentration of ten times higher as compared with that in non ultramafic rocks[1]. Nickel is one of the essential elements needed by plants in small amount with normal content range of between 0.1 and 10 mg kg^{-1} [2]. However, the presence of metal Ni can become toxic for plants, through among others inhibition of mitosis and enzymatic activities, decrease of growth and photosynthesis and induction of oxidative pressure[3]. All metals (including Ni) in dissolved form in soils can be absorbed by microorganisms and plants[4]. One of the microorganisms which have some roles in absorption of nutrients and metal is arbuscular mycorrhizal fungi.

Arbuscular mycorrhizal fungi are obligate symbionts which perform symbioses with 97% of land plant families[5] and are generally found in terrestrial ecosystem. Xavier and Boyetchko[6] explained that AMF have important role in ecosystem recovery as biofertilizer, bioprotectans and biodegraders. Interaction between arbucular mycorrhizal fungi and plants could increase the tolerance and growth of plants in several conditions of disturbed sites, including sites which are contaminated with heavy metals[7,8]. In many cases, plants with mycorrhiza increase the absorption of heavy metals, which are transported from root to shoot (phytoextraction), whereas in other case, AMF contributes some roles in immobilization of heavy metals in soils and roots (phytostabilization)[9,10].

Arbuscular mycorrhizal fungi are found in nickel mining land in Southeast Sulawesi (Sultra)[11]. The AMF is also found to colonize roots of Ni hyperaccumulator *Phyllanthus favery* in New Caledonia[12] and *Berkheya codii* in South Africa[13]. Besides that, application of AMF could increase growth, biomass and plant nodulation (particularly leguminous plant) in soil media of nickel post mining land[7,14]. The Ni hyperaccumlator plants *Berkheya codii* which were inoculated with AMF, were reported to be able to absorb high Ni content of 6.567-13.204 μg g^{-1} of shoot dry weight[13].

One of the local leguminous tree species of Sultra which was reported to have symbioses with AMF and was developed in nickel post mining land is kayu kuku [*Pericopsis mooniana* (Thw.) Thw]. Exploration and multiplication of local AMF from rhizosphere of kayu kuku, both in nickel post mining land and other habitats, need to be conducted to support species conservation and effectiveness test of the AMF. Local Arbuscular mycorrhizal fungi which were isolated from rhizosphere of kayu kuku were proven to be able to increase growth, biomass, root nodules and nutrient absorption in soil media of Inceptisol[15]. Therefore, those local AMF need to be tested in nickel containing media to support post mining land rehabilitation. Muleta and Woyessa[16] explained that AMF effectiveness under condition of heavy metal stress was strongly affected by: (1) Strain and ecotype of AMF, (2) Type and ecotype of plants and (3) The metal and its availability. Several studies reported that AMF species from land contaminated with metal, possessed great tolerance and adaptation for heavy metal toxicity[17,18].

The objectives of this study were studying the effectiveness of local mycorrhizal fungi which were isolated from kayu kuku rhizosphere, in affecting the performance of plants, biomass and absorption of nutrient and heavy metal nickel of kayu kuku plants in soil media of nickel post mining sites.

MATERIALS AND METHODS

Time and location: This study was conducted for 5 months (February-July, 2014) in the nursery area of PT. Vale Indonesia Tbk in Kolaka district, Sultra. Chemical analysis of the media and absorption of nutrients and metal, were conducted in Laboratory of Soils and Plants of SEAMEO BIOTROP Bogor.

Materials and equipments: Materials being used in this study were among others seeds of kayu kuku, soils and sand, polybags (15×20 cm), water, KOH, HCl, trypan blue, aquadest, inoculum of AMF from trapping result and mycofer, plastic boxes measuring 40×30×15 cm, gibberelin and sodium hypochlorite 5%. Equipments being used in this study were among others: Digital weighing apparatus, scissor, centrifugation apparatus, compound microscope, ruler, caliper, camera and writing materials.

Research procedure

Collection and germination of seeds: Fruits of kayu kuku were collected from mother trees of kayu kuku in village of Bali Jaya, subdistrict of Lamedai, District of Kolaka. Collections were conducted by climbing the trees and subsequently, the fruits were air dried for 4 days. Afterwards, the seeds were taken out from the fruit manually. Before kayu kuku seeds were used for study, they were stored in refrigerator at

temperature of 4°C. Before being germinated, all seeds were sterilized by soaking in sodium hypochlorite (5%) for 5 min. After the sterilization, the seeds were rinsed several times with clean water. After that, there were seed treatments by slicing the side of the seeds and soaking in gibberelin of 0.05 ppm for 6 h. Seeds were germinated in plastic boxes measuring 40×30×15 cm which contain sterile zeolite media (2 mm). Nursery medium being used in this study were soil: sand (sterile): compost (2:1:1).

Inoculation of AMF: Arbuscular mycorrhizal fungi were obtained from the result of trapping in first stage study. Before inoculation of AMF, polybags (measuring 15×20 cm) were each filled with planting media as much as 1 kg. The AMF was inoculated as much as 5 g for each polybag by inserting into the planting hole, together with 4 weeks old kayu kuku seedling. Seedlings which were not inoculated, were used as control. Seedlings were grown and watered everyday and observed for 5 months. Maintenance of the seedlings (planting stocks) was conducted by controlling the weeds and pest and pouring of terabuster of 2 mL L^{-1} of water, with pouring volume per plant as much as 100 mL per week. Measurement of temperature and humidity was conducted very day, using hygrothermometer.

Study design: This study (experiment) was designed as Block Randomized Design (BRD) which comprise 8 treatments, namely control (M0), AMF from Urban Forest of Governor Office (M1), AMF from campus environment of Halu Oleo University (M2), AMF from nickel post mining land of PT. Vale Indonesia Tbk, from Pomalaa Kolaka (M3), AMF from Lamedai Kolaka Nature Reserve (M4), AMF from plantation forest of Bali Jaya Village (M5), AMF from Natural Forest Tanggetada Kolaka (M6) and exotic AMF (mycofer) (M7). Each treatment was replicated 5 (five) times and each replicate comprise 5 plant units, so that altogether the there was a total number of 200 plants.

Parameters being observed

Observation of planting stock growth: Planting stock height (cm), was measured by using ruler from stem base, up to the highest growint point of the main stem. Planting stock diameter (mm) was measured with caliper at 1 cm above media surface. Measurement of height and diameter was conducted every 2 week. Leaves were observed in terms of number of leaves (blades), leaf length, leaf width and their increments. Counting of number of root nodules was conducted at the end of observation. Planting stock dry weight was measured after eposure to oven temperature of 70°C for 2×24 h. Three parts of the tissue: Roots, stems and leaves were measured separately. Shoot-Root Ratio (SRR) was measured as ratio of dry weight of shoot part and that of root part in the end of the study. The SRR 1-3 and SRR 2-5 were categorized as good SRR[19,20]. Index of Planting Stock Quality (IPSQ) was measured by the formula:

$$\text{IPSQ} = \frac{\text{Dry weight of shoot+dry weight of root}}{\text{(Height/diameter)+(dry weight of shoot/dry weight of root)}}$$

Good quality planting stocks are those with IPSQ value ≥0.09[20].

Analysis of content and absorption of nutrients: Contents of C, N, P, K, Mg, Ca and Ni were measured. Content of C was measured using Walkley and Black method; those of N with Kjeldahl method; P, K, Ca, Mg and Ni with method of HNO_3-$HClO_4$. Absorptions of C, N, P, K, Mg, Ca and Ni, were measured by multiplying the content with dry weight of the planting stock tissues.

Transport Factor (TF) of Ni: (C_{aerial}/C_{root}) was measured as ratio of C_{aerial} [metal concentration in shoot part (leaves and stem)] and C_{root} [metal concentration in root]. Increase/decrease of Ni content was measured with the following formula[21]:

$$\text{Ni content (\%)} = \frac{\text{Ni uptake of mycorrhizal plants} - \text{Ni uptake of nonmycorrhizal plants}}{\text{Ni uptake of nonmycorrhizal plants}} \times 100$$

Observation of AMF colonisation and mycorrhizae Inoculation Effect (MIE): The AMF colonization = [Σ viewing area with mycorrhiza/Σ total observed viewing area]×100%[22]. The MIE = [dry weight of plant with mycorrhiza-dry weight of non mycorrhiza plant/dry weight of plant with mycorrhiza] ×100%[23]. Spores were extracted from 50 g of wet soil with sieving and decanting method[24] followed by centrifugation of supernatant which was obtained from addition of 50% sugar solution[25]. The AMF spores obtained from extraction were observed and counted under dissecting microscope with 35x magnification.

Data analysis: Observation results from each observation units were initially analyzed using analysis of variance (F-test). If test results showed significant difference, then there were tests of treatment differences using Duncan Multiple Range Test (DMRT) at 95% confidence level.

RESULTS

Colonization of AMF, mycorrhizae inoculation effect (MIE) and number of spores: In general, all treatments of AMF isolates, colonize kayu kuku roots with a range of 29-46%

(Table 1). Kayu kuku planting stocks possessed very high dependence on mycorrhiza, with (MIE) >75%, except for planting stocks which were treated with mycofer (M7), which have lower MIE (66%). For the variable number of spores, treatment of AMF from PT. Vale Indonesia Tbk (M3) produced the greatest number of spores, namely 99 spores which were significantly different from other treatments.

Growth of planting stocks: Results of analysis of variance show that AMF application affected significantly growth parameters of 5 months old kayu kuku planting stocks (Table 2). Treatment M4 (Lamedai Nature Reserve AMF) exhibited higher average height of planting stock (28.2 cm) and were significantly different from those of M0 and M1.

For diameter variable, leaf lengths and widths of all AMF exhibited higher average than those of control (M0) with increase by 27-38, 91-118 and 82-100%. Number of leaves of kayu kuku was higher than those of treatment M3 (28 blades) and treatment M6 was not significantly different from other treatments, except treatment M0, M2 and M7. Planting stocks treated with Lamedai NR AMF (M4) exhibited the greatest No. of root nodules, namely 33 nodules, while the lowest was in control treatment (7 nodules), or the No. of root nodules in planting stocks treated with M4 increased 4.7 times as compared with those of control treatment (planting stock growth performance could be seen in Fig. 1).

Table 1: Effects of treatment on root colonization, Mycorrhizae Inoculation Effect (MIE) and No. of spores in 5 months old kayu kuku (*Pericopsis mooniana*) planting stocks

Source of AMF inoculum	Colonization (%)	MIE (%)	No. of spores/50 g
M0	30±2.56^{b}	-	0000±0.00^{E}
M1	33±4.77^{a}	79±2.77^{a}	6700±6.01^{Cd}
M2	29±4.79^{a}	76±1.49^{a}	2930±3.7^{1B}
M3	40±10.45^{a}	80±2.39^{a}	3300±1.20^{A}
M4	46±7.36^{a}	80±2.96^{a}	8300±1.33^{C}
M5	36±7.38^{a}	80±2.82^{a}	8700±2.08^{C}
M6	38±7.97^{a}	76±4.67^{a}	5000±2.67^{D}
M7	37±4.73^{a}	66±2.89^{b}	3700±1.20^{D}
Pr>F	0.0012	0.0079	<0.0001

Mean values which are followed with letters which are not similar in the same column, are significantly different at DMRT test level 0,05. M0: Control (treatment without AMF), M1: Governor office AMF, M2: Halu Oleo University Campus AMF, M3: PT. Vale Indonesia Tbk AMF, M4: Lamedai Nature Reserve AMF, M5: Bali Jaya village AMF, M6: Tanggetada Natural Forest AMF and M7: Mycofer

Biomass of planting stocks: In general, treatment by local mycorrhiza (M1-M6) significantly affected planting stock dry weight and were significantly different from those of treatment M0 and M7, except for variable of root dry weight (Table 3). For variable of Root Dry Weight (RDW), planting stocks inoculated with AMF from Lamedai NR (M4) and Tanggetada Natural Forest (M6) exhibited the highest RDW with increase of 4 times each as compared with control. However, the two treatments were not significantly different with other treatments, except with control treatment (M0) and mycofer.

Dry weight of shoot (stem and leaves) and Total Dry Weight (TDW) of 5 months old kayu kuku planting stocks were highest for treatment of local mycorrhiza (M1-M6) with increase, as compared with control, respectively as much as 6.2-7.4 times (516-636%) for variable of stem dry weight, 5.4-6.6 times (438-563%) for leave's dry weight and 4.6-5.7 times (365-472%) for total dry weight of planting stock. Shoot-Root Ratio (SRR) of 5 months old planting stocks inoculated with AMF ranged between 2.67-3.96 and SRR of planting stocks without AMF was 1.40. Index of Planting Stock Quality (IPSQ) of kayu kuku was higher for treatment by local AMF and ranged between 0.63 and 0.75. On the other hand, IPSQ of those who were not treated with AMF was categorized as low, with value of 0.21 (Table 3).

Contents and absorption of nutrients: Content of C in the root of 5 months old kayu kuku planting stocks was highest for mycofer (M7) treatment, followed in magnitude by that of Bali Jaya AMF (M5), while the lowest was in planting stocks inoculated with AMF from Tanggetada Natural Forest (M6) (Table 4). Content of C in stems and leaves, respectively ranged between 42.1-51.6 and 41.4-48.3%.

Accumulation of C in the roots, stems and leaves of 5 months old planting stocks was the lowest for control

Table 2: Effects of treatment on increment of growth and number of root nodules of 5 months old kayu kuku (*Pericopsis mooniana*) planting stocks

Source of AMF inoculum	Height (cm)	Diameter (mm)	No. of leaves (blades)	Leaf length (cm)	Leaf width (cm)	No. of root nodules/planting stock
M0	14.3±0.13^{c}	3.79±0.19^{b}	10±0.19^{c}	5.30±0.24^{b}	3.4±0.13^{b}	70±0.58^{g}
M1	26.7±0.84^{ab}	5.02±0.27^{a}	23±1.60^{ab}	11.5±0.98^{a}	6.6±0.42^{a}	17±0.58^{e}
M2	25.3±0.56^{b}	5.01±0.08^{a}	21±2.16^{b}	10.6±0.38^{a}	6.8±0.15^{a}	19±0.58^{de}
M3	27.2±0.56^{ab}	5.23±0.13^{a}	28±0.88^{a}	10.1±0.09^{a}	6.4±0.15^{a}	21±0.57^{cd}
M4	28.2±0.34^{a}	4.81±0.18^{a}	25±0.62^{ab}	11.4±0.49^{a}	6.8±0.31^{a}	33±1.76^{a}
M5	27.5±1.08^{ab}	4.92±0.17^{a}	25±3.87^{ab}	11.6±0.61^{a}	6.7±0.41^{a}	13±0.88^{f}
M6	27.1±0.64^{ab}	5.07±0.03^{a}	26±1.49^{ab}	10.3±0.95^{a}	6..2±0.15^{a}	25±0.33^{b}
M7	26.5±0.85^{ab}	4.81±0.17^{a}	21±1.20^{b}	10.1±0.10^{a}	6.4±0.28^{a}	23±1.15^{bc}
Pr>F	<0.0001	0.0019	0.0004	<0.0001	<0.0001	<0.0001

Mean values which are followed with letters which are not similar in the same column, are significantly different at DMRT test level 0.05. M0: Control (treatment without AMF), M1: Governor office AMF, M2: Halu Oleo University Campus AMF, M3: PT. Vale Indonesia Tbk AMF, M4: Lamedai Nature Reserve AMF, M5: Bali Jaya village AMF, M6: Tanggetada Natural Forest AMF and M7: Mycofer

Fig. 1: Performance of kayu kuku planting stocks and root nodules of kayu kuku, M0: Control (treatment without AMF), M1: Governor office AMF, M2: Halu Oleo University Campus AMF, M3: PT: Vale Indonesia Tbk AMF, M4: Lamedai Nature Reserve AMF, M5: Bali Jaya village AMF, M6: Tanggetada Natural Forest AMF and M7: Mycofer

Table 3: Effects of treatment on biomass, Shoot-Root Ratio (SRR) and Index of Planting Stock Quality (IPSQ) of 5 months old kayu kuku (*Pericopsis mooniana*)

	Dry weight (mg)					
Source of AMF inoculum	Root	Stem	Leaves	Total	SRR	IPSQ
M0	0.42±0.02^{c}	0.25±0.11^{c}	0.48±0.06^{c}	1.16±0.16^{c}	1.40±0.16^{d}	0.21±0.03^{c}
M1	1.19±0.18^{ab}	1.54±0.14^{a}	2.66±0.28^{a}	5.39±0.57^{a}	3.80±0.16^{ab}	0.63±0.09^{a}
M2	1.56±0.27^{ab}	1.67±0.19^{a}	2.98±0.27^{a}	6.21±0.67^{a}	3.67±0.08^{ab}	0.75±0.09^{ab}
M3	1.42±0.15^{ab}	1.69±0.11^{a}	3.18±0.08^{a}	6.29±0.33^{a}	3.96±0.10^{a}	0.69±0.07^{a}
M4	1.68±0.29^{a}	1.83±0.32^{a}	3.13±0.26^{a}	6.64±0.58^{a}	3.87±0.31^{ab}	0.75±0.11^{a}
M5	1.49±0.5^{ab}	1.84±0.04^{a}	2.94±0.13^{a}	6.27±0.19^{a}	3.83±0.06^{ab}	0.74±0.02^{a}
M6	1.66±0.18^{a}	1.65±0.18^{a}	2.58±0.24^{a}	5.90±0.54^{a}	3.23±0.22^{bc}	0.75±0.06^{a}
M7	1.06±0.05^{b}	1.00±0.08^{b}	1.77±0.22^{b}	3.84±0.34^{b}	2.67±0.21^{b}	0.47±0.03^{b}
Pr>F	0.0019	0.0001	<0.0001	<0.0001	<0.0001	0.0006

Mean values which are followed with letters which are not similar in the same column, are significantly different at DMRT test level 0.05. M0: Control (treatment without AMF), M1: Governor office AMF, M2: Halu Oleo University Campus AMF, M3: PT. Vale Indonesia Tbk AMF, M4: Lamedai Nature Reserve AMF, M5: Bali Jaya village AMF, M6: Tanggetada Natural Forest AMF and M7: Mycofer

Table 4: Contents and accumulation of C in 5 months old kayu kuku (*Pericopsis mooniana*) planting stocks

	Content (%)			Accumulation (mg)		
Source of AMF inoculum	Roots	Stems	Leaves	Roots	Stems	Leaves
M0	42.8±0.82^{dc}	51.6±5.69	46.1±6.45	17.9±0.53^{c}	12.6±2.55^{c}	22.00±3.04^{c}
M1	38.2±0.87^{de}	42.6±1.35	42.9±0.39	45.4±6.31^{b}	65.4±3.91^{a}	114.30±12.54^{a}
M2	44.3±2.03^{bc}	42.1±2.01	42.4±1.42	68.1±9.46^{ab}	69.8±6.87^{a}	126.50±13.67^{a}
M3	44.7±0.45^{bc}	44.8±2.67	42.7±0.97	63.1±6.05^{ab}	75.2±3.72^{a}	13.80±4.89^{a}
M4	41.9±0.43^{dc}	44.5±0.51	43.7±0.78	70.5±1.53^{a}	81.7±15.18^{a}	136.70±12.26^{a}
M5	48.8±3.13^{ab}	42.3±0.64	48.3±3.26	72.7±5.66^{a}	77.9±2.73^{a}	141.10±3.42^{a}
M6	33.6±0.74^{e}	43.3±1.19	41.4±1.02	55.8±5.93^{ab}	71.3±5.89^{a}	106.60±10.06^{a}
M7	51.1±2.21^{a}	44.8±2.64	44.8±1.07	54.4±4.88^{ab}	44.5±1.04^{b}	79.70±11.99^{b}
Average	43.2	44.5	44.0	56.0	62.3	107.8
Pr>F	<0.0001	0.2908	0.7298	0.0017	<0.0001	<0.0001

Mean values which are followed with letters which are not similar in the same column, are significantly different at DMRT test level 0.05. M0: Control (treatment without AMF), M1: Governor office AMF, M2: Halu Oleo University Campus AMF, M3: PT. Vale Indonesia Tbk AMF, M4: Lamedai Nature Reserve AMF, M5: Bali Jaya village AMF, M6: Tanggetada Natural Forest AMF and M7: Mycofer

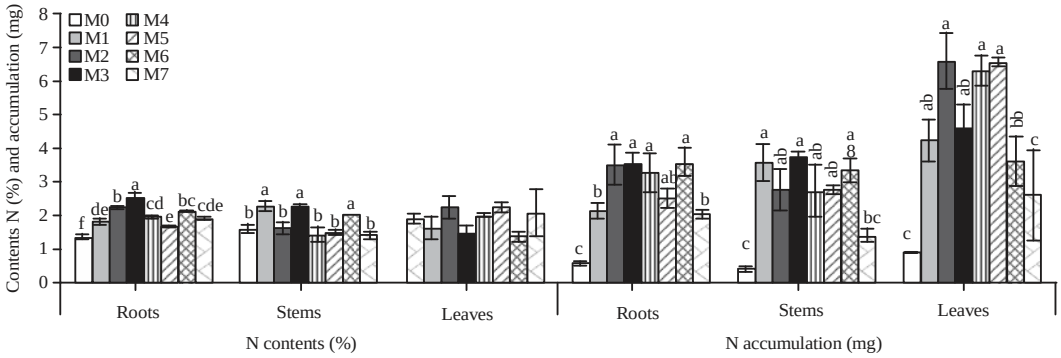

Fig. 2: Contents and accumulation of N in 5 months old kayu kuku (*Pericopsis mooniana*) planting stocks, Average values which are followed with letters which are not similar in the same column, are significantly different at DMRT test level 0.05. M0: Control (treatment without AMF), M1: Governor office AMF, M2: Halu Oleo University Campus AMF, M3: PT: Vale Indonesia Tbk AMF, M4: Lamedai Nature Reserve AMF, M5: Bali Jaya village AMF, M6: Tanggetada Natural Forest AMF and M7: Mycofer

treatment (M0). In roots, planting stocks which were treated with AMF from Bali Jaya (M5) and Lamedai Nature Reserve (M4), absorbed C abundantly and was not different with other treatments, except that of AMF from Governor Office (M1). Planting stocks inoculated with local AMF generally exhibited the highest C absorption, as compared with those of mycofer (M7) and control. From the point of view of plant parts, leaf is categorized as organic material which contain large amount of C (Table 4).

Results of analysis of variance show that AMF treatments gave highly significant effects on content and accumulation of N in roots ($p<0001$) and leaves ($p<0.0001$) and stem ($p<0.0009$) and ($p<0.0001$), whereas content and accumulation of N in leaves were not significantly affected (Fig. 2). Effect on N content in roots of 5 months old kayu kuku planting stocks was highest for AMF treatment. The AMF from PT. Vale Indonesia Tbk (M3) was associated with the highest N content with increase as much as 82% as compared to control. For the stem, planting stocks which were treated with AMF from governor office (M1) exhibited the highest N content (2.31%) and was followed in terms of magnitude by natural forest AMF treatment (M6) and AMF from PT. Vale Indonesia Tbk (M3). Range of average N content in leaves of 5 months old kayu kuku planting stock was 1.39-2.26%. Absorption of N by kayu kuku planting stocks, was highest for AMF treatment, except in stem and shoot, where the treatment by mycofer (M7) did not produce significant differences with control . The highest N accumulation was in leaves (Fig. 2).

In general, AMF treatment increased the contents and absorption of P in 5 months old kayu kuku (Fig. 4) as compared with control. Planting stocks inoculated with AMF from PT. Vale Indonesia Tbk (M3) possessed the highest content of P in roots, namely 0.25% or twofold increase as compared with control. In stem, planting stocks which were treated with M6 and M1 possessed P content above 0.2% and were statistically not different with those of other treatments, except the treatment of Bali Jaya AMF (M5) and control (M0). In terms of leaves, P contents of more than 0.2% were found in treatment of AMF from Tanggetada Natural Forest (M6) and were not different significantly with other treatments, except with treatment of Bali Jaya AMF (M5) and control (M0). Absorptions of P in treatment with local AMF were different with those of mycofer, as well as with control. Increase in absorption of nutrient P by local AMF as compared with those of control, were respectively 440-600% for roots, 900-1000% for stems and 733-900% for leaves of kayu kuku planting stocks. In terms of parts of the kayu kuku planting stocks, leaves accumulate P the most, as compared with those of roots and stems (Fig. 3).

Unlike with the previous nutrient elements, the largest K content was found in control treatment (M0) (Table 5). Planting stocks without AMF possessed K content as large as 3.24, 4.37 and 4.30%, respectively for roots, stems and leaves. However, the highest K accumulation occurred in AMF treatment. Absorption of K by plants with mycorrhiza increased as compared with those of control by, respectively 1.9-2.8 times in roots, 2.4-4.4 times in stems and 2.8-3.9 times in leaves. The largest K accumulation occurred in leaves (Table 5).

Contents of Ca in plant parts were not affected by AMF application (Table 6). There was a tendency of Ca content increase in the upper parts of the planting stocks (leaves)

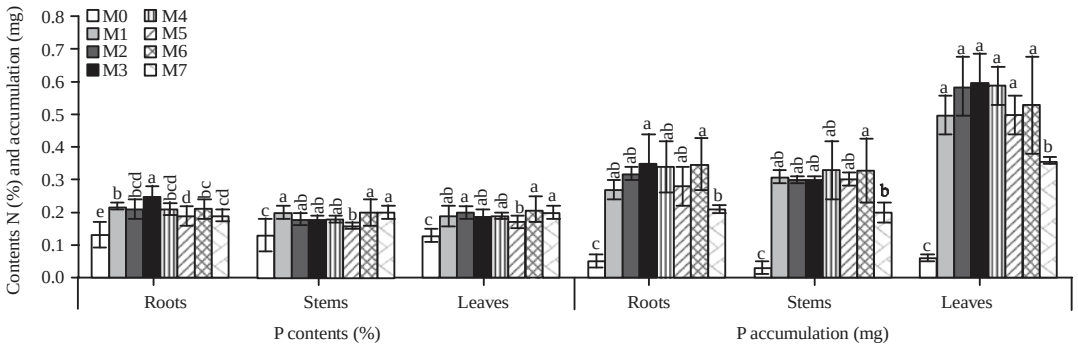

Fig. 3: Contents of P and accumulation of P in 5 months old kayu kuku (*Pericopsis mooniana*) planting stocks, Mean values which are followed with letters which are not similar in the same column, are significantly different at DMRT test level 0.05. M0: Control (treatment without AMF), M1: Governor office AMF, M2: Halu Oleo University Campus AMF, M3: PT. Vale Indonesia Tbk AMF, M4: Lamedai Nature Reserve AMF, M5: Bali Jaya village AMF, M6: Tanggetada Natural Forest AMF and M7: Mycofer

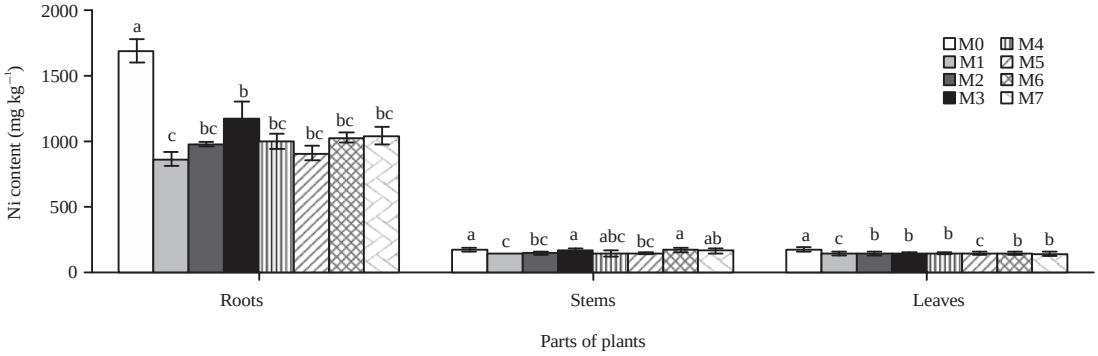

Fig. 4: Contents of Ni in 5 months old kayu kuku (*Pericopsis mooniana*) planting stocks, Mean values which are followed with letters which are not similar in the same column, are significantly different at DMRT test level 0.05. M0: Control (treatment without AMF), M1: Governor office AMF, M2: Halu Oleo University Campus AMF, M3: PT. Vale Indonesia Tbk AMF, M4: Lamedai Nature Reserve AMF, M5: Bali Jaya village AMF, M6: Tanggetada Natural Forest AMF and M7: Mycofer

Table 5: Contents and absorption of K in 5 months old kayu kuku (*Pericopsis mooniana*) planting stocks

	Contents (%)			Absorption (mg)		
Source of AMF inoculum	Roots	Stems	Leaves	Roots	Stems	Leaves
M0	3.24±0.11^{a}	4.37±0.17^{a}	4.30±0.13^{a}	1.36±0.09^{b}	1.13±0.31^{c}	2.02±0.33^{b}
M1	2.18±0.01^{b}	2.73±0.12^{bc}	2.20±0.30^{c}	2.60±0.40^{a}	4.21±0.45^{ab}	5.68±0.19^{a}
M2	2.10±0.18^{b}	2.34±0.20^{bc}	2.21±0.21^{c}	3.19±0.34^{a}	3.85±0.28^{ab}	6.65±1.02^{a}
M3	2.59±0.04^{b}	2.15±0.46^{c}	2.26±0.07^{c}	3.10±0.37^{a}	3.53±0.50^{ab}	7.21±0.39^{a}
M4	2.29±0.02^{b}	2.43±0.36^{bc}	2.58±0.21^{bc}	3.86±0.68^{a}	4.65±1.51^{ab}	8.04±0.89^{a}
M5	2.58±0.17^{b}	2.22±0.21^{bc}	2.36±0.20^{bc}	3.85±0.36^{a}	4.09±0.32^{ab}	6.89±0.39^{a}
M6	2.23±0.08^{b}	2.95±0.41^{b}	2.81±0.41^{bc}	3.69±0.35^{a}	4.93±0.93^{a}	7.43±1.79^{a}
M7	2.63±0.03^{b}	2.75±0.28^{bc}	3.24±0.40^{b}	2.79±0.16^{a}	2.75±0.33^{bc}	5.63±0.55^{a}
Average	2.48	2.74	2.75	3.06	3.64	6.19
Pr>F	<.0001	0.0001	0.0021	0.0081	0.0080	0.0232

Mean values which are followed with letters which are not similar in the same column, are significantly different at DMRT test level 0.05. M0: Control (treatment without AMF), M1: Governor office AMF, M2: Halu Oleo University Campus AMF, M3: PT. Vale Indonesia Tbk AMF, M4: Lamedai Nature Reserve AMF, M5: Bali Jaya village AMF, M6: Tanggetada Natural Forest AMF and M7: Mycofer

where the ratio of content in leaves and that in roots were above the value 1 (1.8). In the root parts, Ca content ranged between 0.15 and 0.24%. Absorption of Ca in root was not significantly affected by AMF treatment. Planting stocks treated with AMF from Tanggetada natural forest (M6) absorbed Ca the most (0.62 mg/stem dry weight) in stem

Table 6: Contents and accumulation of Ca in 5 months old kayu kuku (*Pericopsis mooniana*) planting stocks

	Contents (%)			Absorption (mg)		
Sources of AMF inoculum	Roots	Stems	Leaves	Roots	Stems	Leaves
M0	0.22±0.03^{a}	0.34±0.01^{a}	0.51±0.06^{a}	0.09±0.02	0.09±0.02^{c}	0.34±0.08^{c}
M1	0.16±0.01^{a}	0.34±0.03^{a}	0.27±0.09^{a}	0.20±0.04	0.53±0.08^{ab}	0.68±0.14^{bc}
M2	0.24±0.06^{a}	0.27±0.03^{a}	0.36±0.09^{a}	0.39±0.13	0.46±0.10^{ab}	1.01±0.21^{bc}
M3	0.17±0.02^{a}	0.27±0.04^{a}	0.22±0.07^{a}	0.25±0.05	0.44±0.03^{ab}	0.70±0.21^{bc}
M4	0.16±0.02^{a}	0.23±0.06^{a}	0.36±0.09^{a}	0.27±0.07	0.46±0.18^{ab}	1.13±0.32^{a}
M5	0.17±0.01^{a}	0.23±0.06^{a}	0.33±0.09^{a}	0.25±0.02	0.42±0.10^{ab}	0.96±0.24^{abc}
M6	0.15±0.02^{a}	0.37±0.03^{a}	0.27±0.05^{a}	0.26±0.05	0.62±0.11^{a}	0.67±0.10^{ab}
M7	0.17±0.02^{a}	0.27±0.04^{a}	0.39±0.09^{a}	0.18±0.02	0.27±0.05^{bc}	0.66±0.09^{ab}
Average	0.18	0.29	0.34	0.24	0.41	0.77
Pr>F	0.1579	0.1534	0.2785	0.0998	0.0500	0.0695

Mean values which are followed with letters which are not similar in the same column, are significantly different at DMRT test level 0.05. M0: Control (treatment without AMF), M1: Governor office AMF, M2: Halu Oleo University Campus AMF, M3: PT. Vale Indonesia Tbk AMF, M4: Lamedai Nature Reserve AMF, M5: Bali Jaya village AMF, M6: Tanggetada Natural Forest AMF and M7: Mycofer

Table 7: Contents and accumulation of Mg in 5 months old kayu kuku (*Pericopsis mooniana*) planting stocks

	Contents (%)			Absorption (mg)		
Source of AMF inoculum	Roots	Stems	Leaves	Roots	Stems	Leaves
M0	0.97±0.10^{a}	0.53±0.12^{a}	0.70±0.14^{a}	0.40±0.03	0.14±0.04	0.32±0.04^{d}
M1	0.69±0.02^{a}	0.44±0.09^{a}	0.38±0.07^{a}	0.83±015	0.70±0.18	0.97±0.07^{c}
M2	0.69±0.09^{a}	0.41±0.08^{a}	0.49±0.05^{a}	1.05±0.15	0.68±0.15	1.42±0.01^{ab}
M3	0.81±0.18^{a}	0.39±0.12^{a}	0.37±0.05^{a}	1.18±0.36	0.64±0.15	1.18±0.17^{bc}
M4	0.68±0.09^{a}	0.39±0.11^{a}	0.51±0.05^{a}	1.18±0.34	0.79±0.36	1.59±0.22^{a}
M5	0.65±0.06^{a}	0.46±0.06^{a}	0.40±0.06^{a}	0.97±0.13	0.85±0.12	1.17±0.13^{bc}
M6	0.71±0.09^{a}	0.49±0.12^{a}	0.44±0.06^{a}	1.18±0.02	0.85±0.24	1.07±0.06^{bc}
M7	0.68±0.02^{a}	0.41±0.10^{a}	0.52±0.09^{a}	0.72±002	0.43±0.14	0.89±0.08^{c}
Average	0.74	0.44	0.48	0.94	0.64	1.08
Pr>F	0.4124	0.9644	0.1130	0.1683	0.1604	0.0232

Mean values which are followed with letters which are not similar in the same column, are significantly different at DMRT test level 0.05. M0: Control (treatment without AMF), M1: Governor office AMF, M2: Halu Oleo University Campus AMF, M3: PT. Vale Indonesia Tbk AMF, M4: Lamedai Nature Reserve AMF, M5: Bali Jaya village AMF, M6: Tanggetada Natural Forest AMF and M7: Mycofer

and were not different with those of other treatments except mycofer (M7) and control (M0). In the leaves, planting stocks which were inoculated with inoculum from Lamedai NR (M4) exhibited the highest Ca absorption and were not different with those of treatments M7, M6 and M5 (Table 6).

Analysis of variance showed that AMF treatments did not affect significantly Mg content in the whole parts of the planting stocks and Mg absorption in the roots and stem (Table 7). Ranges of average content of Mg in roots, stems and leaves were 0.65-0.97, 0.39-0.53 and 0.37-0.70%, respectively. On the other hand, absorption of Mg in roots and stems were, respectively 0.40-1.18 mg/root dry weight and 0.14-0.85 mg/stem dry weight. For Mg absorption variable, in the leaves, treatments of AMF from Lamedai NR (M4) exhibited the highest absorption of Mg (1.59 mg/leaf dry weight) followed in magnitude by treatment of Halu Oleo University AMF (M2).

Contents and absorption of Ni: In general, the largest content of Ni occurred in roots. Plants which were not treated with AMF exhibited the largest content of Ni in all parts of the planting stocks (Fig. 4). The AMF treatment could reduce the Ni content in kayu kuku planting stock tissue. Arbuscular Mycorrhizal Fungi (M1) could reduce the Ni content by 50% in roots, 43% in stems and 51% in leaves, as compared with control (Fig. 4).

The lowest Ni accumulation occurred in non mycorhiza treatments in the three parts of the planting stocks. Plants subjected to control treatment and Bali Jaya village AMF treatment were those which accumulate the largest amount of Ni in leaves (Fig. 5).

Comparison between Ni content in stem and leaf tissues and those in root tissues is indicated by the value of Transport Factor (TF). In general, the values of TF of Ni , both in stems and in leaves were <1. The values of TF of Ni in stems ranged between 0.10-0.16, which were greater than TF in leaves, which ranged between 0.09-0.12 (Fig. 6).

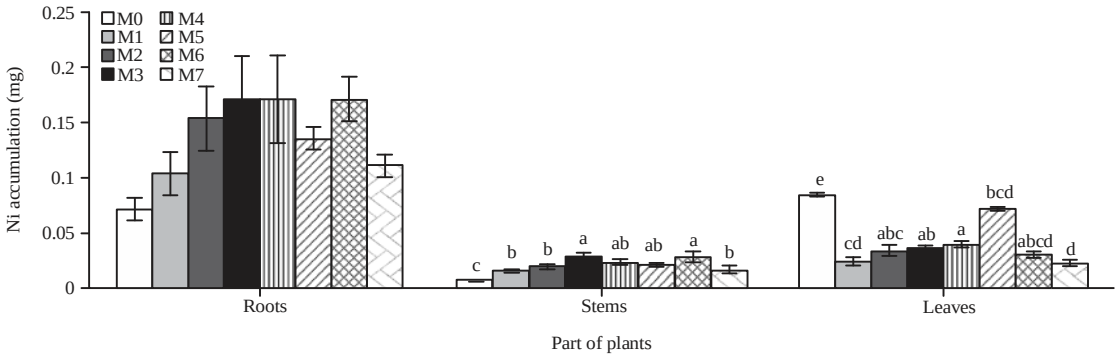

Fig. 5: Accumulation of Ni in 5 months old kayu kuku (*Pericopsis mooniana*) planting stocks, Average values which are followed with letters which are not similar in the same column, are significantly different at DMRT test level 0.05. M0: Control (treatment without AMF), M1: Governor office AMF, M2: Halu Oleo University Campus AMF, M3: PT. Vale Indonesia Tbk AMF, M4: Lamedai Nature Reserve AMF, M5: Bali Jaya village AMF, M6: Tanggetada Natural Forest AMF and M7: Mycofer

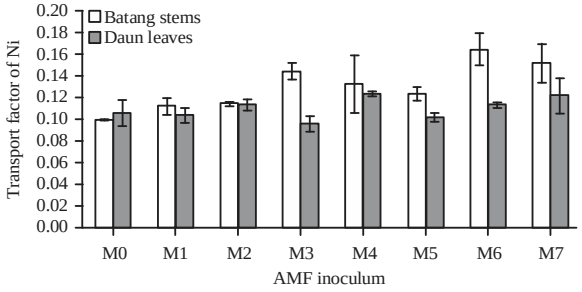

Fig. 6: Values of transport factor of Ni in stems and leaves of 5 months old kayu kuku (*Pericopsis mooniana*) planting stocks

DISCUSSION

Rooting system of 5 months old kayu kuku planting stocks were colonized by AMF (Table 1). Colonization in AMF treatment ranged between 29-46%, whereas plant roots which were not treated with AMF were also colonized by AMF. Presence of AMF structure in roots of plants which were not treated with AMF, was probably due to colonization by natural AMF existing in the soil medium, by the help of wind or water being applied. Presence of AMF structures, such as internal and external hyphae and vesicles could contribute something for growth and biomass of kayu kuku planting stocks in the media of nickel post mining sites. Improvement of kayu kuku growth by AMF could occur through increasing the P absorption, soil quality improvement and limiting the Ni content in plant tissues[16].

Results showed that kayu kuku planting stocks have high dependence on mycorrhiza (Table 1). On the basis of categorization of dependence on mycorrhiza which was developed by Habte and Manajunath[23], kayu kuku is categorized as species which has very high dependence on AMF, particularly all local AMF (76-80>75%), except mycofer (66<75%). The high values of MIE for several local AMF indicates that the growth and survival of kayu kuku plants, depend very much on their symbioses with AMF. The high values of MIE are manifestation of increasing growth and biomass of plants with mycorrhiza. Muleta[26] explained that level of dependency of plant species on AMF varies among plants (particularly in relation with root morphology), soil and climate. Muleta[26] explained further that rooting systems which have little branching and poor in root hairs, would depend more on AMF for supporting their growth and plant development. Besides that, soils with high level soil fertility and sufficient moisture could reduce the dependency of plants on AMF. Therefore, the very high dependence of kayu kuku on AMF is probably due to root characteristic with little branching (as in most other legumes) and also due to its growth media which contain heavy metals and hence was not supportive for plant growth. Dependence of leguminous species on AMF had been reported also for species *Acacia mangium*[27].

Treatment of AMF, both with mycofer and local AMF could increase plant growth as compared with those of control (Table 2). Besides growth, AMF also contributes toward increasing the planting stock biomass (Table 3). Planting stock biomass which were inoculated with local AMF (M1-M6) were higher than those treated with mycofer (M7) and control (M0). Effectiveness of local AMF for kayu kuku in soil media contaminated with nickel, was possible due to several phenomena, namely: (1) Local AMF were suitable with the existing condition, (2) Local AMF were suitable with root exudates produced by kayu kuku roots and (3) Genetically, the local AMF possessed ability to absorb water and nutrients for

their host plants. Increase in plant growth and biomass was related much with absorption and improvement of water and nutrient status of plants by AMF under soil condition or media contaminated with metal[5,28,29].

Shoot-Root Ratio (SRR) is the ratio between dry weight of plant's part above ground and that below ground. The higher the SRR, the better would be the preparedness of the planting stocks to be planted in the field. The values of SRR in the treatment of local AMF (3.23-3.96) were better than those of mycofer and control. The appropriate value for SRR is between 2-5 and values approaching 5 are better than those approaching 2[20]. The value of SRR for plants without AMF treatment was 1.40. The phenomenon of low SRR in treatment without AMF occurred because the planting stocks which were under stress condition, often allocate most of their photosynthesis products to lower part organs. This study result is in agreement with statement from Muleta[26] that AMF inoculation was reported to be able to decrease plant shoot-root ratio under condition of media or soil which is contaminated with heavy metal. Indexes of Planting Stock Quality (IPSQ) obtained from this research were 0.21-0.75, which had fulfilled the requirement of IPSQ $\geq$0.09. On the basis of those values of SRR and IPSQ, the kayu kuku planting stocks have been ready to be planted in the field.

Results of this study showed that AMF treatment increased the absorption of C, N, P and K in the three parts of the plants and of Mg in leaf tissues. Specifically for element P, planting stocks which were treated with local AMF, increased their content and absorption of P. Unlike with other elements, K content was higher than those of control. Contents of Ca and Mg in plant tissues were not significantly affected by AMF. Increase in absorption of that nutrients could support growth and plant biomass. Muleta[26] explained that absorption of N, P, K, Ca and Mg increased significantly in plants with mycorrhiza as compared with those without AMF. One important element is phosphorus. Phosphorus which is one of the essential elements, is able to be absorbed by AMF, so that its availability is sufficient for plants[5,29]. Several studies reported that AMF could fulfill 90% of the plant's need for P[26]. Sufficient availability of P could support the formation of root nodules and N fixation by kayu kuku under condition of metal pollution[7,26]. Element P is highly needed by nitrogenase enzyme in nodulation stage and N fixation[30]. Besides that, reduction of Ni contents in planting stock's root with mycorrhiza (reduction by 30-49%) could also contribute toward formation of root nodules. The small No. of root nodules in planting stocks with AMF (Table 2) was possibly due to inhibition of nodulation due to presence of heavy metal, such as Ni. Vivas *et al.*[28] reported that there is tendency that increase in Ni could decrease the No. of root nodules and their size. Increase in the No. of root nodules and ability to fix nitrogen by rhizobia which are affected by AMF is expected to support the availability of plant nitrogen. Study results by Veresoglou *et al.*[30] in several publication showed that AMF inoculation could increase the capacity of nitrogen fixation by leguminous species. Nitrogen is categorized as macronutrients which are highly needed for plant growth and biomass. Therefore, synergy between legume-mycorrhiza-rhizobia could be useful for improvement and development of leguminous species.

Range of Ni in the three parts of the planting stocks were respectively 860-1691, 96-170 and 86-177 mg kg^{-1} (Fig. 4). Based on criteria of normal Ni content for cultivated plants (0.1-1.0 mg kg^{-1}) the species of kayu kuku is categorized as moderate toward Ni (>50 mg kg^{-1}) and not a sensitive species toward Ni (>10 mg kg^{-1})[2,31]. Content of Ni of plant tissue is affected very much by availability of Ni in the soil, plant species, plant parts and environmental condition[32]. The highest Ni content occurred in the root of the plants. On the basis of such data, kayu kuku is categorized as excluder of Ni metal. This fact is supported by the value TF<1 (Fig. 6), where the highest Ni content was in the root part, as compared with that in shoot part. Ability to survive and exclude Ni by kayu kuku species under condition of metal pollution is probably due to mechanism of heavy metal detoxification (particularly Ni) in the form of production of root exudates as metal chelator. Such root exudates are amino acid (histidine) and carboxylic (citrate) acid[31,33].

Plants which are hyperaccumulator of Ni in chelating or absorbing and translocating Ni in plant's body, usually produce ligand or particular chelator to chelate Ni. Bhatia *et al.*[34], explained that transportation of Ni occurred through cytoplasm with the help of organic acid (malate and citrate) and amino acid (histide and glutamine). Results by Montagres-Pelletier *et al.*[35] found carboxylic acid (citrate and malate) in hyperaccumulator plants *Leptoplax emerginata* and *Alyssum murate*. There was found also histidine in *Alyssum lesbiacum* and *Thlaspi goesingense*[36,37]. Citrate was also found in *Thlaspi goesingense*[36]. Besides that, species *T. caerulescens* produced nicotianamine for transportation of Ni[38]. Also, addition of synthetic chelator such as EDTA, increase absorption and translocation of Ni in *Helianthus annuus* L.[39] and *Brassica juncea*[40]. The Ni which is absorbed by plant's rooting system was afterwards be able to be stored in plant tissues. Berazain *et al.*[41] reported that Ni is stored in stems by laticifer tubes and epidermis vacuole tissue of leaves of family Euphorbiaceae. According to Kramer *et al.*[42], Ni is stored in protoplasm of leaves and vacuole.

Content of Ni in roots and leaves of kayu kuku planting stocks was higher in treatment without AMF (control) (Fig. 4). This indicates the excess of Ni in roots and leaves. Excess of Ni in roots and leaves could results in toxicity in kayu kuku planting stocks as evidenced by the presence of necrosis in leaves of kayu kuku planting stocks . Toxicity of Ni in kayu kuku planting stocks could reduce the absorption of nutrient elements C, N, P and K, 193 and 421; 36.359 and 496.70%; 480 and 783.33; 211.50 and 198.02%, respectively (Table 4 and 6, Fig. 4 and 5). These phenomena were similar with those found by Brune and Deitz[43] that toxicity of Ni could reduce absorption of nutrients K, P, Mg in roots and leaves of barley plants and could reduce N in leaves and roots of green peas. Lack of those nutrients could affect the growth and development of kayu kuku, especially in the form of retarded photosynthesis. Toxicity of Ni in roots could probably retard the development of roots, particularly decreasing the root length of kayu kuku planting stocks. Study results by Alam *et al.*[44] showed that root length of *Brassica juncea* plants decreased by 33% with the presence of 100 µm Ni.

Treatment with AMF was able to decrease Ni content in roots and shoots, respectively 30-49 and 29-49% as compared with planting stocks which were not accompanied with AMF. Table 6 shows that sources of AMF gave differing effects on contents and absorption of Ni in plant parts. Planting stocks which were inoculated with FMA exhibited lower content of Ni as compared with control. The low content of Ni in AMF treatment indicated that AMF play a role as Ni phytostabilization. Concentration of Ni was possibly stored in AMF structure existing in plant roots (vacuole, hipha and vesicle)[45], or through compounds excreted by fungi (such as glomalin), could reduce potential content of metal[46] and is absorbed by AMF cell wall.

Muleta and Woyessa[16] explained that effectiveness of AMF in phytoremediation was highly affected by (1) Strain and ecotype of AMF, (2) Type and ecostype of plants and (3) Metal and its availability. Differences in AMF inoculums have some influence on AMF effectiveness in affecting contents and absorption of Ni. Inoculation of AMF (*Glomus mosseae*) could reduce Ni concentration in shoots of bean and clover[47] and *Trifolium repens* as compared with those of control[29]. Unlike with the results of this study, several studies indicated that AMF inoculation could increase the biomass of shoot and high absorption of Ni in hyperaccumulator plants *Berkheya codii*[13], *Phyllanthus favery*[12] and *Helianthus annuus* L[48].

In line with the improvement of nutrient and water status and chelating of heavy metal by local AMF, the growth and biomass of plants with mycorrhiza could be increased. Several studies result showed the same phenomena that under media condition which is contaminated with Ni, local AMF could increase growth and biomass of Ni in hyperaccumulator plants *Berkheya coddii*[13], *Trifolium repens*[29], *Helianthus annuus* L[48], *Albizia saponaria*[14] and *Pericopsis mooniana* Thw.[7].

CONCLUSION

Local arbuscular mycorrhizal fungi were effective in increasing the growth and biomass; absorption of nutrients C, N, P, K, Mg and nodulation and could reduce Ni content in the tissues of kayu kuku planting stocks. Arbuscular mycorrhizal fungi from Lamedai Nature Reserve and PT. Vale Indonesia Tbk were more effective than mycofer. Kayu kuku was categorized as species which has very high dependence (MIE) on local AMF (>75%) and high dependence on mycofer (<75%) and as excluder species (TF<1) and moderate toward Ni (>50 mg Ni/kg dry weight of planting stocks). These results indicates that local AMF symbioses are important in adaptation of kayu kuku species in soils which are contaminated with Ni and are relevant with ecological restoration of degraded land.

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