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THE EFFECT OF APPLICATION *ACAULOSPORA* SP ON THE ROOT GROWTH OF *CANAVALIA ENSIFORMIS* L AT NICKEL POST-MINE LAND

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ABSTRACT

Plant root growth is greatly influenced by the chemical properties of the soil; therefore, excessive heavy metals content in the soil will affect plant root growth. The aim of this research was to study the effect of *Acaulospora* sp as biological agents on the root growth of *Canavalia ensiformis* at nickel post-mining land in Sorowako, Indonesia. The research was carried out using a randomized block design with application of *Acaulospora* sp from different area as treatment, namely: native *Acaulospora*, exotic *Acaulospora* and without *Acaulospora*. The results show that the application effect of native *Acaulospora* increased the number of lateral roots, percentage infected root, root length, root volume, root dry weight, and root:shoot ratio of *Canavalia ensiformis*. The native *Acaulospora* also acceleration of the adaptation process of *Canavalia ensiformis* in the land with high nickel concentration.

Keywords**:** Bioremediation, fungi, heavy metal, mycorrhiza**.**

INTRODUCTION:

The root is the part that cannot be separated from the plant and root is also important for upper part of the plant (Koevoets *et al*., 2016). Potential root growth needs to be fully achieved to obtain the maximum potential of plant growth; more roots result in higher crop yield (Man *et al*., 2016). The concept of equilibrium morphology most often is used in allometric relations (Mokhtari *et al*., 2017 and Tian *et al*., 2017). This concept means that growth in a section of a plant is followed by a growth of the other part (Anfodillo *et al*., 2016).

Root growth is greatly influenced by the chemical properties of the soil, excessive Heavy Metals (HM) content will dent the plant root growth (Hossain and Haque, 2011). Heavy Metals enter the symplasmic or apoplasmic path through plant roots before entering the xylem of roots (Gao *et al*., 2016). A study showed that high concentration of HM caused rigidity and lignification of the cell wall, and caused reduction of roots growth (Gao *et al*., 2016). Lin and Kao (2005), Hossain *et al*. (2012) and Emamverdian *et al*. (2015) found that HM can reduce root length up to 33% in *Brassica juncea* (Alam *et al*., 2007) as well as affecting the cell anatomy structure of root (Lux *et al*., 2011, Gowayed and Almaghrabi, 2013, Asati *et al*., 2016; Tupan and Azrianingsih, 2016, Sachan and Lai, 2017). According to Khan and Khan (2010), level of nickel (Ni) concentration that is toxic to plants ranged from 25 up to 246 ppm of the plant dry weight, dependes on the species and varieties of the plants.

Effects of HM on plant poisoning can be reduced by using an Arbuscular Mycorrhiza (AM), but the response varies between the plants, as a result of genetic variation in the colonization of AM and plant growth response (Garq and Pandey, 2015). Bano and Ashfaq (2013) and Shabani *et al.*, (2015) reported some types of AM that can tolerate HM stress, such as; *Glomus mosseae*, *Glomus intrara-dices*, and several other species of *Glomus*. Nevetheless, the previous studies did not provide information on whether the species of AM was native or exotic.

The main mechanisms how AM eliminates or reduces the effect of HM toxicity in plant is by distributing HM into cortex cell (Berruti *et al*., 2015; Guerrero *et al*., 2016), binding on HM into cell wall or mycelium and storing inside the vacuoles or other organelle (Krishnakumar *et al*., 2013, Rezvani *et al*., 2013, Emamverdian *et al*., 2015), releasing heat-shock protein 90 kD and Glutathione-S-transferase (Hildebrandt *et al*., 2007, Shen *et al*., 2015, Ferrol *et al*., 2016) chelating HM into a matrix of soil through the formation of glycoproteins or producing complex fosfatemamin in the hypha (Aloui *et al*., 2011, Lokhandwala *et al*., 2017), increasing the growth of roots and shoots (Mohammadi, 2011). In addition, AM can reduce HM absorption by dissolving HM through pH changes, then chelation/immobilization of the HM can be carried on by ekstraradis mycelium, glomalin or the AM exudate (Kanwal *et al*., 2015). Bano and Ashfaq (2013) suggested that AM parts that serve as the place of HM accumulation are the hypha and arbuscular. The varied strategies used by AM against toxicity of HM indicates that different types of AM can act specifically or does a remedial function in accordance with conditions applicable in the rhizosphere or plant. Defense mechanism also occurs in plants that are experiencing the HM stress, for instance; growth regulator (Egamberdieva *et al*., 2017, Neto *et al*., 2017), antioxidant enzymes (Sheetal *et al*., 2016), the amino acid as osmoprotectants (Joseph *et al*., 2015) and Ni^+ Chelation with metalloproteins and metallothionines (Sachan and Lai, 2017).

The family of *Canavalia ensiformis* L. (*C. ensiformis*) is Fabaceae, a perennial legume plant commonly cultivated as an annual. The plant grows up to 3 m high with 10-25 cm long trifoliate leaves and a strong root system that have nodules. Flowers are white, mauve, or pink with a red base. Pods are up 40 cm maximum long and contain ellipsoid seeds with 1-2 cm long. *C. ensiformis* is able to grow on sub optimum land (Suharsi *et al*., 2013) and can be used as a heavy metal phytoaccumulator (Souza *et al*., 2013), several studies shown that *C. ensiformis* are tolerant to heavy metal lead (Pb) (Romeiro *et al*., 2007), zinc (Zn) (Andarade *et al*., 2009a), copper (Cu) (Andrade *et al*., 2009b), and cadmiun (Cd) (Rossi *et al*., 2012) thus showing their potential as a phytoextraction plant.

Acaulospora sp is one of the genus of endomycorrhiza that belongs to Acaulosporaceae family which has 32 species (Simanungkalit, 2006). Characteristics of *Acaulospora* sp. are colored; dark brown to dark brown orange, formed; Globose, subglobose. Spore diameter average is 149.3 µm (INVAM, 2016). The preliminary research results showed that the endomikoriza dominated at the nickel post-mining plantation area are genus of *Acalauspora* (75.1%), *Gigaspora* (19.4%) and *Glomus* (5.6%). This research was carried out to examine the effects of *Acaulospora* sp as biological agents against root growth of *C. ensiformis* at nickel post-mining land in Sorowako.

MATERIALS AND METHODS

Research was conducted in the area of Nursery of Vale Indonesia in Sorowako, East Luwu District, South Sulawesi Province, Indonesia, from December 2016 until May 2017, at altitude 419,76 m asl, latitude 2°31’33” S, longitude 121°20’50” E, which has a climate type Af or tropical rainforest climate (Koppen-Geiger), temperature and annual precipitation average was 24.2°C and 2019 mm respectively. The soil was analyzed in the Laboratory of Chemistry and Soil Fertility, Department Soil Science Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia. Soil texture was determined using hydrometer method, pH of soil was measured using pH meter, base cation (BC) with ammonium acetate extracted in pH 7 then was measured with an atomic absorption spectrometer, base saturation calculated with % BC= (bases cation/CEC) x100%, Cation exchange capacity (CEC) with 1 M NH4OAc, C-organic with Walkley and Black method. The concentration of Ni was measured in the laboratory of chemistry, Polytechnic of Ujung Pandang, Makassar, using the manual book of X-Ray Florence Spectrophotometer/Bruker/S2 Ranger. The characteristics of soil properties are shows in Table 1.

Table 1: Overburden soil characteristic of nickel post-mining land, Sorowako.

Characteristic	Number
Texture	Clay Loam
pH	5,62
BC (%)	69
C-organic (%)	1,88
Ni ($cmol.kg^{-1}$)	14.200
Ca ($cmol.kg^{-1}$)	2,26
Mg ($cmol.kg^{-1}$)	3,96
K ($cmol.kg^{-1}$)	0,27
Na ($cmol.kg^{-1}$)	0,12

This research was compiled based on the randomized block design (RBD), consisting of three treatments, namely: native *Acaulospora*; exotic *Acaulospora* and without *Acaulospora* as control, each treatment had three replications, and each repeat consists 10 experimental units.

Native *Acaulospora* was isolated from plant rhizosphere in Ni post-mine land that have been rehabilitated in Sorowako, by using wet filtering method and sucrose centrifugation method (Gerdemann and Nicolson, 1963), (Daniel and Skipper, 1982) in microbiology laboratory, Centre for Research and Development of Environment and Forestry of Makassar, South Sulawesi Province, Indonesia, while exotic *Acaulospora* isolates obtained from sugar cane plant rhizosphere which isolated by Kumalawati (2014). The AM used was only identified morphologically at the *Acaulospora* genus level using manual book from International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM, 2016).

The medium used was overburden with soil in Ni concentrations of 14.200 $cmol/kg^{-1}$ that was incorporated into the polybag as much as 75%. Exotic composite seeds of *C. ensiformis* were grown and inoculated as much as 22 *Acaulospora* spores contained in propogoul. Watering was given every day at 10 a.m., and an organic fertilizer was given when young plants were 7 days after planting (DAP), pest and disease control was done after there are symptoms of an attack, and weeds control was done every week.

The lateral roots number, root length, root volume, root dry weight, root: shoot ratio, and root infects was measured when the plant was 90DAP. The number of lateral roots and root length were measured using a method by Rao and Ito (1998). The measured root volume using a method by Sattelmacher (1986) and Harrington *et al.*, (1994). The dry weight of roots and the root: shoot ratio calculated using a method by Shuurman and Goedewaagen (1971) and Jokanovic *et al*, (2014), and the infection percentage was calculated by using the formula by Sarah and Ibrar (2016).

Data were subjected to a one-way analysis of variance (ANOVA) using Microsoft Excel 2010 and significant differences of treatment means were compared by the Fisher's protected Duncan's procedure and the orthogonal test.

RESULTS AND DISCUSSION

Analyses of variance showed that *Acaulospora* sp treatment significantly affected lateral roots number of *C. ensiformis*. The result of Duncan's test showed that effects of native *Acaulospora* were significantly different with exotic *Acaulospora* and without *Acaulospora*, and the result of the orthogonal test showed that effect of native *Acaulospora* was only different with control (without *Acaulospora*) treatment on lateral roots number of *C. ensiformis*. Furthermore, analysis of variance showed that *Acaulospora* sp had insignificant effects on root length, root volume, dry weight of root and root:shoot ratio of *C. ensiformis*. However, based on average value, native *Acaulospora* was higher than exotic *Acaulospora* and without *Acaulospora*.

The lateral roots number of *C. ensiformis* that was inoculated with native *Acaulospora* increased by 20.07-28.83% (Fig. 1a), with the percentage root infected was 20.59-95.24% (Fig. 2). In this case, the native *Acaulospora* have adapted to environments had higher Ni concentrations so root infection on *C. ensiformis* was not inhibited.

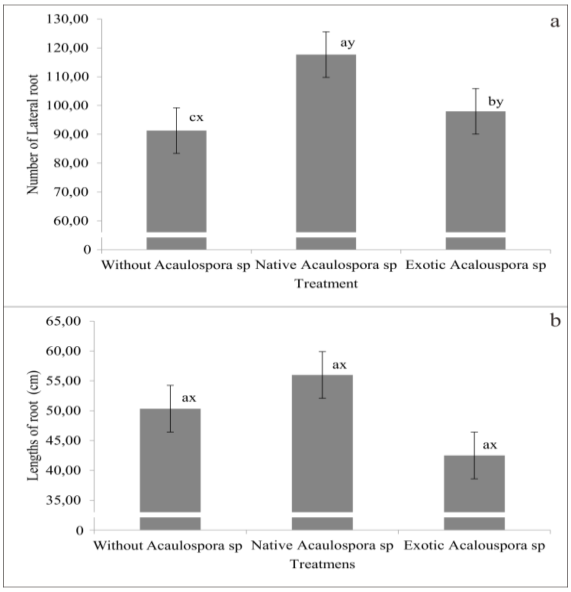

Fig. 1: Average of number of lateral root (a), lengths of root (b) *C. ensiformis* at treatment *Acaulospora* sp. in land post-mining nickel.

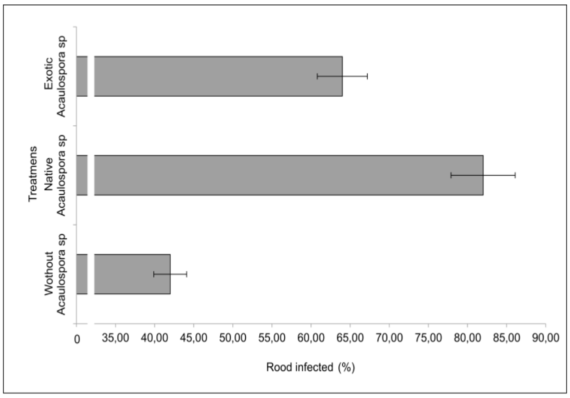

Fig. 2: Percentage of infected *C. ensiformis* root at treatment *Acaulospora* sp. in land post-mining nickel.

Bano and Ashfaq (2013) suggested that plants produced root exudate that contains strigolactone and flavonoids to attract AM, and AM also emits a signal of lipochito-oligo-saccharide to plants. At the time of symbiosis between AM and plant roots, and exchange of signal was not hampered by the presence of heavy metals includes Ni metal, then AM has had high Ni metal tolerance. Amir *et al*., (2013) and Nafady *et al*., (2017) showed some native AM types that are resistant to heavy metals included nickel, namely; *Acaulospora bireticulation, Gigaspora margareta, Glomus lamellosum and Glomus etunicatum.* In addition to adaptation aspect, plants hormones such as auxin tend become signal and contribute toward symbiosis formation of AM and plant roots, and the important factor for lateral roots development which infection location was preferred by fungi (Muller and Guther, 2007, Miransari *et al*., 2012). Furthermore, according to Seregin and Ivanov (2001) and Lai (2010) that lateral roots have different resistance to HM when compared between primary root and root hairs because of existence endodermal barriers and centers cylinder structure characteristics. The formation and development of lateral roots began with the periclinal cleavage that occurs in some pericycle cells group, the resulting cell will divide again in a way periclinal and/or anticlinal for formed a cell group. At the moment of primordial root to grow elongated penetrate roots cortex cell, then young lateral roots will appear on primer root surface (Hidayat, 2010, Cheng *et al*., 2013). The researchers acknowledge, some the plant hormones role and the environment signal effect show that lateral root induction more dominant controlled by auxin hormone that move acropetally and supported by cytokinin hormone which move basipetally (Chang *et al*., 2013, Kazan, 2013, Takahashi, 2013, Bensmihen, 2015; Voβ *et al*., 2015).

Colonization of AM on plants roots cause (1) higher antioxidant enzymes activity (superoxide dismutase, katalase, and guaiacol peroxidase) increase antioxidant molecules concentration (karotenoid, prolin, and α-tocopherol) in plant, (2) increased of enzyme activity at Glutathione-ascorbic cycle result in higher ascorbate:dehydroascorbate (AsA:DHA) and glutathione:glutathione disulfide (GSSG:GSH) ratio, (3) increased glyoxalase system by stimulating enzyme activity of glyoxalase I and glyoxalase II, (4) increased concentrations of cysteine, glutathione, thiol non-protein, and activity of glutathione-S-transferase, which facilitates of HM absorption to non-toxic complex (Garg and Kaur, 2012; Hashem *et al*., 2015; Nahar *et al*., 2015, Huang *et al*., 2016, Przepiora *et al*., 2016, Sharma *et al*., 2017). Sun and Tang (2013), Casarrubia *et al.,* (2016), suggests that AM can change the root morphology and profiles of volatile organic compounds that were emitted by plant roots, so it has potential to help plants adapt and changing the soil environment. But according to Hanlon and Coenen (2011) and Foo *et al*., (2013), that auxin hormone only needed by AM when infecting the to plant roots and not needed for post-infection root development.

The auxin hormones in addition to be a signal when the formation of symbiosis between MA and plant roots (Muller and Guther, 2007; Miransari *et al*., 2012 and Etemadi *et al*., 2014) and dominant controller of lateral root development (Moriwaki *et al*., 2011, Lavenus *et al*., 2013 and Fusconi, 2014), auxin hormones also play a role in lengthening the plant roots besides hormone of ethylene, cytokinin, gibberellin, and ABA (Takatsuka and Umeda, 2014).

The root length mean indicates that native *Acaulospora* can extend the *C. ensiformis* roots 11.26-31.76% of the *C. ensiformis* root length with exotic *Acaulospora* treatment and without *Acaulospora* (Fig. 1b), the same result was also obtained by Aguilar *et al*. (2016) when using *Agave inaequidens* applied with eight isolate native AM, at *Ocimum basilicum* L in salt stress conditions (Enteshari and Hajbagheri, 2011), and *Sorghum bicolor* that inoculated with *glomus* sp (Sun and Tang, 2013). It is likely not to be separated from the role of lateral roots and external hypha native *Acaulospora* that provides wider contact area wider between the soil particles and surface of lateral root or external hypha in absorption of nutrient elements and water through physical and chemical mechanism processes (Pierre *et al*., 2014) to supply the *C. ensiformis* needs in increasing of production assimilating as primary product from metabolism process and phytohormone as one of secondary products allocated on root part to does elongate.

The primary root zone was divided into several parts, namely; (1) the proximal meristem zone, the zone can produce root cells because cytoplasmic colloids content that higher; (2) the elongation/differentiation zone, the zone of cells elongation with rapid without growth to the vertical direction; (3) the transition zone, located between the zone of proximal meristem and elongation/differentiation, the cells in this zone showed growth in the direction of vertical and horizontal. This zone was also allegedly to serves as buffer from cell division to elongation of cell (Verbelen *et al*., 2006, Alarcon and Salguero, 2017) and, according to Zhang and Hasenstain (1999) and Cristobal *et al*, (2015) that silver ions (Ag+, an antagonist of ethylene perception) and I-α-(2-aminoethoxyvinyl) glycine (AVG, a chemical inhibitor of ethylene biosynthesis) can spur primary root elongation.

The average value of root volume showed that native *Acaulospora* can escalate *C. ensiformis* root volume 20.51% of the *C. ensiformis* root volume applied with exotic *Acaulospora* or without *Acaulospora* trieatments (Fig. 3a). Research results of Habibzadeh (2015) also show that AM application on *Cucumis sativus* L increases roots volume and provides a positive correlation coefficient value in dry weight of plant canopy. Inoculation with *Glomus mosseae* and *G. versiforme*, significantly increases the lateral roots number, root projected area, surface area, volume, and total root length (mainly 0-1 cm root length) of *Poncirus trifoliata* (Zou *et al*., 2014). AM as obligate symbiotic urgently requires the carbon compound produced out of plants photosynthetic activity (Berruti *et al*., 2015; Konvalinkova and Jansa, 2016). According to Graham (2000) that symbion AM can increase the assimilation production total for host plant growth and allocated 20% to AM as the energy source, this too causes the root dry weight median value increased 5.73-16.77% (Fig. 3b) and root: shoot ratio 3.44-30.18% (Fig.3c) to native A*caulospora* application at *C. ensiformis.* Pellegrino *et al*, (2011) also found that native AM isolates were more effective than exotic AM isolates in improving the productivity and quality of *Trifolium alexandrinum* and *Zea mays* in the field, the increasing of dry weight of shoots, an efficiency of photosystem II, stomatal conductance and glutathione accumulation that higher also shown by *Asteriscus maritimus* on the high salinity levels (Estrada *et al*., 2013). Quaryi *et al.* (2016) recommended that in determining the phytostabilization process to select lower cost by combining native MA and exotic plants that have rapid growth.

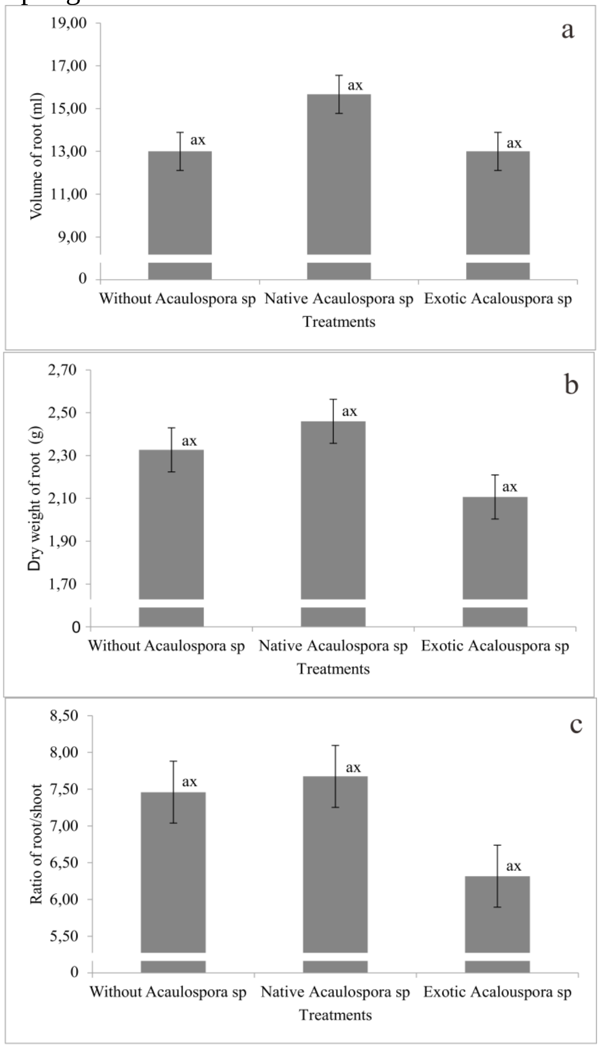

Fig. 3: Average of root volume (a), dry weight (b), and the ratio of root/shoot (c) *C. ensiformis* at treatment *Acaulospora* sp. in land post-mining nickel.

CONCLUSION

The effect of native *Acaulospora* plays an important role in protecting *C. ensiformis* roots in nickel post-mine land. Association of *C. ensiformis* roots with native *Acaoulospora* was able to increase the lateral root number 20.07-28.83% with percentage root infected 20.59-95.24%; root length 11.26-31.76%; root volume 20.51%; dry weight root 5.73-16.77%, and a root-shoot ratio 3.44-30.18%.

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