**Soil Drive Nutrient as New Method for Tin Mining Remediation**

Anton Muhibuddin1, Sri Nurhatika2 and Verra Claudya Vallafanie2, Minnatul Karim Akanda3, Tjuk Eko Hari Basuki4

1 Agriculture Faculty, University of Brawijaya

2 Biology Departments, Faculty of Science, Institute Technology of 10th Nopember

3 Faculty of Agriculture, University of Bangla, Bangladesh

4 Indonesia Ministry of Agriculture

**ABSTRACT**

A contaminated soil by tin usually is a big problem in Indonesia. Uniformity is essential for processing soil at a normal quality and to ensure conformity to specify clean up levels. Revegetation efforts were carried out by using rubber and jatropha combination which infected by arbuscular mycorrhizal (VAM) fungi applied using the soil drive nutrient (SDN) method showed good result in the previously research.

This research purposes to know the effectivity of soil drive nutrient (SDN) method to promote the growth of soybean plants (*Glysine max* L.) in tin mining soil. The results showed that the cropping pattern and dose of mycorrhizal gave effect on soybean growth, especially on the trunk diameter. Result also showed that mycorrhizal infection on soybean roots significantly influenced by the SDN method and dose of mycorrhizal that applied. Both of these are expected to have a positive impact on soybean production and resistance to environmental stress and disease attack. Viceversa, height and number of plant leaves untill 28 days were not affected by the SDN method and the dose of mycorrhiza applied.

Keywords: *Glysine max* L., Growth, Mycorrhyzal, Soil Drive Nutrient (SDN) Method.

**INTRODUCTION**

**Background**

The country which is the largest producer of tin in the world is Indonesia. Tin production in Indonesia is spread across many regions which consist of Karimun Island, Kundur Island, and Singkep Island, part of Sumatra Island, Bangka Belitung Islands and Riau Islands, to the west part of Borneo Island. Because of mining activity, all districts in Bangka Belitung Islands have changed their methods in utilizing their land. Use of the land for mining tends to rise in the last 10 years. Area of mining grows every year with rate of increase around 1,315 ha/year where from 2000 to 2004, pace of growth on mining field is 1,215 ha/year and from 2004 to 2010 the growth reached 1,381.67 ha/year (Yulita, 2011).

Soil condition in quarry where tin was mined is very worrisome. A case that shows the effect made by mining is the creation of tailing. Tailing is a gathering formed from mining material's residue in alluvial mine which is created when they were being cleaned of (Inonu dkk, 2011). Roughly 80%-90%, tin tailing is sand tailing and the rest is mud tailing (slime tailing). Sand Tailing has features such as soil with large sum of sand component and huge soil's pores, (Sitorus & Badri, 2008), soil's condition become clay sandy to dry sandy, (Hasnelly, Z., dkk, 2013), soil has nutrient component (fosfat dan nitrogen), soil with organic component and boilogical component, and soil with very low pH (Fery, 2013). Moreover, tin tailing has very high proportions of heavy metal which is: 3.040ppm Fe; 15, 8 ppm Mn; 1, 9 ppm Cu; 6, 29 ppm Pb; 0, 02 ppm Cd; 0,37 ppm Co; and 1,43 ppm Cr in 1 year old tailing (Kusumastuti, 2005).

Result of those mining life is the ex-tin mine sites became barren. The necessity of food especially soy is increasing while the land availability that can be used for agriculture is decreasing. Indonesia requires at least 2 Million hectare agriculture fields to provide need for soy nationally. However, the number (supply of soy) is declining even to this day; therefore soy production is reduced to 800 ton per year.

Tin mined land is a marginal terrain that needs to be reverted and reused optimally through revegetation. Successful revegetation on tin mined area is affected by chosen plant types (Prayudyaningsih, 2014). Plants used on revegetation must be plants that are able to tolerate lands critical condition, grow rapidly, resist drought of water, and capable to grow on low nutrient land (Nurtjahya, 2003), since, according to Permenhut RI (law by ministry of forestry) No. P.4/Menhut-II/2011, those plants can adapt to their surrounding climate and soil.

Ruber plant (*Hevea brasiliensis)* and *jarak pagar* (*Jatropha curcas* L.) are few plants that qualified to life and highly adaptable to soil with critical condition, lack of water and nutrient (Tjahyana & Ferry, 2011; Kartika dkk., 2014) therefore they have potential to be used for revegetate mined site in Bangka (Tjahyana & Ferry, 2011). Moreover, those plants can life on acid pH (Verheye, 2010; Hariadi, 2005) and fit to accumulate many kind of metal.

The success of land revegetation is also affected by microorganism to suply nutrient to plants. There is no more top layer (top soil) as part of land after being used for mining. Often, this layer doesn't contain *propagul* mychorrizal. Therefore, inoculation on mycorrhizal to plants is very essential (Simanungkalit dkk., 2006; Nurhalimah, S., dkk., 2014).

Mycorrhizal is a microorganism which has symbiosis mutualism relationship with plant's root. Moreover, mycorrhizal contributed to immobilization on heavy metal in soil and root's rhizosphere that made mycorrhizal increase the same Phytostabilization as plant. (Babu & Reddy, 2011; Puspitasari, D., *et. al*, 2012).

Mycorrhizal application to plants requires a new method which maintains composition of nutrients in the soil (Muhibuddin, A., 2009). Bowl methods is a new method that involve mycorrhizal and plants as it's host using *tumpang sari* (poly culture) by measuring it's planting time and structure of plant's root to build strong and wide (plentiful) hyphae to store nurtients and water consequently increasing critical land's quality and make it into productive land.

According to these descriptions, researcher wants to analyze effects of mycorrhiza by applying *metode cawan* (bowl method) to increase soy plant's growth (*G. max* L.) on land with critical condition that was used to mine tin.

This research aims to figure out correlation between cropping pattern and dose of mycorrhiza to growth of soy plant (*G. max* L.) on land that was used for mining tin.

The result of this research is expected to (1) Solving soy plant's productivity crisis in Indonesia especially using mycorrhiza with bowl method and (2) Utilizing critical land that has been used tin mine tin using bowl method to increase growth and productivity of soy plant on the greater scale.

**METHODS**

**Time and Place**

This research was conducted in Bangka Belitung Province. Analizing infection in soy plant's root was done in Botany Laboratories, Biology Major, Mathematic Faculty and Science, Institute Teknologi Sepuluh Nopember (Institute Technology of 10th November), Surabaya. The research was started on April 2015 and finished on March 2017.

**Method**

**Land Preparation**

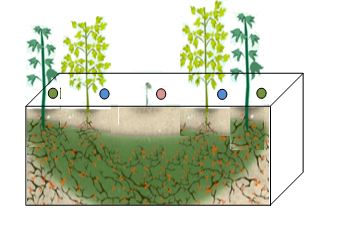
Before conducting the research, it is necessary to prepare the land that will be used for this research. Firstly, the land is cleared from weeds. After the land is free from weed, the farm is dug to make sections (terrace) with 1m x 1m wide, 60cm deep, and 50-60cm space between sections (Subardja dkk, 2007).

**Preparation of Planting medium and seeds** *Havea brasiliensis* **and** *Jatropha curcas* **L.**

Planting medium used in this research is sandy soil medium. Terrace (section) that has been ready is covered under plastic then sterile sand soil is put over it until full. Soil sterilization is done in fumigate with formalin 5% (Astiko, 2009) then rest it for 1 week. Next, sandy soil medium in the terrace is conditioned the same as tin mined land with addition of heavy metal. The added heavy metal is in the form of powder and scattered to topsoil layer then mixed evenly. The type of heavy metal contents are 3,040 ppm Fe; 15,8 ppm Mn; 1,9 ppm Cu; 6,29 ppm Pb; 0,02 ppm Cd; 0,37 ppm Co; and 1,43 ppm Cr (Kusumastuti, 2005). Next, Organic materials are added in top soil layer evenly using compos fertilizer. Ratio of the compos fertilizer to be added to sandy soil is 2: 1 (Inonu, 2011).

**Planting physics nut seeds (J. curcas L.), rubbers (*H. brasiliensis),* and planting soybeans (*G. max* L.)**

This study uses three types of crops such as rubber (H. brasiliensis) as the main bowl plant (I), jatropha (J. curcas L) as the second plant saucer (II), and soybean (G. max L.) as a major crop in the bowl cropping pattern. All of those 3 plants are planted in sand medium in different period and placed with 10cm gap between them. + 2-4 weeks old H. Brasiliensis and J. curcas L are prepared. After a week when organic materials have been given to the planting medium they are planted. Planting mediums are perforated sufficiently and 20gram of mycorrhizal *Glomus* sp. is inoculated to it. Then rubber seeds are planted then buried again with dirt. After 15 days from rubber seeds planting time, seeds of jatropha (J. curcas L.) are planted to mediums that have been inoculated with 20 gram of mycorrhiza *Glomus* sp. Soybean seeds are planted to medium which have been inoculated by mycorrhiza with treatment of 0 gram, 40 gram, and 8 gram after 1 month jatropa's seeds are transplanted. 1 week before soybean seed, organic materials are added. On non bowl planting method there is no bowl plant used, they use soy beans that have been inoculated using mycorrhiza with 0 gram, 40 gram, and 8 gram treatment.



**Picture 1** Structure of plant's position following treatment variable listed.

Legend: Bowl Plant 1; Bowl Plant 2; Main Bowl Plant

**Nurturing the plants**

Nurturing the plants is done by watering them every day, each morning or afternoon in addition of weeding when weeds are found.

**Observation Variables**

**Soy plant morphology observation (*G. max* L.) as the main plant.**

Observation on soy plant morphology is done every week for 4 months.

**Plant Height (cm)**

Plant's height examination measured using thread and ruler from plant's lowest point of growth to its highest point of growth which is the last sprouted leaf. (Sastrahidayat, 2011).

**Stem diameter (cm)**

Measuring main stem's diameter (cm) is done by caliper to stem with 5 cm height above ground level every week start from 7 days after planting until 50 days after planting (Wijaya *et.al.*, 2010).

**Leaf Quantity**

Counting the number of leaf is done every month by counting every leaf (Lestari, 2008), both healthy leaf and dry leaf, (Sastrahidayat, 2011).

**Percentage on Soy Plant Root infection (*Glysine max* L.)**

Calculation on percentage of root infection *Glomus* sp***.*** on soy plants is done on 5 highest growth and lowest growth soy plants by making *preparat* (object used for observation by microscope) on semi-permanent root in advance

Plant's root is taken and then rinsed cleanly using aquades. Roots that has been rinsed is stored Formalin Acero-Alkohol (FAA) to be used for fixation before painting. Afterward, roots will be soaked in 10% KOH and heated at 121ºC using autoclave for 15-20 minutes and then washed using water. Roots subsequently bleached using hydrogen peroxide alkali, and once more washed using water. Next, the root is acidified with HCI 1%, and they are soaked in trypan blue paint that has 0,05 % w/v concentration and apply lactoglycerol until they are submerged, thereafter use autoclave at 121ºC for 15 minutes. The paint is then removed and soaks the root in lactoglycerol. Afterward, observe it under microscope (Alkareji, 2007).

Slice of the root is observed under microscope with 100-250x. Percentage number in infection on inoculum mycorrhizal *Glomus* sp. is calculated from the quantity of infected roots of 10 root pieces which observed from each plant. Infected plants can be identified by vesikel or arbuskul on their root's cortex. Percentage of infection on inoculum mycorrhiza *Glomus* sp. is formulated using Alkareji formula (2008):

% infection = (∑ Infected root/ ∑ totall root observed) x 100%

**Research Plan and Data Analysis**

**Research Plan**

Random Group Factorial Research will be used. Observation variable in this research is mycorrhiza and bowl plants which are consist of 3 different dose of mycorrhiza such as 0 gr (A1), 40 gr (A2), dan 80 gr (A3) and also bowl plant (B1) or no bowl plant (B2) wherein each treatment is done 3 times. These are treatment that will be used:

Rubber plant is used as bowl plant 1, Jatropha is used as bowl plant 2, and soybean is used as main bowl plant. Observation parameter being inspected on soybean composed of height of the plant, diameter of the stem, and % root infection.

**Data analysis**

Data analysis is conducted to determine effects of the treatment variables in the research while implementing 5% (0,05) ANOVA Two Way then calculated using SPSS as tools. Whenever there is actual effect to the treatment variables, there will be further comparison test that utilize Duncan test and T test using SPSS software.

**RESULT AND DISCUSSION**

**The Growth of Soybean Plant (*Glysine max L*)**

Based on the results of ANOVA *Two Way*, it is discovered that the interaction of planting and dose does not affect growth of plants, with the value of *p* is 0.681 (*α=*0.05). Dose factor shows there is no effect against on soybean high, with the *p* value of 0,209 (*α=*0.05), similiarly, by a factor of planting, the *p* value of 0,762 (*α=*0.05).



Graph 2. The growth of soybean plants with croping pattern and its dosage

Description:

m1 (mycorrhiza 0 gr dose), m2 (mycorrhiza 40 gr dose), m3 (mycorrhiza 80 gr dose), c (bowl), nc (non bowl).

Based on picture 2 seen that soybean plant grow in each week although the croping pattern and mycorrhiza suggest that doses have no effect agains soybean plant. Soybean plant at planting bowls pattern or non bowls inoculated with mycorrhiza of different dose showed almost the same height of the observation day 1 to observation day 8. The best of soybean plant height was 16.6 cm on the planting pattern bowls with a dose of mycorrhiza 0 gr, while the lowest soybean plant by 15 cm planting pattern on non bowls with doses of 40 gr. It was suspected, the soybean plant at planting pattern bowls with a dose of mycorrhiza 0 gr, 40 gr, dan 80 gr gave the same response with soybean plant at planting pattern non bowls with a dose of mycorrhiza 0 gr, 40 gr, and 80 gr when grown on media that was conditioned as the land of the former tin mines



**m1c**

**m2c**

**m3c**

**m1nc**

**m2nc**

**m3nc**

**A**



**B**

**Figure 3.** Soybean Height (*Glysine max* L.) with Planting pattern and its dosage.

Description:

m1 (mycorrhizal 0 gr dose), m2 (mycorrhiza 40 gr dose), m3 (mycorrhiza 80 gr dose), c (bowl), nc (non bowl).

The existence of the planting pattern of bowls consists of rubber plants and jatropha plants resulted in plants of soybeans could be experiencing high growth better than the soybean plants at planting pattern bowls. Rubber plants and Jatropha plants are a hypercumulator plant that can accumulate heavy metals in excess of the amount (Kumar *et al.,* 2008). Plants in bowls that have been inoculated soybean plants can help mycorrhiza in overcoming heavy metal impurities in planting area. Mycorrhiza has effect on the absorption of heavy metals (translocation and accumulation in plant tissue) (bai *et al*., 2008). Mycorriza *Glomus* sp. was able to increase tolerance against the absorption of heavy metals (Adewole *et al*., 2010).

Absorption of heavy metals by fungi is a passive mechanism of immobilizations on the surface of the hypha mycorrhiza including proess such as adsorption, ion exchange, complexation, deposition, and crystallization Mycorrhizal are known to be able to absorb and accumulate metals in biomass and host plant roots. Mycelium of intra and extracellular mycorrhiza is potentially in the absorption of metals (Jones *et al*., 2000) through the surface area absorption and its scope in the ground. Most metals appear to be bound to the cell wall components such as cittin, cellulose, and melanin of mycorrhiza. Khelat agents such as EDTA (Chen *et al.*, 2004) and glomalin (Khan, 2006). Mycorrhiza activity played a major role in the mobilization of metals bound by ground components (Göhre &Paszkowski, 2006).

Heavy metals in growing media have been overcome by the presence of a plant in bowls. In addition, the bowl plant symbiotes with mycorrhiza will form a strands - a tangle of roots are very long and spread to the entire territory of the planting the existence of plant in the bowl with mycorrhiza in it has a role in increasing the absorption of ions with low mobility level, such as phosphate (PO43-), ammonium (NH4+) (Suharno and Santosa 2005) and other soil nutrient elements which increases the surface area in contact with the ground as well as the absorption roots to 47 times to ease the access to perform nutrient elements in soils (Suharno & Sancayaningsih, 2013) This condition apparently did not affect the height of the plant of soybeans at planting bowl pattern. It was suspected due to observations of the growth of soybeans was 28 days where at that age, soybean plant is still experiencing a phase of vegetative that is organ vegetative growth stages of the roots (Saputro, 2011).

The roots of soybean plants aged 28 days could be possible still hadn’t been able to reach nutrient elements in soils, one elements of P which had been provided by the plant bowl resulting in the growth of soybeans at planting bowl pattern was not optimum. The formation of hormones growth on a plant due to the absorption of elements of P which acted as one of the elements forming the ATP or energy in a metabolism proccess. Therefore if the plants could do maximum absorption of P then ATP production was also sufficient for the metabolic processes that would later produce growth hormones that were essential for the growth of plants (Sastrahidayat, 2011).

State of soybean plant in the planting bowl pattern is unlike the case with soybean plant in the planting non-bowl pattern. Soybean plant is not a hypercumulator plant. In addition, soybean plant at planting non-bowl pattern working alone in addressing the effect of Heavy metals in growing area. On heavy metal contaminated land, most of plants cannot adapt to the habitat (Khan 2006; David 2012).

Heavy metals get into the plant through the roots cells by diffusion on or through non-specific transporter, if the concentration is high. At this concentration, heavy metals disrupt work activities of enzymes by modifying the structure of the protein or replace important elements that lead to symptoms of deficiency. The membrane plasma was highly susceptible to the toxicity of heavy metals when permeability and functions were influenced by changes in the intrinsic membrane proteins such as H+-ATPase. Otherwise, the production of the type of reactive oxygen oxidative tissue damage cause plants which occurred due to the response of the high level of heavy metals. As a consequence, symptoms resembling chlorosis, slow gowth, browning root which lowers the effectiveness, against influential photosystems, disruption of cell cycle, etc (Leyval *et al*. 2002). It was the condition that caused soybean plant at planting bowl pattern did not experience optimum growth.



**Figure 4.** Rubber plant's root structure (*Hevea brassiliensis*) and Jatropha Plant (*Jatropha curcas*) as bowl plants.

**The number of leaves of soybean plant at planting bowl pattern and planting non-bowl pattern.**

Based on the results of ANOVA *Two Way*, it is known that the interaction of planting patterns and doses did not affect the growth of the number of soybean plant leaves, the *p*-value of 0.624 (*α=*0.05). The number of soybean leaves was not affected by dose factor with the *p* value of 0.187 (*α=*0.05), as well as planting factor, the p value of 0.335 (*α=*0.05).



**Figure 5.** Number of soybeans leaves (*Glysine max* L.) with planting pattern and its dosage.

Description:

m1 (mycorrhiza 0 gr dose), m2 (mycorrhiza 40 gr dose), m3 (mycorrhiza 80 gr dose), c (bowl), nc (non bowl).

Based on picture 5 we can see that the number of leaves had been increase evey week eventhough the number of leaves were not affected by cropping pattern and the dose of mycorrhiza applied.

The addition of mycorrhizal in the plant bowl will maximize its potential in soils containing heavy metals PbThe physiological processes play a role in the accumulation of heavy metal throughout the life cycle plant. The first process was the interaction of rooting zone on rizosferik (*rizosferic)),* where occurs the process of processing elements in the soil of a form that can not be absorbed into a form that can be absorbed by involving a number of root exudates produced. Hiperakumulator (hyperacumulator) plants have a higher capacity for changing the metal in the root zone into a form that is available. Beside, hyperaccumulating plant has the ability to accelerate the dissolution of metals in the rhizosphere by releasing the kelat (chelate) to the metal that was specific to the rhizosphere by root then do the metal translocation from root to canopy. Secuestration and complexation are the process which undertaken to determine the form of the metal that will be accumulated and to know which part of tissue where it will be stored (Salt, 2000)

Mycorrhiza can help plant the chalice as hyperaccumulator plant in the absorption of nutrients such as N, K, Mg, Fe, Mn, Cu, and Zn, which are materials that play a role in the formation of chlorophyll (Rossiana, 2003)

The presence of chlorophyll will increase the photosynthesis process that will affect the number of leaves and leaf area. Transportation of photosynthesis to the roots determines the ability of roots to absorb and acquire nutrients. Mycorrhizal hyphae have an external tissue that has a size finer than root hairs. The hyphae are allowed to enter into the pores of the soil most small (micro) so that it can absorb water under conditions of very low ground water. Cells infected root. Mycorrhiza has a size that will be growing due to their extracellular hyphae that expand the surface absorption of nutrients (Delvian, 2005).

The cropping bowl pattern and the dose of mycorrhiza do not affect the amount of soybean leaves. This is presumably due to the planting and observation of the soybean crop is still very briefly about 28 days. The planting time is the vegetative stage for soybean (Saputro, 2011) to form the vegetative organs of the roots, stems and leaves. Root length greatly affects the absorption of nutrients in the soil. The length of the roots of soybean plants in the vegetative phase of 28 days, suspected to be too short (Saputro, 2011) to be able to take nutrients in the soil that has been provided by the plant saucer and was instrumental in the formation of the leaves.

The formation process of leaves inseparable from the role of nutrients such as nitrogen and phosphate which is contained in the soil medium danyang is available to plants. Both of these nutrients play a role in the formation of new cells and the main component of organic compounds in plants such as amino acids, nucleic acids, chlorophyll, ADP, ATP. If the plants are deficient in both these nutrients then the metabolism will be disturbed, and the formation of the leaves will also be hampered. The Mg element is also very helpful in the transportation of nutrients, especially of P (Nyakpa et al, 1988). The most influential nutrient in the growth and development of the leaf is nitrogen, a high nitrogen concentration generally produce more leaves. (Lakitan, 1996). Sufficient of nitrogen soil can increase protein synthesis for cell division and enlargement which causes the increase in the number of leaves and the increase in cell size so that the growth of plant and leaf number are increased (Susilo, 1991 in Fatima, 2008).

The number of leaves of soybean plants in the cropping pattern non bowls also showed results similar to soybean crops in cropping pattern bowls. Soybean plants on non bowl pattern that had been inoculated mycorrhiza did not produce the amount of leaves that was better than the soybean crop in the bowl pattern.

Fitter and Hay (1991) in Siahaan et al (2014), stated that heavy metals can interfere with metabolic processes in plants, thereby these was disrupting the formation of plant cells and tissues at the root meristem. Decreased tissue growth at the root can cause decreased growth in the top of the plant, such as leaf. Plants are not in a group of hyperaccumulator like soybean crop will rapidly die because of impaired metabolism while already using mycorrhiza as a biological fertilizer. (Widyati, 2008).

The overload presence of heavy metals Pb to the growing media of soybean cropping pattern non bowl can cause a limited amount of the nutrients in plant tissue which led to the development and growth of plants decreases. ons nutrient cations such as K +, Ca 2+, Mg 2+, Mn 2+, Zn 2+, Cu 2+ and Fe 3+, and anion NO3- the absortion was inhibited by Pb to plant roots (Mishra and Dubey, 2005).

**Soybean Plant Stem diameter (*Glysine max* L.) cropping bowl Pattern and cropping non-bowl Pattern**

Based on ANOVA Two Way analysis, it is known that the interaction of cropping pattern and dose of mycorrhiza was not affected to stem diameter with *p* is 0.094 (*α=*0.05). As well as dose factor did not affect to the stem diameter with *p* is 0.024 (*α=*0.05). Next experiements was T test at the cropping pattern that showed (Attachment 3.3b) cropping pattern had better influence to the stem diameter of soybean plant.

**Table 1. The Effect of Mycorrhiza Dosage on Plant Stem Diameter**

|  |  |  |
| --- | --- | --- |
| Dose | Plant Pattern | The Average of Stem Plant (mm) |
| 0 | Bowl | 1.56 |
| Non Bowl | 1.2 |
| 40 | Bowl | 1.23 |
| non Bowl | 1.13 |
| 80 | Bowl | 1.83 |
| non Bowl | 1.2 |

Based on table 1, it showed that the stem diameter size at the soybean plant was affected by cropping pattern existence. Cropping bowl pattern could help soybean plant got bigger diameter size than using cropping non-bowl pattern method. By existence of the bowl plant which was inoculated by mycorrhiza would give influence for nutrient and water to be absorbed by plant for their living. Good root system will support the absorption of nutrients and water in the soil is better anyway, so the metabolic processes that occur in plants will run normally. Water absorption by the roots can affect the diameter size of the plant stem (Sari et al, 2010). Nitrogen plays a role in improving the development of stem both horizontally and vertically (Yulius et al, 2009). The growth of stem diameter was influenced by the content of P element, the element K played an important role in the activity of cell division and the development of meristematic tissue of plants which resulted in the enlargement of the stem (Herdiana et al, 2008).

Cell enlargement was largely a water absorption event into vacuoles which expanded. The concentration of dissolved materials in the vacuole is high and there are hundreds of dissolved materials, including Cd, Ni and Pb. The water pressure causes the growth by encouraging walls and membranes become wider (Salisbury & Ross, 1995).

Mychorrhiza was one type of fungus that can stabilize the soil structure. In the process of soil formation, mycorrhiza assisted in the formation of soil aggregates. Soil aggregate was a conglomeration from soil composing particle which has certain size and shape; organic materials such as plant left over, organic compond such as Fe and Alluminium oksida, root of plant, fungus hiphae and other microba (Chenu *et al.*, 2000; Six *et al.*, 2001; Astiko, *et. al*., 2013) Roots and the fungal hyphae function as "net" that collect mineral soil, organic matter and so forth. Roots and microbial exudates such as polysaccharides and glomalin (glycoprotein produced hyphae of fungi) provided the "glue" that binds to soil particles apart to be "netted" (Rillig & Mummey, 2006). In addition to helping glue the aggregates, soil aggregates create and maintain soil pores to provide the level of infiltration and aeration which are favorable for plant growth. This has resulted in soybean plant in the cropping bowl pattern had a diameter larger than the soybean plant in the cropping non-bowl pattern.

The roots of soybean plants in the cropping non-bowl pattern help these plants in overcoming heavy metals in the planting medium. Soybean roots ability to absorbing nutrients and binding of the soil aggregates in order to establish good aeration to the growing media is severely limited. It was suspected, cause the presence of heavy metals in the planting medium that inhibits the metabolic system of plants that interfere with the formation of plant cells and tissues in the root meristem (Fitter & Hay, 1991 in Siahaan et al, 2014). Other than that, soybean plant at the cropping non-bowl pattern must work by itself to get nutrient from soil and water for the living of plant. The absence of non-bowl plant makes nutrients and water in the soil can not be used directly by the soybean plant because of the role of bowl plant is trapping nutrients and water that could later be used / taken directly by plants (Muhibuddin, 2015) .

**Soybean plant's Root Infection (*Glysine max* L.) with bowl planting patern and bowl planting patern**

Based on *Anova Two Way* the results, known that the interaction of cropping bowl pattern and mycorrhiza dose significantly affect the% root infection with AP value of 0.05 (α = 0.05)

Likewise, the dose and the planting factors significantly influence% infection of plant roots with p value of 0.00 (α = 0.05).

**Table 2**. Effects of mycorrhiza's dosageincorelation with % Plant Root's Infection

|  |  |
| --- | --- |
| **Dosage (g)** | **Avarage % Plant Root's Infection** |
| 0 | 41.667a |
| 40 | 56.667b |
| 80 | 71.667c |

Description: Avarage with different letter shows significant difference on *Duncan* tes(p = 0,05).

**

Not treatment

Bowl treatment

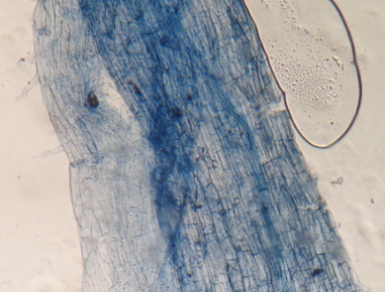
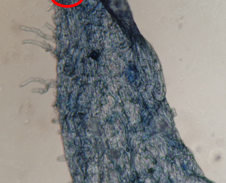
**Graph 6.** Planting Method Interaction and its Dosage on Plant's Root Infection

Based on Table 1, Figure 6, it is known that the amount of infection of root of plant with increasing doses of mycorrhiza and type of cropping patterns. T test results stating that the cropping bowl pattern is the best planting way than cropping non-bowl pattern. At a dose of 0 g, 40 g and 80 g with cropping bowl patterns showed the best results than the dose of 0 g, 40 g and 80 g without cropping bowl pattern.

Infection of plant roots in the cropping bowl pattern with doses of 0 amounted to 56.66%, a dose of 40 g of 70%, and a dose of 80 grams of 76.66%, while in the cropping non-bowl pattern with doses of 0 g of 26.66%, a dose of 40 grams of 43.33%, and a dose of 80 grams of 66.66%. This occurs because of the mycorrhizal symbiosis between the roots of plants.

Mycorrhizal symbiosis occurs when get into or perform root infection. The infection process begins with the germination of spores in the soil. Hypha that grows will penetrate into the roots and develop in the cortex (Talanca, 2010). According to Djazuli (2011), the application of mycorrhiza increases the population of spores and% root infection.

Mycorrhiza root infection was characterized by the presence of hyphae, or vesicular arbuscular (Figure 7). This is according to Irawati research (2004) in Nurhandayani et al (2013) that the presence of mycorrhizae in root characterized by hyphae, or vesicular arbuscular fill the root cortical cells.



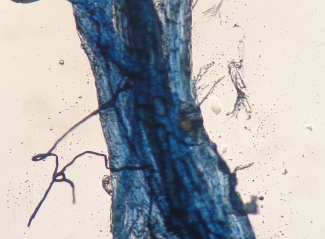
aa

c

b

d

b



**Figure 7**. Root Infection

Legend: Arbuskular (a), Hifa (b), Spora (c), dan Vesikel (d)

Cropping patterns and dose mycorrhiza affect root infection. Infection of the roots can be detected when mycorrhizal colonies around the hair roots is spotted. Degree of infection varies depending on the number of mycorrhizae. This is proven by the difference in percent of infection in different doses of treatment, either by bowl cropping and non bowl cropping. The more mycorrhizal applied to the roots of the plant, the higher the rate of infections and mycorrhizal colonization (Marwani dkk, 2013). Research by Fitriatin dkk (2003), prove that inoculated plants on 50 g of mycorrhiza (per plant) have higher percentage of root infection compared to plants without mycorrhiza inoculation. This shows that mycorrhizal inoculation can improve crop root infection. Percentage of mycorrhiza infection when applied with 40 gr and 80 gr and implementing bowl method is categorized as high and very high. *The Institute of Mycorrhizal Research and Development* classify mycorrhiza infection with 51-75% infection as class 4 (high) and 76-100% infection as class 5 (very high) (O’connor *et al.,* 2001).

b

Cropping pattern also affect the number of percentage of root infection. This can be seen from 0 gr dose of mycorrhiza, whether bowl planting pattern is employed or not, roots in both pattern are infected. This is because in bowl method roots of soybean plant interact with bowl plants which are jatropha and rubber plant. Rubber plant and jatropha are inoculated using 20 gr mycorrhizal then 2-3 weeks after those soybeans are planted. The existence of 20 gram inoculation mycorrhiza makes rubber plants and jatropha plants become infected by mycorrhizal which generate longer roots for both bowl plant and spread across entire planting area. This occurrence is matching on research made by Jannah (2011) which stated that mycorrhizal mushroom infection is able to change structure, increase length of roots, root system and its quantity because of mycorrhizal's hifa that is formed. The cropping process when bowl crops have been infected by mycorrhiza that induces soybean plant to be infected.   
It is alleged that the mycorrhiza on the roots of the bowl plants have spread throughout the entire planting area, resulting in entire area containing mycorrhizal spores that infect the roots of soybean plants. Bowl plant method is similar to *tumpang sari* method (intercropping). Intercropping is applying several kinds of plants which has relatively close age in the same land and time and organized into lines (Warsana, 2009). Intercropping or plant rotation could also increase mycorrhiza population (McGonigle & Miller, 1993 *dalam* Musfal, 2010).

Non bowl method with 0 gr dose experience symbiosis with mycorrhizal that cause 26.66% root infection on soybean plant.   
Land used to grow soybean plants with non bowl cropping pattern is sterile sandy soil. In general, soil which is fumigated using formalin 5% become sterile soil.   
Infection on soybean roots in 0 gr sterile soil with non bowl method is reputedly because of their pH, phosphate and soil microbes. Mycorrhizal's growth needed not only exact pH but also need P content to be low (Bowen, 1982 *in* Mujoko and Wahyuningsih, 1998). Microba's existance is expected to increase competition on utilizing of root's exudates. According to Graham (1982) *in* Mujoko & Wahyuningsih, (1998), root's exudates affect colony formation before infection occurs. Thus, microbes’ competition in low sterilized soil which causes the mycorrhizal infection made the soil become a high sterilized soil. In addition, it is estimated that NPK content in the non sterilized compos fertilizer on the beginning of the experiment made the environment around soybean plant's roots compatible and supports mycorrhizal's root infection by nature (Marwani dkk, 2013).

**CONCLUSION AND SUGGESTION**

**Conclusion**

1. Planting pattern and mycorrhiza dosage do not affect height of soybean plant.

2. Planting pattern and mycorrhiza dosage do not affect quantity of soybean plant's leaf.

3. Planting pattern affects stem diameter. Best planting pattern for soybean plants stem diameter found on bowl pattern.

4. There is interaction between planting pattern and mycorrhiza dosage to soybean plant's root infection. Best plant infection which is 76.66% on bowl planting method that has 80 gr dosage of mycorrhiza.

## SUGGESTION

It is necesary to do further experiment on vegetatif fase to generatif fase in relation to heitght growthe of soybean plant which implement bowl planting mrthod and without bowl planting method.

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