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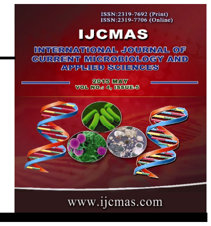

Original Research Article

The Effectivity of Arbuscular Mycorrhizal Utilization and The Level of Watering Toward The Growth and Production of Soybean Plants

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Tomato, Solanum lycopersicum, DNA polymorphism, RAPD markers

A B S T R A C T

The most applied soil microbiology in rizosphere ecological improvement is Arbuscular Mycorrhizal Fungi (AMF). AMF can improve crop growth and production in the widely reported dry area but the combination of AMF utilization along with rhizobium bacteria is still relatively limited. The research aims to study the effectivity of Arbuscular Mycorrhizal Fungi (AMF) utilization and the level of watering upon the improvement of soybean plants production. The research is conducted in factorial experiment form arranged by random group design pattern. The first factor is the dose of AMF inoculation consisting of no inoculation, inoculation AMF 0.025 g, 0.050 g and 0.075 g of each crop, the second factor is the level of watering consisted in 80-100 % watering in field capacity, 60-80 % field capacity and 40-60 % field capacity. The results showed that inoculation of AMF 0,050 g to 0,075 g of each crop can improve crop growth and production, soil fertility, fullfil soybean nutrient needs and improve the efficiency of absorption of N. P and K.

Introduction

Soybean is a strategic commodity after rice and maize and has important role in industry development in Indonesia as standard materials industry of food and animal feed. In the next five years period (2010-2014) the needs of soybean every year has reached about 2.3 million tons of dry beans, but based on ARAM II in 2012, domestic production only reached 783.158 tons or 34.05 % of the national requirement (*BPS 2012*) which had average productivity about 1.2 to 1.3 tonnes per ha. The increase of productivity and production still need to be pursued primarily by the application of competitive innovative technologies (productive, efficient and quality) through integrated crop and resource management methods. Integrated crop management (ICM) is basically a methodology in increasing crop production through intergrated crop and resource management combined with synergistic effect of technology (*Wasmo, 2012*).

Soybeans in Indonesia are grown in paddy fields or dry land after rice cultivation in the form of monoculture or intercropping. The pattern of rainfall distribution in Indonesia, particularly in soybean planting center about 4-7 months, with the result that soybean crop is often having production failure or low productivity due to drought condition. Decreasing in water availability generally produce limited total nutrient absorption and mineral nutrient concentration in plant tissues. The important effect of water deficit is limited mineral nutrient absorption by root and limited transportation to shoots (*Alizadeh and Leila, 2011*). The limits of nutrition and water availability for plants will lead to lower production and productivity at last.

One of components of integrated crop management is utilization of soil microbes. As growing plant medium (Rizozfer), soil is a component of the overall ecosystem and can not be separated from the sanity of the ecosystem. In agriculture, healthy soil has good physical, chemical and biological condition to support high crop productivity and sustainability (*Nasaruddin, 2012*). Rhizosphere, influenced by compounds released by roots intensively and is food for soil microorganisms (*Zare et al , 2011*). The effective bacteria which can colonize root is called "Rhizobacteria". Plant Growth Promoting Rhizobacteria (PGPR) or Rhizobacter can stimulate plant growth (*STURZ and Nowak, 2000*), protect the parts of the plant on the ground against viral diseases, fungi and bacteria (*Kloepper et al., 1992*), accelerate germination, stimulate the growth of roots and shoots (*Yeole and Dube, 1997*), increase the chlorophyll content of leaves (*Singh et al, 2003*), increase plant tolerance to drought and salt and can delay leaf senescence (*Lucy et al, 2004*). One type of PGPR bacteria which have been used widely in plant cultivation system is Rhizobium bacteria particularly on soybean.

Soybean has mutual symbiotic along with rhizobium bacteria through forming of root nodules (nodules). The existence of Arbuscular Mycorrhizal Fungi (AMF) symbionts with legume roots have a very important role toward the activity of rhizobium bacteria in the root nodule formation (*Provorov et al, 2002; Barea et.al 2002a, 2002b*). Arbuscular Mycorrhizal Fungi (AMF) improves plant tolerance to water deficit through the transformation of plant fisilogy (*Ricardo Aroca et al.,2008*). Most of Mycorrhizal have been improving plant survival and fitness through mechanisms such as increasing water and nutrient absorption (*Marschner and Dell 1994; Peterson et al, 2004; Pasqualini et al, 2007; Plassard and Dell, 2010*). Mycorrhizal fungi makes symbiotic coherence with host plant. Most of the experiments have shown that Arbuscular Mycorrhizal Fungi (AMF) are able to transform the water coherence of their host plant (*Huixing Songs,2005*). During growth, they have fast coherence with root and play important role in concentrating and transferring soil nutrition for plant. Instead, plant supplies sugar as a source of nutrients.

Root systems of plants colonized by the roots can increase the absorption of mineral nutrients and improve soil structure and fertility. Arbuscular Mycorrhizal Fungi (AMF) penetrates the root and grow extensively between cortical cells, expands surface absorption dynamically and extends the reach of root absorption (*Bhosale and BP Shinde 2011*), binds the particles and improves micro and macro aggregation (*Aliabadi Farahani et al. 2008*). By understanding these interactions, particularly in regions with tropical climate conditions and limited fertilizer resources and simple culture system is very important in order to improve production and productivity of soybean plants.

Conduct the research about the effectivity of utilization in Mycorrhizal Fungi and water supply growth level and production of soybean plants. This research generally aims to know the effectivity of Arbuscular Mycorrhizal Fungi (AMF) dan watering level toward the improvement of nutrient availability, effectivity of nutrient uptake, growth and production of soybean.

Materials and Methods

The experiment was arranged 2 factors factorial based on random group design pattern. The first factor was AMF inoculation which consisted of 4 levels: without inoculation as a control (c0), AMF inoculation 0.025 g per tree (c1) , 0.050 g per tree (c2), 0.075 g per tree (c3). The second factor consisted of watering level which consisted of 3 levels: watering 80-100 % of field capacity (a1) , 60-80 % of field capacity (a2) and 40 -60 % of field capacity (a3). We did the observation to measure the influence of treatment toward growing plants medium characteristics, the levels of N, P_2O5 and K_2O, dry weight and plant biomass and production components . As the observation result, we did correlation and ANOVA test analysis, in order to determine the best treatment, we did follow-up testing by comparing two average values, using the Honestly Significant Difference (HSD) test.

Result and Discussion

Growing plants medium characteristics

Laboratory analyses at the last experiment of growing plants medium showed that treatment of AMF at low level of water availability (40-60) % of field capacity showed the charasteristics of growing plant medium in nutrient levels higher than the treatment of high levels of water availability. The higher nutrient level at low level of water availability due to lower absorption and utilization level of nutrients by plant roots than completed water availability plant. On the other hand, mycorrhiza-infected plants has effect in increasing of nutrient solubility in soil by AMF activity. AMF inoculation has linear positively correlated with fertility charasteristics except for CN ratio, it has negatively correlated with AMF inoculation (Figure 1). The reduction of CN ratio caused by increasing N level in soil released by plant root due to rhizobium activity and the release of N soil by mycorrhizal activity.

Correlation analysis showed that AMF inoculation correlated highly significant with CEC, organic C, soil pH, levels of N, P_2O_5, K_2O and correlated negatively with the C/N soil ratio. In any increase of 0.025 g AMF dose will increase 0,48 cmol (+) kg^{-1} CEC soil, 0.17% organic C, 0.77 soil pH, 0.071 % N levels, 0.099 ppm P_2O5 level of soil and 0.099 cmol () kg-1 K_2O level and decreasing 0.24 CN of soil ratio.

Levels N, P_2O_5 and K_2O of Leaves

The result on Table 1 shows the treatment interaction of AMF 0.050 g to 0.75 g per crop by watering level about 40-60% of field capacity response the highest N level of leaves, but P2O5 has the highest level on treatment interaction AMF 0.050 g to 0.075 g per crop by watering level 60-80% of field capacity. Treatment interaction of 0.075 g AMF per crop by watering level 40-60% of field capacity (m2a1) gives the highest level K2O of leaves compared to other treatment interaction rbuscular mycorrhizal fungi inoculation per crop positively correlated and is quadratic on N content and P_2O_5 of leaves, but positively correlated and has linear side toward K_2O content of leaves (Figure 2). The increase of AMF inoculation doses, will increase the level of N content and P_2O_5 of leaves up to reach a maximum N in the amount of 1.46% at 0.066 g doses and a maximum P_20_5 in the amount of 0:53% AMF inoculation doses are 0.053, while K_2O content of leaves will increase 0:58% for each dose increasing of inoculation AMF 0.025 g.

Dry weight of crop and dashed-root ratio

The result of the study did not show any interaction between AMF inoculation with watering level (Tabel 2). The treatment of 0.025 g to 0.075 g per crop AMF inoculation showed the heavier biomaas compared to non AMF inoculation. In treatment of 0.075 g per crop AMF inoculation dry weight of crop tends to come down 0.050 g per crop AMF inoculation produced the highest dry weight of the leaves, dashed, and the largest dashed-root ratio.

Treatment watering level 80-100% of field capacity produces the highest total biomass compared with 40-60% and 60-80% watering level of field capacity. Root dry weight were not significantly different between 80-100% and 60-80% watering level of field capacity.

Production components

The study results did not show an interaction between AMF inoculation with the watering level to the soybean crop production components. The treatment of AMF 0,050 g per crop inoculation put out higher number of production branches, 100-seed weight pod and seed number per crop. AMF inoculation treatment did not significantly affect to the harvest index, but AMF inoculation treatment per crop showed the highest harvest index. 80-100% watering of field capacity showed the average number of production branches, number of pods, number of crop seeds, 100-seed weight, dry weight per crop and the highest harvest index.

Soybean plant is one of the important food crops which depends on Rhizobium inoculation and it's culture. Thus, inoculation of Arbuscular Mycorrhizal Fungi (AMF) is a co - inoculation forms tripartite interaction between soybean plant as host plant, with rhizobium bacteria and AMF as bioinoculan. Rhizobium bacteria is one of PGPR bacterial strains that have been widely used in the growth repairement and crop production. The result of the study indicates rhizobium inoculation and AMF at different levels of water supply is able to improvesoil fertility,increasethe level of NPK nutrients insidethe leaf tissue and improve growth perfomance and component of soybean plant production.

The combination of more than one microorganism in promoting plant growth through various mechanisms (*Bashan et al, 2004; Rodriguez et al, 2006*). Their interaction with AMF is involved in the promotion of plant growth and protection (*Sanchez et al, 2004*). Mycorrhizal colonization affect soil microbial interactions through modification of root exudates by mycorrhizal fungi can act as a carbon absorbentwhich is enough for various metabolic processes. It can induct qualitative and quantitative changes from exudate into rhizosphere which allows changes of rhizosphere fertility characteristic and even the change in microbial populations in the rhizosphere finally happened (*Johansson et al, 2004*). the improvement of soil fertility charasteristics is happened because AMF redeposites nutrients within the soil and modifiesrhizospherewhich is benefecial for host plants through the formation of hyphae and Glomulin of AMF (*Richardson et al, 2009*). AMF and glomulin products contributedirectlyto the formation of soil structure (*Rillig and Mummey, 2006*), increase soil aggregate stabilization, the content of C organic and maintain the presence of N soil (*Rillig, 2004*) .

The increaseof nitrogen uptake shown in the levels of nitrogen leaf get higher due to an increase in the amount of available nitrogenfor plants in the rhizosphere root (*Van der Heijden, 2010*). Various form of available N can be utilized from the AMF activity result, thereby it increases N absorption (*Govindarajulu et al, 2005 Hodge et al. (2001*) demonstrated the ability of AMF to decompose organic matter and obtain N from organic sources. They also showed that AMF increases the diffusion rate of N to the host plant. Therefore , the micorizalplant has additional access to the source of N compared with non –micoriza plant (*Govindarajulu et al. 2005*) showed that the AMF transfers large amounts of N from the soil to the root system. Extraradical hyphae of AMF take unorganic N then it is transferred to intraradical hyphae in the form of the amino acid, arginine. Hyphae intraradical decompose amino acid to obtain carbon compound and subsequenty N - ammonium is released into the host plant (*Adeleke, 2010*). The presence of AMF at legume plants will encourage the increase of nodulation and N fixation activity by rhizobium (*Barea et al., 2005; Demir and Akköprü 2007*). Therefore, the amount of N released into the rhizosphere get more and the amount of absorbed nitrogen get higher (*Barea et al., 2002*), reported that Rhizobium on infected alfa with AMF is higher than on the plants without Mycorrhiza (*Karandashov and Bucher, 2005)* reported an increased ability N_2-fixing in Mycorrhizal plants compared to non- mycorrhizal plants. Combined inoculation of G. Clarum and B. japonicum increase nitrogen fixation in soybean compared to single inoculation Bradirhizobium japonicum (*Antunes et a ., 2006*) .

The most important role of AMF inoculation is an increase in the availability and uptake of P_2O5. Simbyosis of *Rhizobium bacteria* can support the AMF in increasing solubility of phosphate through the production of phosphatase enzymes and organic acids produced by plants and bacteria to increase the absorption of phosphate. The concentration of available P in the soil solution is the result of the phosphatase enzyme activity produced by hyphae intrardicula of AMF (*Arthurson et al., 2011*). External hyphae of AMF can help plants to find P which is not available to be available outside the rhizosphere area (*Krishnamoorthy, et al., 2011*). synergetic interaction between AMF with Rhizobium bacteria modify root architecture togetherby extending absorptive surface to be larger and increase mycelium development so that the amount of adsorbed ion P is higher in the plants infected with AMF (*Gamalero et al., 2004*).

AMF infected plants, can not only improve the absorption and nutrient content N and P in the plant tissue, but many researchers reported the occurenceof increased K nutrient levels (*Kim et al., 2010; Nasaruddin 2012*), Mn and Zn (*Roesti et al., 2006, Yusran et al. 2009*), Cu, Fe, Mg (*Kim et al. 2010*) in the leaf tissue. The research results of Nasaruddin 2012 reported that AMF Dual inoculation with A. chroococcum on cocoa plants can increase kalim uptakereached above 150 %, depending on the inoculation dose.

Table.1 The average level of N, P2O5dan K2O of leaves (%) at different levels of watering and AMF inoculation

Water (% field capacity)	Arbuscular mycorrhiza inoculation dose				NP BNJ α 0.05
	m_0 (0,000 g)	m_1 (0,025 g)	m_2 (0,050 g)	m_3 (0,075 g)	
Leaf N content					
a_0 (80-100)	1.21 c^y	1,29 b^y	1,40 a^z	1,39 a^z	
a_1 (60- 80)	1.26 c^x	1,41 b^x	1,45 a^y	1,43 ab^y	0,02
a_2 (40- 60)	1.27 c^x	1,39 b^x	1,53 a^x	1,53 a^x	
NP BNJ α 0.05		0.02			
Leaf P_2O_5 content					
a_0 (80-100)	0.35 d^z	0,44 b^y	0,54 a^y	0,41 c^z	
a_1 (60- 80)	0.40 d^y	0,05 c^x	0,58 a^x	0,56 b^x	0,01
a_2 (40- 60)	0.45 c^x	0,49 b^x	0,49 b^z	0,53 a^y	
NP BNJ α 0.05		0.02			
Leaf K_2O content					
a_0 (80-100)	1.40 b^x	1,40 b^x	1,39 b^x	1,46 a^y	
a_1 (60- 80)	1.32 b^y	1,35 b^y	1,49 a^x	1,47 a^y	0,04
a_2 (40- 60)	1.27 c^z	1,40 b^x	1,39 b^x	1,59 a^z	
NP BNJ α 0.05					

Note: The numbers followed by the same letter on the line (a, b, c) and column (x, y, z), not significant at the level of 95%

Table.2 The average of biomass and rry weight (gram) and the ratio of dashed-root at CMA inoculation treatment and watering level

Treatment	Dry weight (grams) and the dashed-root ratio					
	Leaf	Rod	Root	Dashed	Biomass	Dashed/Root
Inoculation of arbuscular mycorrhizal fungi (AMF)						
m_0 (0,000 g)	10,11	12,95 b	2,52 b	23,06 c	25,57 b	9,58
m_1 (0,025 g)	11,46	15,46 a	3,21 a	26,92 b	30,13 a	8,40
m_2 (0,050 g)	14,60	15,12 a	2,58 b	29,72 a	32,29 a	14,69
m_3 (0,075 g)	12,82	14,11 a	2,96 a	26,93 b	29,89 a	9,17
NP BNJ α 0.05	ns	1,70	0,32	2,61	2,44	ns
Provision of water level (% field capacity)						
a_1 (80-100)	13,60	17,78 a	3,21 a	31,46 a	34,67 a	10,09
a_2 (60 –80)	13,37	14,73 b	3,14 a	28,10 b	31,24 b	9,30
a_3 (40 –60)	9,17	10,72 c	2,10 b	20,41 c	22,51 c	13,28
NP BNJ α 0.05	ns	1,91	0,29	2,36	2,70	ns

Note: The numbers followed by the same letter in a column are not significantly different at the level of 95%

Figure.1 Correlation of AMF with the CEC, organic C and C / N ratio (A) and AMF correlation with pH levels of N, P2O5 and K2O (B)

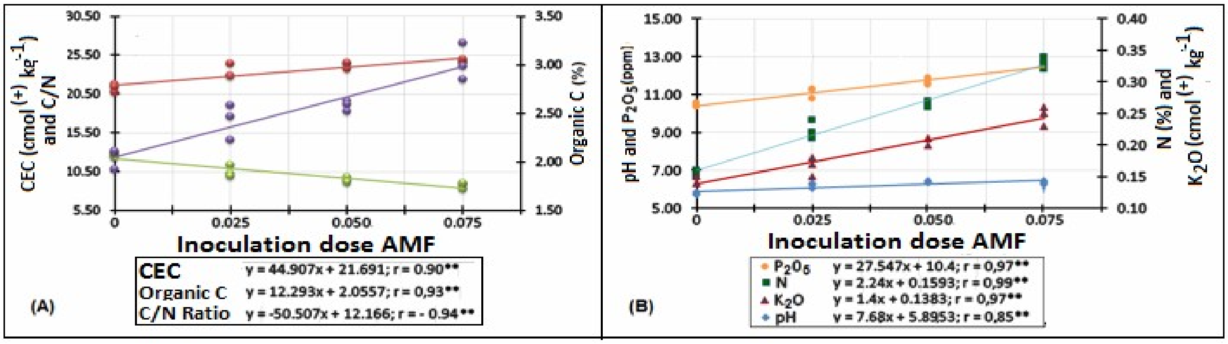
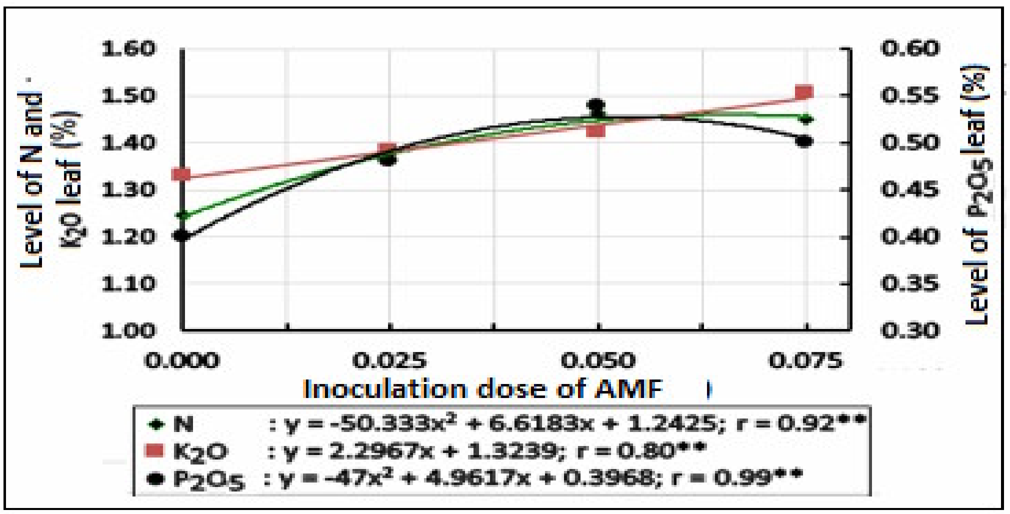


Figure.2 Correlation of AMF with levels of N, K2O with P2O5



The average percentage of the levels of N, P_2O_5 and K_2O leaves in water supply levels 80-100 %, is lower than at 60-80 % and 40-60 % field capacity. Decreased levels of uptake athigher water supply treatment probably happened due to the possibility of soil pores will be filled with water that causes the available number 02 for plant is limited, therefore limited root respiration activity impact in the reduction of uptake level N, P_2O5 and K_2O.

Inoculation AMF and Rhizobium bacteria can increase dry weight, bimass and soybean plant production to be better compared without AMF inoculation. This is because micorizal plant experiences the higher improvement of soil fertility, water absorption rate and mineral nutrients especially N, P and K than plants without AMF. Finally, availability of N, P and K in leaf development is an important step that determines the growth and production (*Thangadurai, et al., 2010*). Enough amount of avaliable nutrients in the rhizosphere can repair plant growth (*Afzal and Asghari, 2008*). In addition, inoculation CMA encourages the development of bacteria consortium symbiotized with AMF can produce phytohormones such as auxin and gibberellins which can stimulate plant growth (*Tini Surtiningsih, 2013*). It will improve the production at last .

Nutrients N, P and K will increase the rate of photosynthesis, produce flowers and fruit formation will occur optimally. Levels of N, P and K in the leaves will continue to decline in line with the formation of flowers and fruit, because the element is moved to the reproduction to support the development of flowers and pods. AMF inoculation can increase the number of pods, pod weight and dry weight of seed and finally it will increase production as well. The nutrient levels of N, P and K in infected leaf can improve the rate of leaf photosynthesis and subsquently improve the growth and production of soybean.

Arbuscular Mycorrhiza Fungi (AMF) can be symbiotic along with Rhizobium bacteria on soybean crop synergistic for improving the growth and production of soybean. Inoculation of Arbuscular Mycorrhiza Fungi (AMF) on soybean crop can improve the availability and absorption of plant nutrient. Inoculation of Arbuscular Mycorrhiza Fungi (AMF) can improve the growth and production of soybean. Activity of Arbuscular Mycorrhiza Fungi (AMF) on low level water availability is more effectively compared to plants that undergo sufficient water.

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