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Effect of *Azotobacter* sp. and N Fertilizer on the Growth of Oil Palm Seedling Inoculated with *Ganoderma* sp.

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ABSTRACT

Excess application of nitrogen fertilizer causes soil nutrient imbalances and reduce the number and soil microbial diversity. This condition could induce soil born dieseses such basal stem rot caused by *Ganoderma* sp. A study was conducted to enhance the plant tolerance to *Ganoderma* sp. through introduction of microbial community especially non-symbiotic N fixing bacteria, *Azotobacter* sp. Plant materials used were 4 months old of germinated oil palms, while *Ganoderma* sp. was isolated from Bekri, Lampung. There are seven treatments tested i e *Azotobacter* sp. + *Ganoderma* sp. (+A+G), *Azotobacter* sp. + 30% N fertilizer of recommended dosage + *Ganoderma* sp. (+A+30N+G), *Azotobacter* sp. + 60% N fertilizer of recommended dosage + *Ganoderma* sp. (+A+60N+G), *Azotobacter* sp. + 100% N fertilizer of recommended dosage + *Ganoderma* sp. (+A+100N+G), 100% N fertilizer of recommended dosage + *Ganoderma* sp. (+100N+G), *Ganoderma* sp. inoculation (positive control, +G), and non-inoculated *Ganoderma* sp. (negative control, -G). The result showed that *Azotobacter* sp. enhanced the height of plant inoculated with *Ganoderma* sp. when accompanied with N fertilizer of 30 to 100% of recommended dose. Moreover, +A+100N+G seedling had significantly higher fresh and dry weight of shoot compared to those of +G seedling or +100N+G seedling.

Key words: *Elaeis guineensis*, microbial community, oil, soil-born diseases, soil nutrient

INTRODUCTION

Nitrogen is the highest amount of nutrients added as fertilizer in soil compared to other macro nutrients such as P and K. Long term and high dose of N fertilizers, especially in the form as urea, is believed to change the soil nutrient balance and soil characteristics. The development of nutrient imbalances could cause various problems including decrease in soil bearing capacity against soil pathogens. This resulted in decline of population of microbial antagonists that play a role in the control of soil borne pathogens. In general, N fertilization promotes vegetative growth, lowering the concentration of carbon-based secondary compounds (phenol, terpenoids) and increasing N compounds such as alkaloids (Rasmussen *et al.* 2008). In addition, excess of nitrogen input could decrease the defense of plants. Nitrogen enrichment can

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increase growth, and differently affect the shoot and root defense (Rasmussen *et al.* 2008; Jamieson e*t al.* 2012). Excessive N fertilization can lead to 40% endophytic depletion and can increase the attack of certain plant diseases. Olese *et al.* (2003) suggests that increased N fertilizers may increase the attack of powdery mildew on wheat crops. However, excessive N fertilization impacting plant disease attacks and also has an impact on environmental pollution.

Azotobacter sp. is known as non-symbiotic N-fixing bacteria, Gram negative, obligate aerobic, and soil dwelling. Its role in N fixation is extensively researched because it can associate with various plant species. Besides known as N fixers, this bacterium is also known as a producer of IAA hormone which can enhance the growth of plant roots. Romero *et al.* (2013) suggests that this bacterium can survive in dry conditions because of its ability to form cysts that are controlled by the sigma factor RpoS. In addition, it is expected that application of *Azotobacter* improve the quality of oil as has been done on the plant *Brassica carinata* cv. Peela Raya. Addition of *Azospirillum* and *Azotobacter* in combination with a half-dose of chemical fertilizers enhance the growth, yield and quality of Ethiopian mustard oil (Nosheen *et al.* 2013).

In oil palm plantations at this time, there is a very important disease known as basal stem rot caused by *Ganoderma* sp. Widiastuti *et al.* (2013) reported that there was a decline in microbial diversity, especially in area with severe attack of *Ganoderma* sp. However, in all samples taken from selected area, *Azotobacter* sp. Were consistently found. *Azotobacter* associations with plants are thought to alter the nutrient status of N crops as well as exudation of plant roots in both the number and components of exudates which may further alter the rhizosphere microbial community (Lakshmanan *et al.* 2014). This change can be through changes in the physical properties of soil chemistry and or indirectly affect the microbial community of antagonists (Kloepper *et al.* 2004). Research on the characterization of *Azotobacter* sp. of various habitats have been carried out including estate crops (Widiastuti *et al.* 2010). This study aims to determine the effect of microbial community improvement through inoculation of *Azotobacter* bacteria on the growth performance of oil palm inoculated with *Ganoderma* sp.

MATERIAL AND METHODS

The experiment was conducted in Microbiology Laboratory and Greenhouse of Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB). Planting materials used were germinated oil palm seeds from Indonesian Oil Palm Research Institute (IOPRI), Medan, grown for 4 months in sterilized sand medium, before they were transplanted in sterilized soil medium obtained from Ciomas, Bogor. Sterilization was done by autoclave for 60 minutes at 121 °C and pressure 1.2 atm. Planting medium was filled in polybags 30x40 cm^2 in size. N fertilizer was given in the form of urea with a dose of 5 g referred as 100% standard. *Ganoderma* sp. was inoculated following the method of Widiastuti *et al.* (2011) prior to inoculation of *Azotobacter* sp. Inoculant of *Azotobacter* sp. with the concentration of 10^8 colony forming units (cfu) in 10 mL water for each seedlings, applied at the beginning of planting the seeds, while the application of N fertilizer had been adjusted to the standard time of fertilization that is every 2 weeks (Lubis 2000). Plant maintenance had been done by watering using tap water every day. Visual symptoms of *Ganoderma* attack and seedling growth, nutrient N content of plants and soil were observed at 8-month after planting.

Morphological observations of *Azotobacter* sp. was conducted using scanning electron microscopy (SEM). The experimental design used in this study was randomized block design to test the 7 treatments, namely inoculation of *Azotobacter* sp. + *Ganoderma* sp. (+A+G), *Azotobacter* sp. + 30% N fertilizer of recommended dosage + *Ganoderma* sp. (+A+30N+G), *Azotobacter* sp. + 60% N fertilizer of recommended dosage + *Ganoderma* sp. (+A+60N+G), *Azotobacter* sp. + 100% N fertilizer of recommended dosage + *Ganoderma* sp. (+A+100N+G), 100% N fertilizer of recommended dosage + *Ganoderma* sp. (+100N+G), *Ganoderma* sp. inoculation (positive control, +G), and non-inoculated *Ganoderma* sp. (negative control, -G). The data obtained were tested by simple statistic and continued with Duncan Multiple Range Test (DMRT) with 5% test.

RESULT AND DISCUSSION

Gram Characterization and Biochemical Properties of *Azotobacter* sp. Gram staining showed that *Azotobacter* sp. is Gram-negative bacteria and SEM observation had revealed that the isolate produced polysaccharides. Mandal *et al.* (2008) suggest this isolate is oval in shape that the formed polysaccharides are useful for protecting the activity of nitrogenase from reactive oxygenase, thereby increasing the activity of fixation. According to Samiran *et al.* (2012) exopolysacharida applications produced by *Azotobacter* sp. can be useful as growth promoting substances, increase plant resistance to drought stress, improve soil aggregates and increase soil microbial community interactions to inhibit pathogens. In addition, *Azotobacter* sp. also produce cyst at dormant stage. Based on the analysis reduction of acetylenic (ARA) activity of nitrogenase *Azotobacter* sp. used in this study was 0.727 µmol hour-1.

The Growth of Oil Palm Seedling

The performance of oil palm seedlings growth after 8 months is presented in Figure 1 while the rooting of the plants is presented in Figure 2. The result of statistical analysis shows that the height of oil palm seedlings +G lower than that of –G although not significantly different (Table 1). The application of *Azotobacter* sp. in addition to 30%N, +60%N, and +100%N significantly yield higher palm seedling growth compared to those +G, while +A+G did not yield significantly higher growth compared to those +G. These results indicate that inoculation of

Table 1 Growth of oil palm seedling in each treatment tested

Treatment*	Seedling height (cm)	Leaf number	Fresh weight (g)		Dry weight (g)	
			Shoot	Root	Shoot	Root
A+G	29.9 ab	5.3 a	113.0 ab	23.7 a	55.3 ab	8.4 a
A+30N+G	34.9 c	5.6 a	81.3 ab	20.67a	39.6 ab	6.6 a
A+60N+G	33.2 bc	6.2 a	67.7 ab	13.8 a	323 ab	4.6 a
A+100N+G	32.7 bc	5.5 a	132.7 b	23.6 a	63.8 b	6.6 a
100N+G	30.8 ab	6.3 a	57.7 a	13.6 a	24.8 a	6.8 a
G	28.1 a	5.8 a	65.0 a	197 a	29.4 a	7.9 a
- G	31.5 abc	5.9 a	51.4 a	147 a	26.4 a	6.3 a

*A: *Azotobacter* sp; G: *Ganoderma* sp.: 30N: 30% recommended doseage of N; 60N: 60% recommended doseage of N fertilizer; 100N: 100% recommended doseage of N fertilizer.

Figure 1 Oil palm seedling performance in each treatment. From left to right: *Azotobacter* sp. + *Ganoderma* sp. (+A+G), *Azotobacter* sp.+ 30% N fertilizer of recommended dosage + *Ganoderma* sp. (+A+30N+G), *Azotobacter* sp. + 60% N fertilizer of recommended dosage + *Ganoderma* sp. (+A+60N+G), *Azotobacter* sp. +100% N fertilizer of recommended dosage. + *Ganoderma* sp. (+A+100N+G), 100% N fertilizer of recommended dosage + *Ganoderma* sp. (+100N+G), *Ganoderma* sp. inoculation (positive control, +G), and 7) non inoculated *Ganoderma* sp. (negative control, -G).

Figure 2 Oil palm root performance in each treatment. From left to right: *Azotobacter* sp. + *Ganoderma* sp. (+A+G), *Azotobacter* sp. + 30% N fertilizer of recommended dosage + *Ganoderma* sp. (+A+30N+G), *Azotobacter* sp. + 60% N fertilizer of recommended dosage + *Ganoderma* s. (+A+60N+G), *Azotobacter* sp. +100% N fertilizer of recommended dosage + *Ganoderma* sp. (+A+100N+G), 100% N fertilizer of recommended dosage + *Ganoderma* sp. (+100N+G), *Ganoderma* sp. inoculation (positive control, +G), and non inoculated *Ganoderma* sp. (negative control, -G).

Table 2 Nitrogen status of soil and leaf and *Azotobacter* population in soil

Treatment*	Population of *Azotobacter* (cfu)	N concentration (%)	
		soil	leaf
A+G	5.0 x 10^3	0.19 b	2.09 a
A+N30+G	2.0 x 10^2	0.21 bc	2.26 b
A+N60+G	3.0 x 10^2	0.20 b	2.61 bc
A+N100+G	4.0 x 10^2	0.21 bc	2.64 bc
N100+G	1.0 x 10^2	0.27 c	2.98 c
G	2.9 x 10^3	0.23 bc	1.86 a
- G	3.2 x 10^2	0.12 a	1.82 a

*A: *Azotobacter* sp.; G: *Ganoderma* sp. N30: 30% recommended doseage of N; N60: 60% recommended doseage of N fertilizer; N100: 100% recommended doseage of N fertilizer.

Ganoderma sp. slightly inhibited the growth ofseedling height and N nutrient adequacy did not seem to be fulfilled from the fixation of N *Azotobacter* sp. alone but should be combined with addition of N fertilizer. Better growth of plants inoculated with *Azotobacter* sp. and N fertilizer appears to be able to increase plant resistance to *Ganoderma* sp attack. In addition, Basidiomycetes among which *Ganoderma* sp. is within this Division, known as a major agent in the decomposition of cell wall polymers. Laboratory test results indicate that an increasing of inorganic N concentration can suppress the transcription of fungi genes necessary for lignin and lignocellulose metabolism (Li *et al.* 1994) and may even lead to a change in the composition of the fungi basidiomisetes (Edwards *et al.* 2011). These results indicate that N fertilization may inhibit *Ganoderma* sp. activity. Further Elsenlord *et al.* (2014) suggested that the presence of N in the soil significantly reduced the population and the diversity of genes involved in depolymerization of starch (12%), hemicellulose (16%), cellulose (16%) and chitin 15% and 16% lignin which generally had an effect on weathering and accumulation of organic matter. However, this possibility does not occur in the presence of N fixation due to fixation by *Azotobacter* which is different in addition to other functions of *Azotobacter* sp. Rasmussen *et al.* (2008) suggested that in Lolium perenne plants infected with endophytic nitric microbes showed a decrease in some amino and magnesium acids, while the water-soluble carbohydrate content, fats, some organic acids and chlorogenic acids, increased. On the other hand, the application of N fertilizer causes an increase in organic and lipid acids while water-soluble carbohydrates, kholorogenic acids, and fiber decreases. These results indicate thar there is differences in metabolism between N fertilized plants and infected endophytic fungi.

Widiastuti *et al.* (2013) showed that there was low correlation between population of *Azotobacter* sp. and the attack rate of *Ganoderma* sp. among the observed microbial parameters analyzed such as total microbes, total fungi, phosphate solubilizing bacteria and total cellulolytic fungi. In addition to N fixers *Azotobacter* sp. also produces indole acetic acid hormone known as plant growth promoting (Widiastuti *et al.* 2010). With the presence of *Azotobacter* sp. the plant gets sufficiency supply of nitrogen and IAA that promote plants to survive *Ganoderma* sp. attacks. This can be seen in Table 1, the height of palm fertilized with N 100% of recommended dosage was 30.75 cm while the treatment of N fertilizer at various doses combined with *Azotobacter* sp. causing an increase in plant height of 2-4 cm. The addition of N 100% fertilizer alone (+N100+G) resulted in seedlings heights that are not significantly different from those given by *Azotobacter* sp. only (+A+G). This result indicates that the supply of N from fixation by *Azotobacter* sp. had been sufficient to support the superior performance of oil palm seedlings.

The seedlings of oil palm treated with +A+N30-100 that are inoculated with *Azotobacter* sp. accompanied by N fertilizer appears to show leaf breakage (Figure 1). These results are thought to be due to the precence of IAA hormone produced by *Azotobacter* sp. which is not found in the application of N fertilizer (urea) alone, especially at low doses of N fertilizer. Although, the number of leaves was not different in all treatments tested (Table 1), however, +N100+G gave the highest number of leaves, while +A+G yielded the lowest number of leaves. The relatively short observation period may not be sufficient to show the effect of treatments to the number of leaves.

Treatment of +A+N30 and +A+N60, yielded fresh and dried shoot biomass unsignificantly different from those +G, but on the +A+100N+G resulting in fresh and dry biomass weight significantly higher than that +G. Between 30% doses and 60% of N fertilizer there was no significant difference in fresh and shoot biomass weight compared to those 100N dose. These results suggest that a higher dose of N may be required to produce fresh and dry weight of plant infected with *Ganoderma* sp.

The performance of oil palm seedlings is presented in Figure 2. In general, oil palm seedlings did not show symptoms of *Ganoderma* sp. Infection such as decay, however, inoculation of *Ganoderma* sp. reduced the development of rooting. *Azotobacter* inoculation alone results in better rooting but the best rooting treatment is +A+30N+G and +A+60N+G. Addition of *Azotobacter* sp. does not significantly affect fresh and dry weight of roots biomass but there is a tendency that all *Azotobacter* sp. application results in higher fresh roots biomass compared to +N100+G. These results indicate that the application of N fertilizer inhibits root growth. Application of *Azotobacter* sp. seems to promote root growth, thus decreasing the need of N fertilizer. The optimum N fertilizer is 60%.

Soil and Leaf nutrient Status as well as *Azotobacter* Populations in Rhizosphere of Oil Palm Seedlings

Population of *Azotobacter* sp. in soil with the inoculation treatment of *Ganoderma* sp. was higher than without inoculation of *Ganoderma* sp. It is generally shown that *Azotobacter* sp. were found in all rhizosphere of oil palm seedlings, although the highest population was found in +A+G treatment. The cause of high population in this treatment can not be explained using available data in this study.

The result of N soil analysis indicated that the soil N content in +G treatment is significantly higher than that of -G. The cause of this can not be explained in this study. The same results were found on soil in oil palm seedlings of +A+30N+G, +A+60N+G, and +A+100N+G compared to that –G. However, the soil N content in these plant rhizosphere was not significantly different compared to those +G treatment.

Decrease in dosage of N fertilizer up to 70% but accompanied by *Azotobacter* sp. application did not cause any significant difference to soil N levels. These results indicate that there is influence of *Azotobacter* sp. although this influence is not significant. Inoculation of *Azotobacter* sp. seemed to cause an increase in soil N as a result of N_2 fixation activity by *Azotobacter* sp. However, it seems that N_2 fixation activity did not give the same influence on soil N levels as that of 100% N fertilizer application.

The result of N leaf analysis showed that N content of leaf of oil palm seedlings +G were not significantly different to –G. These results suggest that inoculation of *Ganoderma* sp. had no effect on leaf N content. However, +A+100N+G, +A+60N+G, and +A+30N yield significantly higher N leaf compared to that –G. These results also show that a reduction in the dose of N fertilizer 40% accompanied by *Azotobacter* sp. (+A+60N+G) produce the same N leaf content with +N100+G. It supposed that *Azotobacter* application increase the efficiency of N fertilizer application.

These results indicate that addition of *Azotobacter* sp. can improve the efficiency of N fertilizer but in high N addition the N leaf content does not significant difference. *Azotocbacter* is a free-living N-fixing bacteria. The magnitude of N fixation capability is strongly influenced by micro-environments including soil N levels as well as root exudates produced by plants as a source of carbon and energy for the activity of fixation N. The fixing ability of N_2 *Azotobacter* sp. is 10 mg N_2 g^{-1} carbohydrate (glucose). The main sources of carbon are sugars, alcohols, salts, and organic acids. This bacterium grows 48 hours at a temperature of 30 °C, pH 7-7.5, with a smony colony, opaque, slightly convex (low convex), and viscid. Carvalho *et al.* (2014) suggests that these bacteria produce increased yield and growth because other functions besides their ability to fix N also produce IAA, gibberellin and cytokinin. In high N conditions, the activity of *Azotocbacter* sp. less optimum and even at high N the possibility of plants producing different root exudates and not supporting the development of *Azotobacter* sp. However, inoculation of *Azotobacter* sp. still need N fertilizer application. The results of Kiba *et al.* (2011) suggests that there is interaction between nutrients N with phytohormon which ultimately affect the growth and development of plants. Both phytohormon and nitrogen simultaneously alter the physiology and morphology of plants. According to Adesemoye *et al.* (2008) *Azotobacter* sp. including in plant growth-promoting bacteria (PGPR) that can induce root hair growth and increase root surface area so that roots absorb more nutrients to meet the need of plants.

CONCLUSION

Addition of *Azotobacter* sp. affect the growth of oil palm seedlings inoculated by *Ganoderma* sp. Although shoot N content did not show any significant differences between plants with and without *Azotobacter* sp. but the treatment of +A+100N+G significantly increase fresh and dry weight biomass compared to those +100N+G.

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