Effectiveness of *Trichoderma viride* (T2) to the Growth of *Acacia mangium* Seedlings

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***Abstract*—Increasing the growth of *Acacia mangium* could be conducted by inoculatin of *T. viride* (T2) that is combined with the use of mature manure. This study aimed to improve the effectiveness of *T. viride* (T2) and manure to the growth of *A. mangium* seedlings. The treatments consisted of the media (soil and sand sterile) without treatment as a control, media that were inoculated T2, media that were supplemented mature manure, and the media that were given mature manure and inoculated T2. This study observed during two months. The results showed that the seedlings in media containing manure and T2 had a height (22.57 cm) and diameter (2.67 cm). It was higher than the control treatment and the treatment were only applied T2 or sole manure. In this study, the seedling growth was influenced by the increase in nutrient content (organic C: 1.38%, nitrogen: 0.18% and phosphorus: 144.35 ppm) and the ability of T2 in utilizing nitrogen for its growth and development, which was indicated by the high of T2 spore density.**

Index Terms*—*Acacia mangium*, manure, *Trichoderma viride

I. Introduction

The growth of forest more emphasis on the utilization of natural fabrics that can assure the sustainability of the product and the environment. It can minimize the usage of chemicals, such as chemical fertilizers. It too becomes the provision of sustainable forest management that must be fulfilled in stokckholders of forests in Indonesia to receive certificates of SVLK (Standar Verifikasi Legalitas Kayu or standard of wood legality verification) and PHPL (Pengelolaan Hutan Produksi Lestari or management of sustainable forest product). One example of the use of natural materials is the use of manure for increasing plant growth (in this case is *A. mangium*).

A. mangium is a plant that has been a favorite plant in Indonesia because of multi-product and a short crop rotation. In the cultivation of *A. mangium* have almost no obstacles at every the stage of growth. Even though the quality and quantity of *A. mangium* still need to be improved because the decline of land quality may occur every year and the high threat of stem rot disease on subsequent crop rotation.

The use of *Trichoderma* as a biological agent in various plants has been established as a pesticide [1]-[3] and biofertilizer [2]-[4]. The role of *Trichoderma* is indicated by the production of phytohormone compound and providers of essential nutrients for plants [5].

Trichoderma applications that are aggregated with the role of manure as a growing medium become very significant in the cultivation of *A. mangium* in West Kalimantan. This is imputable to the character of land that is dominated by ultisol and acidic soil. The ultisol is a nutrient-poor soil, thus, necessary the addition of nutrients and introduction of soil microbial such as *Trichoderma*. The use of *Trichoderma* local isolates is crucial in West Kalimantan because a local strain has a better ability to adapt to the soil environment.

This study aimed to improving the effectiveness of *T. viride* (T2) and manure to the growth of *A. mangium*. The ability of *Trichoderma* as decomposers of manure can optimize their role as the biofertilizer agens to increase the growth of *A. mangium*. Livestock animals are not much cultivated by the forest communities of West Kalimantan become a limiting factor in the supply of manure for fertilizer, so that the manure application can be combined with the use of soil-borne fungi such as *Trichoderma*.

II. Material and Methods

A. *T. viride (T2) Inoculum*

T. viride (T2) (isolates collected in the lab. Silviculture Tanjungpura University) were cultured on PDA for 5 days. T2 isolates (5 plug) were cultured on rice medium (1000 ml) which has been sterilized, and incubated for 3 weeks. Then it was inoculated in planting media of (*A. mangium*).

B. *Experiment Site*

A. mangium seeds were sown in sterile sand medium. After eight days later, seedlings were moved to the planting medium that had been processed. Previously. Ultisol soil, sand and manure were sterilized. Soil and sand in the ratio 3: 1 wt / wt, were mixed into a polybag (20 x 20 cm) as a planting medium. The treatments consisted of: 1) without manure and T2 or control; 2) sole manure (50 g / polybag) (P); inoculation of T2 (5 g inoculum / polybag) (T); 4) manure (50 g / polybag) + T2 (5 g / polybag). Each treatment was repeated 5 times. Watering was done 2 times a day (sunrise and afternoon). The treatments were created with a completely randomized design.

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C. Analysis of Data

Variable of observations consisted of height and diameter of seedlings, soil analysis (C / N ratio and P_2O_5) and spore density of T2. The data were examined using analysis of variation (ANOVA), succeeded by Duncan test.

III. Results

A. Effect of T2 and Manure to the Growth of *A. mangium* Seedlings

Effect of manure and *T. viride* (T2) can be measured in the growth of plants (seedlings height and diameter). Fig. 1 showed that the manure and inoculation T2 significantly affected to the high (ρ> 0.05) and diameter (ρ> 0.05) seedlings in two months of observation

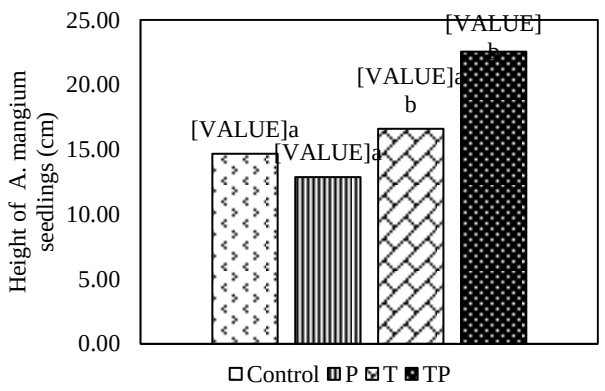

Figure 1. Effect of T2 and manure to the height of *A. mangium* seedling.

The application of manure + T2, seedlings were higher (22.57 cm in height) (Fig. 1) and larger (2.69 cm in diameter) (Fig. 2) than the treatment of sole manure or T2. But treatments that sole manure (P) and sole T2 (T), did not show any significant differences with the control. The diameter growth is secondary growth that can significant effect to the older age seedlings. In Fig. 2 indicated that the combination of T2 and manure to accelerate the growth of secondary *A. mangium* seedlings (in the seedling age of 3 months

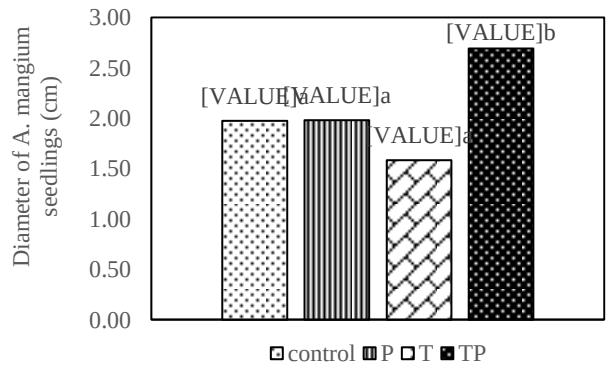

Figure 2. Effect of T2 and manure to the diameter of *A. mangium* seedling.

B. Effect of T2 and Manure to Soil Nutrition

Increased growth of seedlings in the treatment of T2 + manure (TP) was affected by increasing nutrient C, N, and P. Table I showed that the increasing of N content in treatment TP (0.18%) were consistent with the increasing of C-organic content (1, 38%). The ranges of C / N ratio was 7,50 – 8,00, lower than the C / N ratio standard by SNI (10 – 20) [6]. N content at higher control (K = 0.16%) than the media were given manure (P = 0.08%) and T2 (T = 0.06%). While the content of P on the lower control (K = 3.75) compared to all treatment

TABLE I. Effect of T2 and Manure of Nutrient in Treatment Medium

Treatments	Nutrient with treatment medium			
	C-organic (%)	N total (%)	C / N ratio	P2O5 (ppm)
Control (K)	1.27	0,16	7,94	7,35
P	0,63	0,08	8,00	66,44
T	0,45	0,06	7,50	5,55
PT	1,38	0,18	7,67	144,35

There was an increase phosphorus (66.44 ppm) in planting medium by the addition of manure, than controls (3.75%) and treatment of sole T2 (5.55 ppm). Media with the addition of manure + T2 had the highest availability of phosphorus (144.35%) than the other discourses. This suggests that phosphorus is essential for seedling growth.

The treatment of manure and T2 had highest of the T2's growth (3,60 x 10^6 spores/ml) than the others (Fig. 3). *T. viride* (T2) density in control treatment (2,25 x 10^6 spores/ml), sole manure (2,55 x 10^6 spora/ml) and sole *T. viride* (T2) (2,45 x 10^6 spores/ml) did not significantly different. *T. viride* (T2) in control and sole manure treatment might be caused by contamination when *T. viride* (T2) inoculated.

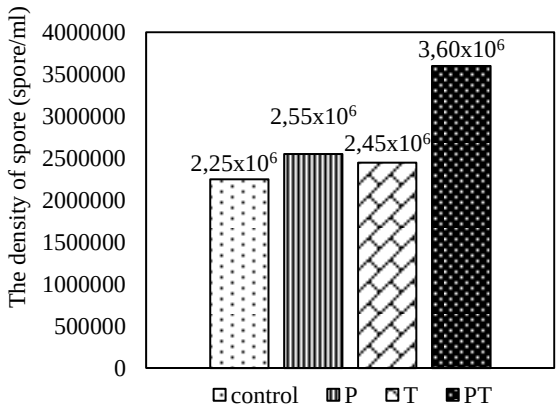

Figure 3. Effect of T2 and manure to the growth of *T. viride* (T2).

IV. Discussion

The previous research explained that *T. viride* (T2), *T. hamantum* (T3) and *T. virens* (T5) had not shown a significant effect on the height and diameter of *A. mangium* seedlings [7]. Likewise with this survey, where

inoculation of sole T2 was not significantly different with the control treatment (Fig. 1 and Fig. 2). Inoculation T2 with the addition of manure showed the most effective to increase the growth seedlings than all treatments. It proved that *T. viride* (T2) required nutrients for growth and development through the manure in TP's media treatment. The treatment of sole T2 without the addition of fertilizer, had a low nutrient content (Table I). [5] Explained that the gain in plant growth was not correlated with root colonization *Trichoderma*. It indicated that T. *viride* (T2) only colonized in rhizospher and use manure, which was added to the media as a substrate and source of nutrients. *Trichoderma* will decompose complex compounds in the manure into compounds available to plants. Thus the role of T2 on plant growth was not quite so straightforward as endophytic *Trichoderma*, which is capable of producing a compound that can stimulate the growth of plants [8].

The content of C and N in application of sole manure (P) was lower than the control treatment (Table I). Nevertheless, it did not provide a significant difference to the growth of seedlings (Fig. 1 and Fig. 2), as well as an increase in availability of phosphorus in the treatment of sole manure (P). The addition of manure also did not affect the growth of okra (*Abelmoschut esculentus* L.) [9], tomato and mustard [10]. This suggests that there was some other factor as the outcome of interaction between T2 with manure thereby affecting seedling growth of A. *mangium*.

Terms of the value of the C / N ratio in accordance with the Indonesian National Standard (SNI) is graded from 10 to 20 to be considered stable. [11] suggested that *Trichoderma* that were isolated in mature compost had 8 of C / N ratio. So do the C / N ratio in this study (Table I) and a closed range. Lower C / N ratio indicates that the microorganisms (in this study is T2) capable of effectively decomposing compost (mature manure) [12]. All the same, the decomposition activity by *Trichoderma* does not add compost N final since N is used for protein synthesis *Trichoderma* [11], Despite the fact that the value of N manure inoculated T2 was higher than in the growing medium which is only given manure. Lower C / N ratio (Table I) than the SNI C / N ratio standard indicated that *T. viride* (T2) would release available nitrogen to *A. mangium* seedlings through mineralization of manure. Nitogen which was released into ammonium sulphate, sodium nitrate and ammonium nitrate increased growth and conidia product of *T. pseudokoningii* [13]. So media with the addition of manure could increase the population of *T. viride* (T2) (3,60 x 10^6 spores/ml) in treatment of manure and T2 (PT).

V. Conclusion

Inoculation of *T. viride* (T2) could improve the growth of *A. mangium* seedlings even though it was significant yet. The effect of *T. viride* could be increased by adding manure. It was able to improve nutrition and the population of *T. viride* in soil.

VI. Recommendations

The effect of *T. viride* (T2) and manure to the growth of seedlings should consume a long time of observation. It is caused by forest plants such as *A. mangium*, has a long crop rotation. Therefore further study needed on the dosis of *T. viride* (T2) and manure effective to increase the growth of *A. mangium* on the ultisol soil in West Kalimantan.

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References

[1] I. Chet, A. Viterbo, Y. Brotman, and T. Lousky. Enhancement of plant diseases resistance by the biocontrol agent *Trichoderma*. Life Science Open Day. [Online]. Available: https://www.weizmann.ac.il/Biology/open_day_2006/book/Abstracts/Ilan_Chet.pdf

[2] R. Suryantini and R. S. Wulandari, "Evaluation of effect *Trichoderma* sp. as biocontrol agents to a nursery diseases and acacia seedlings (*Acacia mangium* Wild.)," Report of young lecturer research, Forest Faculty, Tanjungpura Univ., Pontianak, Indonesia, 2007.

[3] R. Suryantini, "Effect of inoculation time of *Trichoderma reesei* to pathonenicity of damping off pathogen and development of mycorrhizae to the growth of tusam (*Pinus merkusii* Jungh. Et de Vriese) seedlings," Thesis, Forest Faculty, Gadjah Mada University, Yogyakarta, Indonesia, 2003.

[4] R. A. Hill, A. Ambrose, N. A. Sajali, M. Yatim, R. B. Valdez, F. Agbayani, J. Bungang, R. F. Minchin, and A. Stewar, "Bioprotection of *Acacia mangium* using *Trichoderma* in Malaysia," in *Exploiting Microbial Diversity for Sustainable Plant Production*, S. M. Zydenbos, T. A. Jackson, Eds., Christchurch: Microbial Products, New Zealand Plant Protection Society, 2010, pp. 51–55.

[5] C. Altomare, W. A. Norvell, T. Bjbrkman, and G. E. Harman, "Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22," *Applied and Environtal Microbiology*, vol. 65, no. 7, pp. 2926-2933, July 1999.

[6] Anonimous. Spesification of compost from an organic waste, SNI: 19-7030-2004. [Online]. Available: http://www.healthy-rice.com/snikompos.Pdf

[7] R. Suryantini, R. S. Wulandari, and S. Rifanjai, "Dual inoculation of *Trichoderma* spp. (local isolates) and *Glomus* sp. in *Acacia mangium* seedlings," *Journal of Bioscience* (Submitted), The submission id. JBSC-D-16-00594.

[8] R. Azormi, B. Hajieghrari, and A. Giglou, "Effect of *Trichoderma* isolates on tomato seedling growth response and nutrient uptake," *African Journal of Biotechnology*, vol. 10, no. 31, pp. 5850-5855, April 2011.

[9] R. A. Tiamiyu, H. G. Ahmed, and A. S. Muhammad, "Effect of sources of organic manure on growth and yields of okra (*Abe;moschus esculentus* L.) in Sokoto, Nigeria," *Nigerian Journal of Basic & Applied Science*, vol. 20, no. 2, pp. 213-216, November 2012.

[10] M. M. Haque, G. N. M. Ilias, and A. H. Molla, "Impact of *Trichoderma*-enriched biofertilizer on the growth and yield of mustard (*Brassica rapa* L.) and tomato (*Solanum lycopersicon* Mill.)," *The Agriculturists*, vol. 10, no. 2, pp. 109-119, November 2012.

[11] L. L. M. A. Lopez, R. G. Reyes, and D. G. Alvinda, "Evaluation of two species of *Trichoderma* as compost activator and biocontrol agents," *Journal of Agricultural Technology*, vol. 11, no. 2, pp. 525-537, 2015.
[12] S. Goyal and S. S. Sindhu, "Composting of rice straw using different inocula and analysis of compost quality," *Microbiology Journal*, vol. 1, no. 4, pp. 126-138, September 2011.
[13] A. Q. Rajput, M. A. Khanzada, and S. Shahzas, "Effect of different substrates and carbon and nitrogen sources on growth and shelf life of *Trichoderma pseudokoningii*," *International Journal of Agriculture and Biology*, vol. 16, no. 5, pp. 893-898, March 2014.

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