Research article

Effect of Arbuscular Mycorrhizae on the Growth and Health of Eucalyptus Pellita Seedlings

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Abstract.
Nursery productivity is an important factor in plantation forest sustainability. Seedling quality contributes significantly to plantation health. Fertilization regimes and pest and disease control are critical. However, environmental concerns have arisen as a result of the use of chemical fertilizers and pesticides. The ideal is to manage nurseries in an environmentally friendly manner while maintaining productivity. The utilization of plant growth-promoting microbes (PGPM) is seen as one option in this effort. It has been demonstrated elsewhere that arbuscular mycorrhizae (AM) can improve the growth and health of host plants. In this study, the effect of two AM products on the growth and health of *Eucalyptus pellita* seedlings in reduced fertilizer regimes was investigated. The results showed that the AM products were unable to compensate for reduced fertilizer regimes (both to 50% and 25%), in terms of seedling height and diameter, in *E. pellita*. Further research is needed to determine whether using AM products can reduce the need for fertilizers and/or pesticides.

Keywords: Biomass, forest sustainability, nursery, plant growth promoting microbes

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1. Introduction

Nursery productivity is one key component in plantation forest sustainability. Establishment of a healthy plantation starts with the availability of high-quality tree stocks. Seedling quality contributes significantly to plantation health. In this perspective, proper management of nursery is very critical. This includes irrigation, fertilization regimes, pest and disease control, etc. The use of chemical fertilizers and pesticides has posed concerns related to environmental issues. It would therefore be more desirable to manage nursery in an environmentally friendly manner but with comparable productivity, with the ultimate goal of improving tree health in plantation forests. The nursery utilization of plant growth promoting microbes is seen as one of the options to this effort [1-10].

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Mycorrhizal fungi form symbiotic association with the root of most plant species [11]. Arbuscular mycorrhizae (AM) are endomycorrhizae that belong to the Zygomycete of order Glomales. Previously the term arbuscular mycorrhiza was designated to the symbiotic association formed by all fungi in Glomales. However, since not all of them form vesicles in the roots, it is now referred to as AM [12]. It has been demonstrated elsewhere that mycorrhizae, both arbuscular and ecto-, can improve health and growth performance of host plants [13-18]. This research aims to investigate the effectiveness of different AM products on the growth and health of *Eucalyptus pellita* tree stocks and possible reduction of fertilizer or/and pesticide regimes.

2. Methodology

This research was initiated in December 2020 at SMF Corporate R&D Nursery. The duration of this experiment was 90 days. *E. pellita* cuttings and two AM products, i.e. AM1 and AM2, were used in this trial. The experiment followed a randomized complete block design (RCBD) with 9 treatments and 3 replicates for each treatment as follows, (1) current nursery regimes of 100 % additional fertilizer recommendation and pesticides (SOP); (2) AM1 with 100 % additional fertilizer recommendation but without pesticides; (3) AM1 with 100 % additional fertilizer recommendation; (4) AM1 with only 50 % additional fertilizer recommendation; (5) AM1 with only 25 % additional fertilizer recommendation; (6) AM2 with 100 % additional fertilizer recommendation but without pesticides; (7) AM2 with 100 % additional fertilizer recommendation; (8) AM2 with only 50 % additional fertilizer recommendation; (9) AM2 with only 25 % additional fertilizer recommendation.

Experimental units were plots containing 4 trays with a total of 384 tubes/tree stocks. Inoculation was carried out by mixing the substrate containing AM spores with the growing media at setting (AM1) or applied 4 weeks after setting (AM2). Pesticide application considered the compatibility with the products, especially AM1. Variables assessed in this experiment were seedling height, root collar diameter, seedling biomass, foliar nutrient content, root-to-shoot ratio, and leaf disease incidence. Growth gains were calculated based on the obtained data. The data were analyzed using SAS Programs. Descriptive analysis was used to outline the growth and health data. ANOVA was used to indicate significant differences (p-value < 0.05), followed by further test using Multiple Comparison Tests to identify the significantly different treatments.

3. Result and Discussion

3.1. Growth Performance

It has been demonstrated elsewhere that AM can improve health and growth performance of host plants [13-15]. Research has also shown significant roles of AM in uptake of soil nutrients, especially of N and P, which can effectively promote the growth of host plants [19]. Growth performance of *E. pellita* in all treatments is presented in Table 1.

TABLE 1: Growth performance of *Eucalyptus pellita* at 90 days after planting.

Treatment*	N	Height (cm)		Root Collar Diameter (mm)	
		Mean**	**Std Dev.**	**Mean****	**Std Dev.**
SOP (100 % AF_P)	58	48.21a	4.79	4.76a	0.58
AM1_100 % AF_NP	57	46.93a	4.68	4.80a	0.73
AM1_100 % AF_P	57	49.21a	3.69	4.97a	0.63
AM1_50 % AF_P	59	40.72b	4.48	4.30b	0.58
AM1_25 % AF_P	60	33.47d	4.38	3.58cd	0.50
AM2_100 % AF_NP	57	47.31a	5.48	4.81a	0.69
AM2_100 % AF_P	59	49.68a	4.48	4.98a	0.60
AM2_50 % AF_P	59	36.59c	4.03	3.90c	0.50
AM2_25 % AF_P	58	31.74d	4.04	3.44d	0.40
R-Square		0.96		0.92	
p-value		<0.0001		<0.0001	
CV		4.00		4.93	

Note : *P: with pesticide; NP: without pesticide; AF: additional fertilizer. **Numbers in the same column followed by the same letter are not significantly different.

In Figure 1 below it can be seen that the growth (height and root collar diameter - RCD) trend of *E. pellita* decreased with fertilizer reduction. Based on the growth gains and distributions at 90 days after planting (DAP), the height and RCD of seedlings in AM1 or AM2 with 100 % additional fertilizer treatments were not significantly different from those in the SOP plots. Similarly, Figure 2 shows that the use of both AM products have not been able to compensate reduced fertilizer regimes (both to 50 % and 25 %) in term of seedling height and diameter. The growth of *E. pellita* seedlings in fertilizer reduction (to 50 % and 25 %) plots, even with the addition of AM, did not match that in the SOP plot. Therefore, the current full dose of additional fertilization (SOP) is considered the optimum regime for the best growth performance of *E. pellita* seedlings. Although it does not match SOP, AM1 performed better than AM2 when combined with reduced additional fertilization (both to 50 % and 25 %) for both height and diameter variables. One challenging treatment would be the combined application of the AM products, especially AM1, with reduction of the additional fertilizer regime to > 50 %.

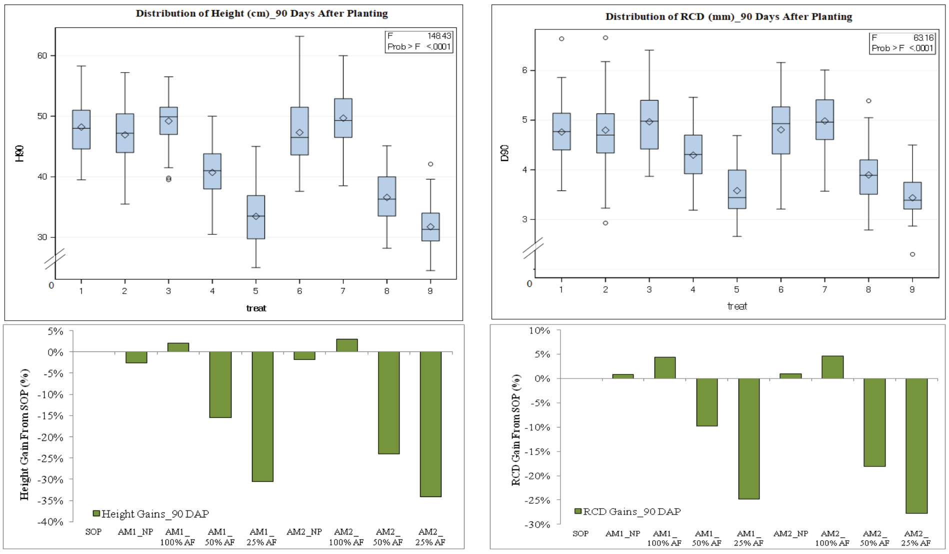

Figure 1: The distribution (top) and the gain (bottom) of *Eucalyptus pellita* height (left) and root collar diameter (RCD) (right).

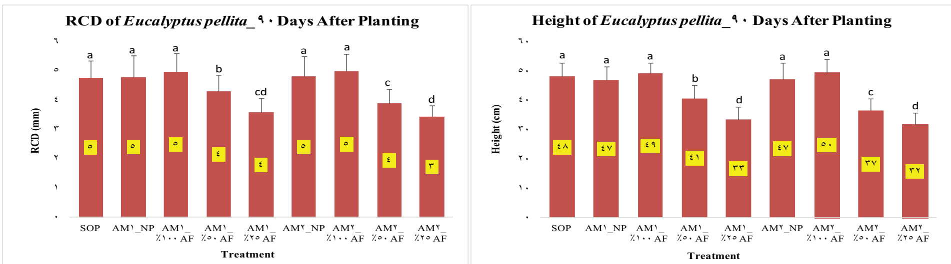

Figure 2: *Eucalyptus pellita* seedling height (left) and root collar diameter (RCD) (right) at 90 days after planting.

3.2. Biomass

The treatment of AM2 with 100 % additional fertilizer resulted in the highest biomass of *E. pellita* seedlings at 7.43 g. Other treatments involving the SOP regime were not significantly different, as presented in Table 2. In general, biomass tends to decrease with fertilizer reduction. The root-to-shoot (RS) ratio in all treatments was less than 1:5 with higher biomass proportions of leaves and stems than that of roots. RS ratio is an important index for assessing plant health and has received increased attention in the last decades as a sensitive indicator of plant stress induced by chemical or physical agents [20]. The low RS ratio might be caused by several factors including size and volume of the container, nutrient rich environment from additional fertilizer or AM application, and light intensity or other environment factors. Low volume containers (plastic pot, tube pot, paper pot) contribute to smaller nutrient reserve and limited root growth. The container size would affect the availability of nutrient to seedlings; thus, a constant and balanced supply of all essential nutrients must be ensured [21].

TABLE 2: Biomass and root-to-shoot ratio of *Eucalyptus pellita* seedlings at 90 days after planting.

Treatment*	Biomass (g)		Root-to-Shoot Ratio	
	Mean**	Std Dev.	Mean	Std Dev.
SOP (100 % AF_P)	6.78ab	1.15	0.17 (1:6)	0.03
AM1_100 % AF_NP	6.97ab	1.07	0.18 (1:6)	0.04
AM1_100 % AF_P	6.82ab	1.18	0.17 (1:6)	0.02
AM1_50 % AF_P	4.41bc	0.75	0.14 (1:7)	0.01
AM1_25 % AF_P	2.72c	0.66	0.14 (1:7)	0.02
AM2_100 % AF_NP	6.28ab	0.97	0.19 (1:5)	0.06
AM2_100 % AF_P	7.43a	0.74	0.16 (1:6)	0.02
AM2_50 % AF_P	3.27c	0.67	0.16 (1:6)	0.02
AM2_25 % AF_P	3.47c	0.96	0.15 (1:7)	0.07
R-Square	0.84			
p-value	<0.0001			
CV	17.34			

Note : *P: with pesticide; NP: without pesticide; AF: additional fertilizer. **Numbers in the same column followed by the same letter are not significantly different.

Plants with a higher proportion of roots can compete more effectively for soil nutrients, while those with a higher proportion of shoots can collect more light energy. When the plant is under stress (especially in reduced fertilizer plots), AM can significantly improve plant nutrient uptake and resistance to several abiotic stress factors [22]. Without the addition of AM or other microbes that help nutrient uptake, plants in nutrient-poor environments tend to allocate a higher proportion to roots [23].

3.3. Nutrient Accumulation

Fertilizer reduction will decrease nutrient intake and results in lower foliar nutrient content, as shown in Table 3. The seedlings treated with 100 % of additional fertilizer dose had higher amount of nutrients (N and P) accumulated in the leaves and the foliar content decreased with fertilizer reduction (both to 50 % and 25 % of the SOP). The nutrients absorbed by seedlings in the AM2 with 100 % additional fertilizer plots were slightly higher than those in the AM1 with 100 % additional fertilizer plots, but both did not match the nutrient uptake in the SOP plots.

AM have shown significant roles in the uptake of soil nutrients, especially of N and P, which can effectively promote the growth of host plants [6]. AM also increase allocation of shoot biomass through increased N and P redistribution to panicles, particularly under low fertilizer levels [24]. In this experiment, the seedlings receiving higher fertilizer doses resulted in higher nutrient accumulation. The use of both the AM products have not been able to compensate reduced fertilizer regimes (both to 50 % and 25 %) in term of nutrient accumulation in leaves. Thus, reducing fertilizer regimes to 50 % or 25 % is not recommended as it would decrease the nutrient intake critical for seedlings growth. Again, trials on combined application of the AM products with reduced fertilizer regimes to > 50 % are worth pursuing.

Table 3: Foliar nutrient content of *Eucalyptus pellita* at 90 days after planting.

Treatment*	**Total Analysis (ppm)**	
	N	**P**
SOP (100 % AF_P)	14420	1757.81
AM1_100 % AF_P	12410	2072.51
AM1_50 % AF_P	8820	1703.92
AM1_25 % AF_P	8400	1534.02
AM2_100 % AF_P	13860	2013.57
AM2_50 % AF_P	9870	1913.43
AM2_25 % AF_P	8190	1588.96

Note : *P: with pesticide; AF: additional fertilizer.

3.4. Plant Health

Leaf diseases caused by different pathogens have been frequently observed on *E. pellita* seedlings in nursery. As presented in Table 4, incidence of the leaf diseases at 90-day-old seedlings in plots with or without pesticide application was not statistically different. This might have been due to the fact that disease incidence was relatively low that the impact of both pest and AM application was not detected. It would have been interesting to compare the data with those in the treatment of nursery SOP but without application of both pesticides and AM products.

Table 4: Incidence of leaf diseases on *Eucalyptus pellita* seedlings at 90 days after planting.

Treatment*	**Incidence (%)***
SOP (100 % AF_P)	5.28ab
AM1_100 % AF_NP	5.10ab
AM1_100 % AF_P	5.05ab
AM2_100 % AF_NP	5.31a
AM2_100 % AF_P	4.65abc

Note : *P: with pesticide; NP: without pesticide; AF: additional fertilizer. **Numbers in the same column followed by the same letter are not significantly different.

4. Conclusion

The results of this study indicate that the application of the tested AM products is not able to compensate reduction of the current nursery SOP (both to 50 % and 25 %) in term of the growth performance of *E. pellita* tree stocks in nursery. A similar result trend is also observed for the parameters of seedling biomass and foliar nutrient content. The effect of both pesticides and AM products on the incidence of leaf diseases is not obvious.

To better elucidate the effect of the AM products, further investigation is required. One challenge is to explore the impacts of the products on the growth performance, biomass, and foliar nutrient content of the *E. pellita* tree stocks in nursery under the reduction of the current SOP fertilizer regime to > 50 %. Data on the incidence of leaf diseases in the treatment of nursery SOP but without application of both pesticides and AM products are also worth obtaining.

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References

[1] Gafur A. Endophytic *Trichoderma* as biocontrol agents of red root rot disease in tropical plantation forests. Paper presented at: Proceedings of the 6th PGPR International Conference; 2019 Aug 18–22; Tashkent, Uzbekistan.

[2] Gafur A. Development of biocontrol agents to manage major diseases of tropical plantation forests in Indonesia: A Review. Environmental Sciences Proceedings. 2020;3:1-7. https://doi.org/10.3390/IECF2020-07907

[3] Gafur A. Plant growth promoting microbes (PGPM) for the sustainability of tropical plantation forests in Indonesia. Academia Letters. 2021;1308:1-3. https://doi.org/10.20935/AL1308

[4] Gafur A, Naz R, Nosheen A, Sayyed RZ. Role of plant growth promoting microbes (PGPM) in managing soil-borne pathogens in Indonesian estate forests. In Mawar R, Sayyed RZ, Sharma SK, Sattiraju KS, editors. PGPM in Arid Ecology: Status and Prospects. 2022; Springer Nature, Singapore, in press.

[5] Hamid B, Zaman M, Farooq S et al. Bacterial plant biostimulants: A sustainable way towards improving growth, productivity, and health of crops. Sustainability. 2021;13(2856):1-24. ttps://doi.org/10.3390/su13052856

[6] Arora H, Sharma A, Sharma S et al. Pythium damping-off and root rot of *Capsicum annuum* L.: Impacts, diagnosis, and management. Microorganisms. 2021;9(823):1-17. https://doi.org/10.3390/microorganisms9040823

[7] Saboor A, Ali MA, Hussain S, El Enshasy HA et al. Zinc nutrition and arbuscular mycorrhizal symbiosis effects on maize (*Zea mays* L.) growth and productivity. Saudi Journal of Biological Sciences. 2021;28(11): 6339-6351. https://doi.org/10.1016/j.sjbs.2021.06.096

[8] Sarkar D, Sankar A, Devika OS et al. Optimizing nutrient use efficiency, productivity, energy use efficiency, and economics of red cabbage following mineral fertilization and biopriming with compatible rhizosphere microbes. Scientific Reports. 2021;11(15680):1-14. https://doi.org/10.1038/s41598-021-95092-6.

[9] Gafur A. Plant growth promoting microbes (PGPM) in the future management of Indonesian estate forests. 2022; KnE Publisher, in press.

[10] Syaffiary S Antonius S, Said D, Nugraha AK, Gafur A. Effect of organic fertilizer products on the growth and health of *Acacia crassicarpa* seedlings. 2022; KnE Publisher, in press.

[11] Bagyaraj DJ. Biological interaction with VA mycorrhizal fungi. Boca Raton: CRC Press Inc.; 1984.

[12] Morton B. Taxonomy of VA mycorrhiza fungi: Classification, nomenclature and identification. Mycotaxon. 1988;32:267-324.

[13] Dehne HW. Interaction between vesicular arbuscular mycorrhizal fungi and plant pathogens. Phytopathology. 1982;72:1115-1119.

[14] Faber BA, Zasoski RJ, Munns DN, Shackel K. A method for measuring hyphal nutrient and water uptake in mycorrhizal plants. Canadian Journal of Botany. 1991;69:87-94.

[15] Azcon-Agular C, Barea JM. Arbuscular mycorrhiza and biological control of soilborne plant pathogen – An overview of the mechanisms involved. Mycorrhiza. 1996;6:457-464.

[16] Gafur A, Schützendübel A, Langenfeld-Heyser R, Fritz E, Polle A. Compatible and incompetent *Paxillus involutus* isolates for ectomycorrhiza formation in vitro with poplar (*populus* x *canescens*) differ in H_2O_2 production. Plant Biology 2004;6:91–99.

[17] Gafur A, Schützendübel A, Polle A. Peroxidase activity in poplar inoculated with compatible and incompetent isolates of *Paxillus involutus*. Hayati Journal of Biological Sciences 2007;14:49–53.

[18] Langenfeld-Heyser R, Gao J, Ducic T et al. *Paxillus involutus* mycorrhiza attenuate NaCl-stress responses in the salt-sensitive hybrid poplar p*opulus* x *canescens*. Mycorrhiza. 2007;17:121–131.

[19] Smith SE, Jakobsen I, Grønlund M, Smith FA. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: Interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiology. 2011;156:1050–1057.

[20] Agathokleous E, Belz RG, Kitao M, Koike T, Calabrese EJ. Does the root to shoot ratio show a hormetic response to stress? An ecological and environmental perspective. Journal of Forestry Research. 2019;30:1569-1580.

[21] Landis TD, Tinus RW, McDonalds SE, Barnett JP. Agriculture handbook No. 674. Washington, DC: US Department of Agriculture Forest Service; 1989.

[22] Begum N, Qin C, Abass M et al. Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. Frontiers in Plant Science. 2019;10:1-15. https://doi.org/10.3389/fpls.2019.01068

[23] Tilman D. The resource-ratio hypothesis of plant succession. The American Naturalist. 1985;125(6):827-852.

[24] Zhang F, Jia-Dong HE, Qiu-Dan NI, Qiang-Sheng WU, Zou YN. Enhancement of drought tolerance in trifoliate orange by mycorrhiza: Changes in root sucrose and proline metabolisms. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2018;46(1):270-276. https://doi.org/10.15835/nbha46110983