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Potential of *Trichoderma* and AMF Mixture with Different Types of Fertiliser for *Durio zibethinus* Murray (Durian) and *Artocarpus heterophyllus* Lam. (Jackfruit) Growth

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ABSTRACT

Over the years, the co-inoculation of *Trichoderma* and arbuscular mycorrhizal fungi (AMF) with different types of fertiliser in a field condition has been understudied. This study explores the potential of *Trichoderma* and AMF mixture (T-AMF) with different types of fertiliser for plant growth with the objectives to: (i) analyse the growth of *Durio zibethinus* and *Artocarpus heterophyllus* fertilised with different types of fertiliser and inoculated with T-AMF and (ii) determine the optimal combination of organic and chemical fertiliser with T-AMF. A randomized complete block design was applied using seven treatments namely, 50 g organic fertiliser (OF) + T-AMF, 100 g OF + T-AMF, 50 g chemical fertiliser (CF) + T-AMF, 100 g CF + T-AMF, 100 g OF, 100 g CF, and control (without fertiliser and T-AMF). The growth analysis of *D. zibethinus* showed the highest mean height was at 100 g OF with T-AMF, stem diameter at 100 g CF with T-AMF, and the number of leaves at 50 g CF with T-AMF. For *A. heterophyllus*, the highest mean height and stem diameter was at 50 g OF with T-AMF and the number of leaves at 100 g OF with T-AMF. The optimal combination of fertiliser with T-AMF for *D. zibethinus*' height was 100 g OF and number of leaves was 50 g CF. The optimal combination of fertiliser with T-AMF for *A. heterophyllus*' height and stem diameter was 50 g OF. The optimal combination for its number of leaves was 100 g OF with T-AMF. This concludes that the application of fertiliser with T-AMF reacted differently to plant species and their growth parameters. The co-inoculation of *Trichoderma* and AMF may present a cheaper and sustainable alternative, especially when the planting scale is huge.

Keywords: Fertiliser, growth, mycorrhizal fungi, sustainable, *Trichoderma*

INTRODUCTION

Natural soil originally consists of various beneficial mycorrhizal fungi (Barman *et al*., 2016). There are seven known types of mycorrhizae namely, endomycorrhiza, ectomycorrhiza, ectendomycorrhiza, ericoid mycorrhiza, arbutoid mycorrhiza, monotropid mycorrhiza, and orchid mycorrhiza. Among these, endomycorrhiza or also known as arbuscular mycorrhizal fungi (AMF) has gained substantial interest for sustainable crop improvement (Begum *et al*., 2019). AMF are the important endosymbionts in plant yield and ecosystem functioning. Among the AMF genera, *Glomus* was found beneficial in accelerating plant growth (Zhang *et al*., 2018). For example, *Glomus intraradices* was found to be a medium of metal transporters i.e., zinc, copper, and iron between the soil and plant (Berruti *et al*., 2016). The metallic compounds are essential for plant growth and survival especially in extreme conditions (Xie *et al*., 2019).

Trichoderma also have shown big potentials as biofertilisers in reforestation (Karličić *et al.*, 2016). These have been demonstrated in studies by Babu *et al.* (2014), Halifu *et al.* (2019), and dos Santos *et al.* (2020). Major *Trichoderma* strains such as *T. koningiopsis*, *T. asperelloides*, *T. atroviride*, *T. virens*, and *T. parareesei* were found contributing to plant growth (Rubio *et al*., 2014; Haddad *et al*., 2017). The *Trichoderma* three major modes of action i.e., mycoparasitism, antibiosis, and competition play important roles in the plant-soil mechanism, thus influencing the plant growth (Dehariya *et al.*, 2015; Joshi *et al.*, 2016; Duc *et al.*, 2017).

A prolonged drought, extreme temperature, and poorly drained soil pose threats to the tree's development (Orwa *et al.*, 2009; Goswami & Chacrabati, 2015; Ketsa *et al.,* 2020). Thus, trees often need catalyser such as fertilisers to promote growth. Organic fertiliser (OF) is preferably used compared to chemical fertiliser (CF) due to environmental awareness. The *Trichoderma*-enriched biofertilisers could serve as prospective replacements for CF and pesticide and indirectly help in conserving the natural environment (Patil & Solanki, 2016; Barua *et al.*, 2018).

Over the years, the co-inoculation of *Trichoderma* sp. and AMF with different types of fertiliser in a field condition has been understudied (Igiehon & Babalola, 2017). The lack of studies justified the need for a field study to imitate the real reforestation site conditions with uncontrolled environmental conditions i.e., weather, temperature, and humidity. *Durio zibethinus* (durian) and *Artocarpus heterophyllus* (jackfruit) were selected for planting due to their high economic value, functional value, and seeds availability and abundance as compared to the natural forest species such as Dipterocarps. These criteria made them suitable for reforestation (Danarto & Budiharta, 2019).

This study explores the potential of T-AMF with different types of fertiliser for plant growth. The objectives are to: (i) analyse the growth of *D. zibethinus* and *A. heterophyllus* fertilized with different types of fertiliser and inoculated with T-AMF and (ii) determine the optimal combination of organic and chemical fertiliser with T-AMF.

MATERIALS AND METHODS

Study Area

The study was conducted at the UNIMAS Real Living Lab, Sarawak, Malaysia. The size of the greenhouse was approximately 4 m x 11 m (width x length), and it accommodated 140 potted plants with 50 cm distance apart. The greenhouse was enclosed with polyethylene shade cloth B70. It was built in an open area without the environmental control throughout the study period. The average temperature and humidity are 32.8 °C and 69.2%, respectively. This may represent the high temperature and humidity level which often are the case in deforested or degraded areas. The minimum and maximum temperature are 25.6 °C and 40.3 °C respectively. On the other hand, the minimum and maximum humidity are 50.5% and 90.4%, respectively.

Experimental Method

The study was carried out for a duration of 16 months. Observation was carried out over a period of 12 months as the plant growth was rapid during this early period as the effect of soil manipulation toward plant growth is clearly visible and detectable. The soil was first mixed with sand and compost with 2:1:1 respective ratio and then treated with fungicide (50% captan). The fungicide treatment works by interrupting the key process of the existing fungus life cycle and killing the fungus-causing-diseases in the soil (Strid *et al.*, 2018). Captan has shown no to little toxicity effect on beneficial AMF species such as *Glomus intraradices* (Shashtri *et al*., 2020) and *Trichoderma* strains such as *T. harzianum* (McLean *et al*., 2001). The active ingredient of captan half-life in soil ranges from one to 24 days.

The *D. zibethinus* and *A. heterophyllus* seeds were then sown in small polyethylene bags (15.24 cm x 22.86 cm) and early fertilisation was carried out using fermented chicken dung plus humic acid fertiliser once a month. After three months and the seedlings have reached the height of approximately 30 cm, the seedlings were transplanted into bigger polyethylene bags (14 cm x 14 cm), with a ratio of 2:1 soil and sand. A total of 140 replicates, with 10 replicates per plant per treatment has been used. The initial seedlings' growth, i.e., height, stem diameter, and number of leaves and environmental factors were recorded. A randomised complete block design (RCBD) was then used to rearrange the plants according to their respective treatments. The treatments were then carried out as shown in Table 1.

The Nutri-Life Platform product, which is commercially sold by Nutri-Tech Solutions Pty Ltd. is used as the T-AMF treatment in this study. The product is a mixture of four beneficial AMF species (*Glomus intraradices*, *G. etunicatum*, *G. aggregatum*, and *G. mosseae*) and *Trichoderma lignorum* strain which was then dissolved in the water at the rate of 100 g per 5 L of water, as recommended by the manufacturer. Approximately 30 mL of this mixture were sprayed into the soil of each polybag. The fertilisation was then carried out after two months and applied twice for one year. The seedlings' growth then was recorded, one month after the treatment until the 12th months.

Table 1. Treatments applied to promote *Durio zibethinus* and *Artocarpus heterophyllus* growth

Symbols	Treatments
A	50 g OF + T-AMF
B	100 g OF + T-AMF
C	50 g CF + T-AMF
D	100 g CF + T-AMF
E	100 g OF
F	100 g CF
G	Control (without fertiliser and T-AMF)

Notes: OF – Organic fertiliser, CF – Chemical fertiliser, T-AMF – *Trichoderma* and arbuscular mycorrhizal fungi

Analysis of Data

Initially, the mean and standard deviation (DV) analysis of height, stem diameter and number of leaves of *D. zibethinus* and *A. heterophyllus* against seven treatments were conducted to describe the mean growth of both plant species resulting from different treatments. Factorial ANOVA analysis was conducted to explore the interaction effect between the treatments and plant species on growth rate i.e., height, stem diameter, and number of leaves. The interaction effect from the factorial ANOVA analysis then shows whether the effect of one of the independent variables on the dependent variable is the same for all values of the other independent variable, and vice versa. The Eta squared value then measures the effect size of the total variance in a dependent variable linked with the association of different groups defined by an independent variable. Separate ANOVA analysis for both plant species then was conducted to determine the effect of treatments on the plant growth. Finally, Tukey post-hoc analysis was conducted to determine the optimal combination of organic and chemical fertiliser with T-AMF for *D. zibethinus* and *A. heterophyllus* growth.

RESULTS AND DISCUSSION

Durio zibethinus and *Artocarpus heterophyllus* Growth

The mean growth analysis of both *D. zibethinus* and *A. heterophyllus* showed that there was a difference in mean height when treated with different fertiliser types and inoculated with T-AMF (Table 2). The highest mean height of *D. zibethinus* was at 100 g OF with T-AMF (Mean = 65.52, SD = 2.58). However, the mean height of *D. zibethinus* at 50 g OF with T-AMF was less than 100 g OF with T-AMF (Mean = 61.52, SD = 6.63). The mean *D. zibethinus* height difference was four cm.

Contrastedly, the highest mean of *A. heterophyllus* height was at 50 g OF with T-AMF (Mean = 113.77, SD = 35.51). The mean height of *A. heterophyllus* at 100 g OF with T-AMF was 102.77 (SD = 31.09). The mean height difference was 11 cm.

The results may indicate that *D. zibethinus* requires more OF as compared to *A. heterophyllus* for maximum height growth. The difference in mean height between plants treated with OF and CF, even though both have been inoculated with T-AMF also may indicates that OF promotes plants' height more than CF. However, the statistical significance of these treatments toward both plants will be further discussed in the next sub-section.

On the contrary, the *D. zibethinus*' mean stem diameter was at its biggest when treated with 100 g CF with T-AMF (Mean = 7.15, SD = 2.85) [Table 2]. There was only a small mean difference between the 100 g CF with T-AMF and 50 g CF with T-AMF (Mean difference = 0.56).

The mean stem diameter of *A. heterophyllus* was at its biggest when treated with 50 g OF with T-AMF (Mean = 8.98, SD = 3.65). Similar to the *D. zibethinus*, the mean difference between the 100 g CF with T-AMF and 50 g CF with T-AMF was also small (Mean difference = 0.69).

Parallel to the *A. heterophyllus*' mean height, the result may indicate that *A. heterophyllus* requires less amount of OF after being inoculated with T-AMF for maximum height growth. On the contrary, *D. zibethinus* needs more CF for maximum diameter growth even though it is inoculated with T-AMF.

The analysis on the number of leaves (Table 2) showed that the highest mean for *D. zibethinus* and *A. heterophyllus* were at 50 g CF with T-AMF (Mean = 37.00, SD = 27.06) and 100 g OF with T-AMF (Mean = 26.75, SD = 12.32) respectively. In contrast to the previous result, the mean number of leaves of *D. zibethinus* was at its highest when treated with T-AMF and less CF (Mean difference = 3.25). Yet, *A. heterophyllus* needs more OF even after being inoculated for maximum number of leaves (Mean difference = 3.08).

The Optimal Combination of Fertiliser with T-AMF for *Durio zibethinus* and *Artocarpus heterophyllus*

The factorial ANOVA analysis (Table 3) showed that there were significant differences between treatments and plant species, and height, $F_{(6, 154)} = 12.44$, $p < 0.05$; stem diameter, $F_{(6, 154)} = 5.57$, $p < 0.05$, and number of leaves, $F_{(6, 154)} = 9.51$, $p < 0.05$. The effect size of treatments and plant species for all growth parameters is large. However, the effect size for height (Eta squared = 0.32) was greater than the stem diameter (Eta squared = 0.18) and number of leaves (Eta squared = 0.27).

The ANOVA result (Table 3) then showed that there were significant differences between treatments and height, $F_{(6, 77)} = 4.26$, $p < 0.05$; and number of leaves, $F_{(6, 77)} = 2.78$, $p = 0.02$ of *D. zibethinus*. Both the effect size of treatments for height (Eta squared = 0.25) and number of leaves (Eta squared = 0.18) was large. However, the effect size for height was greater than the number of leaves. This may indicate that the treatments have bigger effect on the *D. zibethinus'* height.

The ANOVA result for *A. heterophyllus* showed that there were significant differences between treatments and all the growth parameters i.e., height, $F_{(6, 77)} = 16.08$, $p < 0.05$; stem diameter, $F_{(6, 77)} = 6.97$, $p < 0.05$; and number of leaves, $F_{(6, 77)} = 17.87$, $p < 0.05$. All the effect size of treatments for height (Eta squared = 0.56), stem diameter (Eta squared = 0.35), and number of leaves (Eta squared = 0.58) was large. However, the effect size for number of leaves was greater than the height and stem diameter. This may indicate that the treatments have bigger effect on the *A. heterophyllus*' number of leaves.

Tukey post-hoc analysis (Table S1) showed that there were two significant differences between the treatments and height of *D. zibethinus* i.e., 100 g OF + T-AMF and 100 g CF + T-AMF ($p < 0.05$); and control (without fertiliser and T-AMF) and 100 g CF + T-AMF ($p < 0.05$). However, the biggest mean difference in height was between 100 g OF + T-AMF and 100 g CF + T-AMF (Mean difference = 13.53, SE = 3.31). It was also found that there is a significant difference between the treatments and number of leaves namely, 50 g CF + T-AMF and 100 g CF + T-AMF ($p = 0.02$). The mean difference in the number of leaves is quite big (Mean difference = 22.75, SE = 6.86).

There were 12 significant differences between the treatments and height of *A. heterophyllus*. The biggest mean differences were between 50 g OF + T-AMF and 100 g CF (Mean difference = 78.02, SE = 10.63). Additionally, there were eight significant differences between the treatments and stem diameter, with the biggest mean differences between 50 g OF + T-AMF and 100 g CF (Mean difference = 4.92, SE = 1.06). Finally, there were 11 significant differences between the treatments and number of leaves, with the biggest mean differences between 100 g OF + T-AMF and 100 g CF (Mean difference = 23.50, SE = 3.28).

The result showed that plant species and their growth parameters i.e., height, stem diameter and number of leaves, reacted differently to the treatments. In particular, the height of *D. zibethinus* reacted more positively to full amount of OF with T-AMF than the full amount of CF with T-AMF. However, the number of leaves only reacted positively to half of the amount of CF with T-AMF than the full amount of CF with T-AMF.

Table 2. Mean growth of *Durio zibethinus* and *Artocarpus heterophyllus*

Variables			Mean	Std. Deviation	N
Height	*Durio zibethinus*	A	61.52	6.63	12
		B	65.52	2.58	12
		C	60.67	12.37	12
		D	51.98	10.68	12
		E	59.13	4.37	12
		F	55.68	10.43	12
		G	64.84	3.99	12
	Artocarpus heterophyllus	A	113.77	35.31	12
		B	102.77	31.09	12
		C	48.53	8.41	12
		D	68.39	24.82	12
		E	103.43	34.41	12
		F	35.75	6.98	12
		G	92.78	24.75	12
Stem diameter	*Durio zibethinus*	A	6.22	0.84	12
		B	5.02	0.64	12
		C	6.59	3.30	12
		D	7.15	2.85	12
		E	6.60	1.06	12
		F	5.58	2.43	12
		G	5.75	1.01	12
	Artocarpus heterophyllus	A	8.98	3.65	12
		B	8.29	3.25	12
		C	4.26	0.86	12
		D	6.07	2.38	12
		E	7.89	3.08	12
		F	4.06	1.06	12
		G	7.66	2.44	12
Number of leaves	*Durio zibethinus*	A	18.00	9.00	12
		B	14.00	6.00	12
		C	37.00	27.00	12
		D	34.00	27.00	12
		E	23.00	11.00	12
		F	23.00	15.00	12
		G	21.00	7.00	12
	Artocarpus heterophyllus	A	24.00	11.00	12
		B	27.00	12.00	12
		C	4.00	1.00	12
		D	12.00	7.00	12
		E	24.00	10.00	12
		F	3.00	1.00	12
		G	20.00	7.00	12

Note: A - 50 g OF + T-AMF, B - 100 g OF + T-AMF, C - 50 g CF + T-AMF, D - 100 g CF + T-AMF, E - 100 g OF, F - 100 g CF, G - Control (without fertiliser and T-AMF), OF – Organic fertiliser, CF – Chemical fertiliser, T-AMF – *Trichoderma* and arbuscular mycorrhizal fungi

These are different from the *A. heterophyllus* results. Both the height and stem diameter of *A. heterophyllus* reacted more positively to half of the amount of OF with T-AMF than the full amount of CF. The number of leaves then reacted positively to full amount of OF with T-

AMF than full amount of CF. These results may indicate the possibilities to apply T-AMF and minimize the dependency on CF in the plantation sectors.

Previous study by Omomowo *et al*. (2018) also found that the co-inoculation of *G. versiforme* and *T. harzianum* has significantly affected the cowpea growth in Nigeria. Castilo *et al*. (2019) in the Philippines also found a similar result in which the co-inoculation of *Glomus* spp. and *T. harzianum* in the banana plantation field has increased the height, diameter, and root weight of the seedlings as compared to the non-inoculated seedlings. Another study in Iraq also discovered that the co-inoculation of *G. mosseae* and *T. harzianum* improved the fresh and dry weight root of cucumber (Matrood *et al*., 2020). The positive outcomes of the co-inoculation of T-AMF are the results of T-AMF positive interactions (Szczałba *et al*., 2019).

Duaja *et al*. (2019) in a different study in Indonesia also found that the inoculation of *Glomus* sp. with only 75% of the usual recommended amount of phosphorus fertiliser yields a better oil palm growth. Gao *et al*. (2020) then discovered that the co-inoculation of AMF, growth-promoting bacteria, and organic fertiliser can boost the maize growth and yield. These shows the potential of AMF with fertiliser for maximum plant growth.

Nieto-Jacobo *et al.* (2017) and Szczałba *et al*. (2019) suggested that the mechanisms of plant growth promotion by T-AMF vary and are greatly influenced by environmental conditions. The inoculation of *T. viride* and *Funneliformis mosseae* in a phenanthrene-spiked soil has resulted in soil neutralization and lessened the oxidative damage towards the wheat planted (Lagos *et al*., 2021). However, the co-inoculation of T-AMF may also results in no net benefits as shown in Ważny *et al*. (2018). It was found that the co-inoculation of *T. asperellum* and *Rhizoglomus intraradices* in a toxic metal-polluted areas did not produce any biomass yield for *Lactuca serriola* (wild lettuce). These contradicting results may require further studies on the compatibility of T-AMF mixture in a different setting.

Table 3. The interaction impact between treatments and plant species on height, stem diameter and number of leaves

Factors	Dependent variable		Sum of squares	df	Mean square	F	Significance value ($p < 0.05$)	Effect size (Eta squared)
Treatment and plant species	Height		27751.34	6, 154	4625.22	12.44	0.00	0.32 (large)
	Stem diameter		179.28	6, 154	29.88	5.57	0.00	0.18 (large)
	Number of leaves		9887.81	6, 154	1647.97	9.51	0.00	0.27 (large)
Durio zibethinus	Height	Between groups	1683.52	6	280.59	4.26	0.00	0.25 (large)
		Within groups	5076.57	77	65.93			
		Total	6760.09	83				
	Stem diameter	Between groups	38.02	6	6.34	1.57	0.17	0.11 (medium)
		Within groups	310.27	77	4.03			
		Total	348.29	83				
	Number of leaves	Between groups	4714.98	6	785.83	2.78	0.02	0.18 (large)
		Within groups	21731.92	77	282.23			
		Total	26446.89	83	280.59			
Artocarpus heterophyllus	Height	Between groups	65390.74	6	10898.46	16.08	0.00	0.56 (large)
		Within groups	52200.66	77	677.93			
		Total	117591.41	83				
	Stem diameter	Between groups	280.27	6	46.71	6.97	0.00	0.35 (large)
		Within groups	515.78	77	6.70			
		Total	796.04	83				
	Number of leaves	Between groups	6901.79	6	1150.30	17.87	0.00	0.58 (large)
		Within groups	4956.25	77	64.37			
		Total	11858.04	83				

* p-value is significant at 0.05 level

CONCLUSION

This study explores the potential of T-AMF as growth stimulator agents. The first objective which was to analyse the growth of *D. zibethinus* and *A. heterophyllus* fertilized with different types of fertiliser and inoculated with T-AMF has shown that the height of *D. zibethinus* was at its highest when treated with 100 g OF with T-AMF. The stem diameter was at its biggest when treated with 100 g CF with T-AMF. The number of leaves of *D. zibethinus* was at its highest when treated with 50 g CF with T-AMF. For *A. heterophyllus*, the height and stem diameter were at their highest when treated with 50 g OF with T-AMF. The number of leaves was at its highest when treated with 100 g OF with T-AMF. Finally, the second objective which is to determine the optimal combination of organic and chemical fertiliser with T-AMF found that the optimal combination of fertiliser with T-AMF for *D. zibethinus*' height was 100 g OF with T-AMF and number of leaves was 50 g CF with T-AMF. The optimal combination of fertiliser with T-AMF for *A. heterophyllus* height and stem diameter was 50 g OF with T-AMF. However, the optimal combination for its number of leaves was 100 g OF with T-AMF. This concludes that the application of fertiliser with T-AMF reacted differently to plant species and their growth parameters. The co-inoculation of *Trichoderma* and AMF may present a cheaper and sustainable alternative, especially when the planting scale is huge.

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