Growth response of cocoa seedlings (*Theobroma cacao* L.) ICCRI 06 clone with application of manure and *Trichoderma* sp.

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ABSTRACT

The growth of cocoa seedlings (*Theobroma cacao* L.) is influenced by the use of the type of planting medium and the presence of supporting microorganisms, such as the *Trichoderma* sp. This research aims to determine the effect of giving *Trichoderma* sp. and manure on the growth of cocoa seedlings. The research used a factorial randomized block design (RBD) method consisting of 9 treatments which were repeated 3 times, so there were 27 experimental units. Factor 1 is chicken manure by 200, 300 and 400 g/polybag, factor 2 is *Trichoderma* sp. with different level of mixing. The parameters observed were plant height, number of leaves, stem diameter, wet weight of the crown, wet weight of the roots, dry weight of the crown, dry weight of the roots. The research results showed that M3 (400 g/polybag) gave the best results for the parameters of stem diameter, crown wet weight, root wet weight and root dry weight of cocoa plants. Giving *Trichoderma* sp. at a level of 10 mL L^{-1} gave the best results for stem diameter, root wet weight and root dry weight. The research results provide a positive contribution to the application of a combination of chicken manure (M1:200 g/polybag) with *Trichoderma* sp. (P2: 10 mL L^{-1}) on stem diameter and root wet weight.

Keywords: biofertilizer, chicken manure, cocoa seeds, dosage.

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a potential plantation crop commodity to support the economy in Indonesia. According to 2019 Central Statistics Agency data, cocoa productivity in 2019 was able to produce 243,000 tons. Naturally, a variety of factors affect the export of goods to the global (international) market. In the case of cocoa beans, these factors include production volume, local and international prices, and currency rates (Putri & Prihtanti, 2020). The Directorate General of Plantations, Ministry of Agriculture (2023) noted that although there was a decrease in the area of cocoa plantations from 2022 to 2023, namely from 1,442 thousand hectares to 1,389 thousand hectares in 2023, Indonesia's cocoa production reached 692,198 tons.

Significant and sustainable production results cannot be separated from cultivation activities in accordance with GAP *(Good Agriculture Practice)*. Good cocoa breeding techniques are one of the important a*s*pects in cocoa cultivation. The goal is to produce good, quality ready-to-plant seeds that can later produce maximum production. Failure in breeding activities must be suppressed to produce quality seeds. Some of the contributing factors are the use of poor plant materials, less than optimal cultivation technology, plant age and problems with pest and disease attacks. Unhealthy seeds will have a major impact on the productivity and quality of cocoa beans. The low quality of cocoa beans can be overcome by overcoming the problem of low quality cocoa beans through sanitation, pest control, fertilization, pruning, and selecting seed sources with superior clones (Wahyuni & Ndewes, 2023). Cocoa seeds have an important role in farming, because this plant has a long economic life of up to 37 years. Selecting the right seeds is the key to farmer success (Dewi et al., 2023).

The use of superior clones, such as ICCRI 06 clone, in breeding can help overcome reduced production due to disease attacks. The ICCRI 06 clone, the result of crossing Cocoa TSH 858 and KW 162, was proven to be very resistant to two types of detrimental diseases, namely fruit rot and Vascular Streak Dieback (VSD) (Setiawan et al., 2020). Intensive maintenance during the nursery phase can improve the quality of seedlings, one of which is by using appropriate organic growing media (Falieza et al., 2022). The optimal planting medium is one that is able to provide sufficient water and nutrient requirements for the growth of plant seeds. This can be found in soil with good air circulation that has a sturdy aggregate structure, optimal water retention capacity, and adequate root *s*pace (Ahmad et al., 2022).

Using different planting media compositions can affect the growth of cocoa seedlings (Nugroho et al., 2021). Fertilizer from chicken manure, which contains relatively high levels of nutrients, not only acts as a provider of additional nutrients, but is also useful for improving soil structure and increasing the activity of microorganisms (Setiawan, 2022). Analysis shows that chicken manure has an N content of 1.31%, P 1.68%, and K 2.43% (Tarigan et al., 2014). In horticulture such as shallot, the average plant height at 14 and 35 days of planting, tuber diameter, average tuber wet weight per hill, and average tuber dry weight per hill were all significantly impacted by the independent influence of chicken manure.

One strategy for optimizing the use of organic materials in planting media, such as compost, is to add microbes such as the *Trichoderma* sp. (Siswadi et al., 2023).

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There needs to be a combination of environmentally friendly science, one of which is using beneficial microorganisms (Dinata et al., 2021). *Trichoderma* is one of the beneficial fungi that can inhibit the pathogens (Dinata, 2023; Dinata et al., 2023). This not only has antagonistic properties against disease but also acts as a decomposer of organic matter in the soil *Trichoderma* sp. is one of the types of fungi that are found in almost all types of soil and in various habitats which is one of the types of antagonism fungi that can be used as a biological agent controlling soil pathogens. This beneficial fungi can multiply quickly in the root area of plants (Gunawaty et al., 2014). *Trichoderma* sp. is a saprophytic soil microorganism that naturally attacks pathogenic fungi and is beneficial for plants (Inayati et al., 2020; Tyśkiewicz et al., 2022). *Trichoderma* sp. helps speed up the process of breaking down micro and macro nutrients that are really needed by plants (Isnaini et al., 2021). Many studies have shown that *Trichoderma* can be combined with manure such as goat manure (Fatur et al., 2023).

This research will contribute to supporting sustainable agriculture, namely by utilizing chicken manure waste with control using biological agents which is an option that needs to be developed, because it is relatively cheap and easy to do, and environmentally friendly. The development of cocoa seedlings and other commodity, the utilization of local resources is a priority in order to minimize the cost of crop production. Abundant local resources in agricultural areas include the availability of natural materials in the form of livestock waste. Livestock waste is processed into manure. In this case, the utilization of *Trichoderma* sp. can provide additional nutrients, growth regulator compounds for plants (hormonal), and increase soil fertility biologically. Therefore, it is necessary to study the effect of *Trichoderma* sp. combined with chicken manure on the growth and production of cocoa seedlings (*Theobroma cacao* L.).

MATERIALS AND METHODS

This research was carried out from August to November 2023 located at the Research Land of Politeknik Negeri Jember in -8.158846618712836, 113.72312636544673. The tools used are hoes, gembors, machetes, digital scales, buckets, measuring tapes, ovens, rulers, measuring cups, pruning scissors, cutters, label paper, bamboo, seedling tubs, calipers, stationery, signs or boards, and cameras. The materials used are ICCRI 06 clone cocoa seeds from Puslitkoka, *Trichoderma* sp., polybag size 25 x 30 cm, paranet, top soil, chicken manure, fungicide (furadan and dhitane).

The implementation of this research includes preparing the nursery by making a nursery shade, preparing planting media in the form of sand: top soil: chicken manure, preparing cocoa seeds from Puslitkoka, then sowing the seeds for 2 weeks, which are then trans*p*lanted in the form of seeds into polybags, application *Trichoderma* sp. for 5 times, and maintenance of cocoa seedlings.

A. Preparation of Nursery Sites

The first step to take is to prepare a suitable nursery location. The land at the nursery location is leveled and cleaned of weeds and plant roots using a hoe. The beds are made with a width of 1-1.2 m and a length of 2 m and are equipped with forks with a height of 120 cm on the west side and 180 cm on the east side and a paranet is added.

B. Preparation of Planting Media

The planting media used are top soil and chicken manure. The planting medium is sieved and put into each polybag measuring 25 cm x 30 cm with 2 kg top soil. Chicken manure is appropriately weighed according to the treatment, namely, M1: 200 grams/polybag, M2: 300 grams/polybag and M3: 400 grams/polybag. Top soil in polybags is mixed with chicken manure according to the treatment. The planting media was mixed one by one in polybags and furadan was added.

C. Preparation of Cocoa Seeds

ICCRI 06 clone cocoa seeds come from Puslitkoka with the criteria that the seeds are healthy, uniform, large in size, not attacked by pests and diseases, and the skin is not injured.

D. Seed Nursery

The selected cocoa seeds are soaked in 5 grams/l dhitane before sowing. Seeds are planted in finely sifted sand planting medium with a height of 15 cm in a seedbed with the cotyledons not too deep. Next, the surface of the polybag is covered with pieces of straw on top of the seeds that have been immersed and watered every morning and evening.

C. Transplanting Cocoa Seeds

Cocoa seeds that have germinated 2 weeks after sowing are transferred from the seedling tub to polybags with 2/3 of the *sp*routs immersed in the media. Next, rinse with water.

This activity was prepared using a Factorial Randomized Block Design (RAK). The first factor is chicken manure with 3 treatment doses and the second factor is administration *Trichoderma* sp. with 3 treatment doses. This activity used 9 treatments and 3 repetitions to obtain 27 units. Each unit contains 4 plant samples so that the required seeds are 108 seeds. Research data was analyzed using Anova, if it showed significant differences, it was continued with the DMRT test at the 5% level.

Factor 1 is Chicken Manure:
M1: 200 g/polybag
M2: 300 g/polybag
M3: 400 g/polybag
Factor 2 is *Trichoderma* sp.:
P1: 5 ml/l water with 150 ml mixing
P2: 10 ml/l water with 150 ml mixing
P3: 15 ml/l water with 150 ml mixing
Observation parameters observed at 84 days after planting (DAP) include plant height (cm), number of leaves (leaves), stem diameter (mm), root wet weight (g), root dry weight (g), canopy wet weight (g) and canopy dry weight (g).

RESULTS AND DISCUSSION

The results of variance analysis show that there is a significant influence on several parameters on the provision of chicken manure and *Trichoderma* sp. namely the parameters of stem diameter, crown wet weight, root wet weight, crown dry weight, root dry weight. Meanwhile, the real interaction in the treatment combination was in the parameters of stem diameter and root wet weight (Table 1).

Based on the graphic image of the average height of plants aged 84 DAP, it shows that the M3P2 treatment had the highest average growth, namely 30.5 cm and the lowest average value was obtained in the M1P3 treatment, namely 27.3 cm. However, the variance results show that the use of chicken manure and *Trichoderma* sp. has no significant effect on plant height. The nutrient element phosphorus is needed by plants for stem formation and helps stimulate plant vegetative growth such as stem circumference, height and increase in the number of leaves (Setiawan, 2023). The element P (phosphorus) content in chicken manure does not have a significant effect in stimulating vegetative growth, the content is too small so it is not sufficient for the growth needs of cocoa plants.

Plant height of cocoa

One of the keys to the sustainable growth of cocoa plants lies in the leaves. The part of the plant that functions as the location for the photosynthesis process to produce the food needed by the plant itself and as a nutritional reserve is the leaf. The chlorophyll content in leaves is key in the process of plant photosynthesis. The more leaves on the cocoa plant, the higher the photosynthesis results, which in turn, photosynthesis can support optimal growth of the cocoa plant.

The graph of the average number of leaves at 84 DAP in Figure 2 shows that the M3P1 treatment showed the highest average value of 29.10 pieces. Meanwhile, the M2P1 treatment showed the lowest average value of 24.33 strands. This can be caused by using chicken manure doses that are too small and concentrated *Trichoderma* sp. which is too low. Lack of nutrients N, P, K, Mg, S, and Ca can have a negative impact on plant growth, because these nutrients are needed for plant growth and development. The factor of balanced nutrient availability determines plant growth and production (Siahaan, 2022). Photosynthesis from non-leaf parts and shading by non-leaf tissue can also influence the use of sunlight by the canopy of cultivated plants.

Stem diameter of cocoa plant

This parameter shows that the use of chicken manure and *Trichoderma* sp. shows a very significant effect. Giving with levels *Trichoderma* sp. too high does not have a real effect on the stem diameter. This is shown in Table 2 that the highest level of application produces the smallest average stem diameter. Giving *Trichoderma* sp. able to increase the diameter of the stem to a greater extent compared to those not given *Trichoderma* sp. This can happen because *Trichoderma* is able to suppress the growth of pathogens in rhizosfer roots, so that nutrient absorption can be optimal (Yusuf and Firsandi, 2021). *Trichoderma* sp. produces decomposing enzymes that can break down organic material, thus releasing nutrients bound in complex compounds to become available, e*sp*ecially the elements N, P, and S.

Number of leaves of cocoa plant

In the M3 treatment which used chicken manure at a dose of 400 grams, the plant stems had the largest size with an average of 7.47 mm. Meanwhile, the M2 and M1 treatments had means that were not significantly different, this shows that the use of chicken manure at higher doses had a very significantly different effect. The application of chicken manure to the planting medium shows a very significant value, indicating that the cocoa seedlings can develop and grow well. A good planting medium is a planting medium that is able to provide conditions where the roots are able to explore more widely to absorb nutrients in the soil.

Table 1. Summary of Anova Results Of Plant Height Parameters, Number of Leaves, Stem Diameter, Canopy and Root Wet Weight, Canopy and Root Dry Weight at 84 DAP.

Observation Parameters	F. Count		
	M	P	MxP
Plant height	0.45^{ns}	$0,1^{ns}$	0.41^{ns}
Number of Leaves	2.95^{ns}	0.03^{ns}	1.06^{ns}
Stem Diameter	20.36^{**}	5.02^{*}	5.68^{**}
Canopy Wet Weight	5.8^{*}	0.07^{ns}	1.05^{ns}
Root Wet Weight	8.97^{**}	7.15^{**}	3.75^{*}
Canopy Dry Weight	3.32^{ns}	0.48^{ns}	0.56^{ns}
Root Dry Weight	12.56^{**}	6.23^{**}	2.66^{ns}

Note: ns = not significantly different (non-significant), *=significantly different at the 5% level, **= very significantly different (very significant) at the 1% level

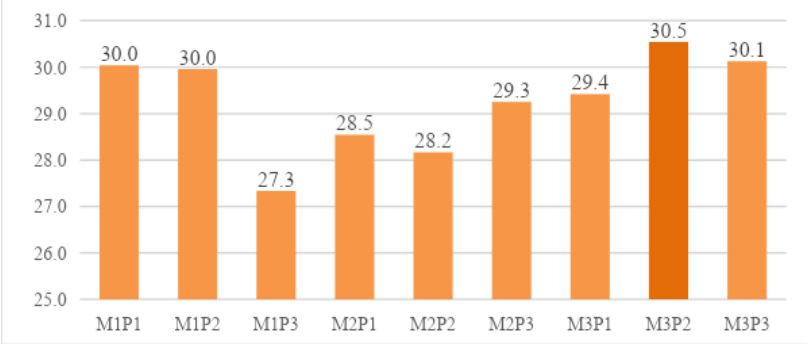

Figure 1. Graph of average height of cocoa plants at 84 DAP.

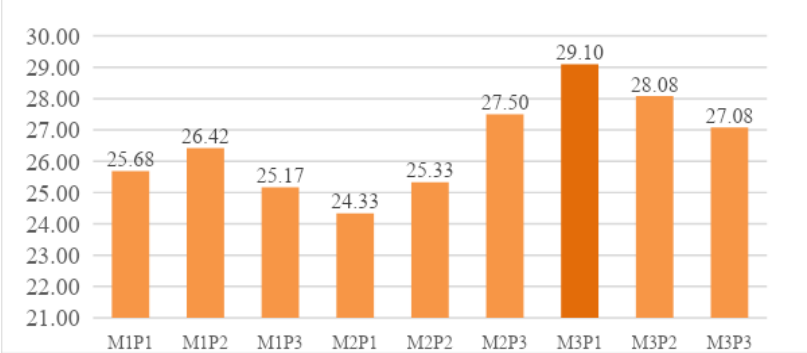

Figure 2. Graph of the average number of leaves for cocoa plants aged 84 DAP.

Table 2. Further test of 84 DAP cocoa plant stem diameter on factor (M) using DMRT level 1%

Treatment	Mean	Notation	DMRT 1%
M2	6.82	a	0.32
M1	6.90	a	0.34
M3	7.47	b	0.35

Note: Values followed by the same letter are not significantly different in the Duncan Multiple Range Test (DMRT) at the 5% level.

Table 3. advanced test of wet weight of cocoa plant crowns 84 DAP on factor (M) using DMRT at 5% level

Treatment	Mean	Notation	DMRT 5%
M1	18.32	a	5.13
M2	19.40	a	5.38
M3	22.88	b	3.24

Note: Values followed by the same letter are not significantly different in the Duncan Multiple Range Test (DMRT) at the 5% level.

Wet weight of canopy cocoa plant

This parameter shows that the wet weight of the cocoa plant canopy has a significantly different influence on the chicken manure use factor (M) with a calculated F of 5.80 which is greater than the F table of 5%. Meanwhile, regarding the application factor *Trichoderma* sp. and the interaction between the two has an influence that is not significantly different. It is suspected that cocoa seedlings are very responsive to media composition.

Based on the table the highest average was obtained in treatment M3. This is because the M3 treatment uses chicken manure at a dose of 400 grams so that the nutrient content needed by cocoa plants is sufficient. The appropriate media composition will increase water and nutrient uptake so that it will trigger the growth of plant organs (Ahmad *et al*., 2022).

Root wet weight of cocoa plant

The figure 3 showes the average wet weight of roots aged 84 DAP, it shows that in the M3P2 treatment the highest average was 9.2 grams, while in the M1P3 treatment the lowest average was 5.9 grams. Based on the table, it shows that the M3 treatment, which uses chicken manure at a dose of 400 grams, has the highest average yield compared to other treatments. The increase in wet root weight along with increasing levels of chicken manure application is thought to be due to the fairly high P content in chicken manure (Tarigan et al., 2014). The formation of healthy plant roots can be influenced by root conditions, good roots are an important factor for plant growth. Availability and absorption of nutrients by plant roots can occur well if root conditions are not disturbed by disease and other attacks. With the addition of *Trichoderma*, apart from being able to protect the roots which can cause wilt disease in plants, it also acts as a decomposer of organic materials from compost. *Trichoderma*

sp is a biopesticide, one of the tactics used for integrated pest control (Agus et al., 2022).

Based on the table, it shows that giving *Trichoderma* sp. can increase root weight gain, where it is shown that treatment P2 is the best treatment which produces the highest average (8.01) in the root wet weight parameter. It is alleged that with *Trichoderma* sp. a level of 10 ml/l is a level that is in accordance with what the plant needs. Planting media is an important factor for plant growth. This is because it affects the performance of the roots in absorbing nutrients in the soil. Factors that can influence the absorption of nutrients and water by roots include humidity levels, fertility, soil friability, soil biota, etc. In this case, a gift *Trichoderma* sp. as a biological agent for controlling disease in soil and also has the potential for fungi that can also support the availability of nutrients. Another thing that is an important factor in plant growth is good nutrient absorption. Maximum nutrient absorption can influence plant growth. Schmidt (2006), stated that *Trichoderma* is a cellulolytic fungi which has good potential for decomposing cellulose and hemicellulose compared to wax and lignin. The use of biological fertilizer is able to maintain the soil environment through fixation of N in the soil, dissolution of P and potassium or mineralization, release of plant growth regulators, and production (Sinha et al., 2014). So it is necessary to maintain a good soil environment. One of the biological materials that can be used to increase plant production is *Trichoderma* sp. The use of *Trichoderma* sp. can be combined with organic fertilizer such as chicken manure.

Based on table 6 on the interaction between the use of chicken manure and *Trichoderma* sp. shows a real difference. In the combination treatment of chicken manure at a dose of 400 grams and *Trichoderma* sp. with a level of 10 ml/L provides an increase in the wet weight of the roots. An appropriate media composition will increase water and nutrient uptake, thereby triggering the growth of plant organs such as shoots and roots (Ahmad et al., 2022).

In the combination of M3P1 treatment, namely chicken manure at a dose of 400 grams and *Trichoderma* sp. with a level of 5 ml/L gave the highest results on the stem diameter parameter. The interaction of the two factors results in stem enlargement, where *Trichoderma* sp. helps decompose organic materials from chicken manure that plants need for growth. According to Yusuf and Firsandi (2021), *Trichoderma* sp. have a high chance of competing for living *sp*ace and food sources first, penetrate cell walls more quickly and enter cells to take up nutrients, and produce antibiotics that can kill pathogenic fungal cells. The availability of nutrients in sufficient quantities causes the metabolic activities of the plant to increase as well as the accumulation of assimilate in the stem area will increase resulting in enlargement of the stem.

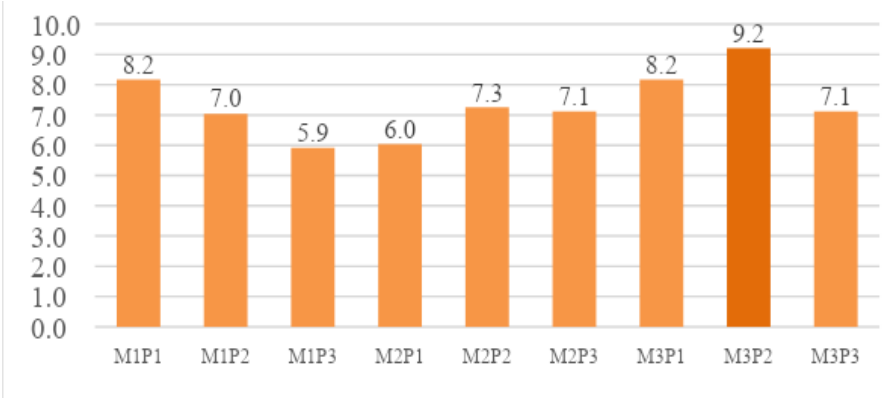

Figure 3. Graph of average wet weight of roots at 84 DAP

Table 4. Advanced test of wet weight of plant roots 84 DAP on factor (M) using DMRT level of 1%

Treatment	Mean	Notation	DMRT 1%
M2	6.89	a	1.9361
M1	7.22	a	2.0191
M3	8.33	b	2.0739

Note: Values followed by the same letter are not significantly different in the Duncan Multiple Range Test (DMRT) at the 5% level.

Table 5. Advanced test of root wet weight 84 DAT on factor (P) using DMRT at 5% level

Treatment	Mean	Notation	DMRT 5%
P3	6.72	a	1.4051
P1	7.71	ab	1.4735
P2	8.01	b	1.5161

Note: Values followed by the same letter are not significantly different in the Duncan Multiple Range Test (DMRT) at the 5% level.

Table 6. Follow-up test of root wet weight 84 DAP on factor (MxP) using DMRT at 5% level

Treatment	Mean	Notation	DMRT 5%
M1P3	5.92	a	1.4051
M2P1	6.04	a	1.4735
M1P2	7.00	ab	1.5162
M2P3	7.13	ab	1.5453
M3P3	7.13	ab	1.5668
M2P2	7.25	ab	1.5823
M1P1	8.17	bc	1.5945
M3P1	8.17	bc	1.6038
M3P2	9.21	c	1.6109

Note: Values followed by the same letter are not significantly different in the Duncan Multiple Range Test (DMRT) at the 5% level.

Dry weight of canopy cocoa plant

Based on the graphic image of the average dry weight of shoots aged 84 DAT, it shows that the M3P3 treatment obtained the highest average value, namely 7.29 grams. According to Susilo et al. (2017) that the high or low dry biomass of plants depends on how much or how little nutrient uptake occurs during the plant growth process. Plant dry weight describes the accumulation of organic compounds that plants have successfully synthesized from inorganic compounds. Based on showing that the use of chicken manure and *Trichoderma* sp. had no significantly different effect on the dry weight of cocoa plant shoots. This is thought to have occurred because the oven process was carried out for too long a time at an inappropriate temperature so that the canopy was too dry when weighed. According to Liu et al., (2014) who stated that the nitrogen content in organic fertilizer is slow releasing so it is difficult for the roots to absorb it as an important element for optimal plant growth.

Root dry weight of cocoa plant

Based on Figure 5, the average dry weight of roots aged 84 DAP shows that the M3P2 treatment obtained the highest average value, namely 1.79 grams. In table 4.1, observations of root dry weight at 84 DAT show that the use of chicken manure (M) has a very significantly different effect on root dry weight and also on application. *Trichoderma* sp. (P) has a very significant different effect on the dry weight of cocoa plant roots. The M3 treatment, namely the use of chicken manure at a dose of 400 g, increases the dry weight of cocoa plant roots. This shows that plants can grow and develop well in this media mixture so that nutrient and water uptake is maximized. The dry weight of plant biomass shows the uptake of photosynthesis or nutrients contained in plant tissue.

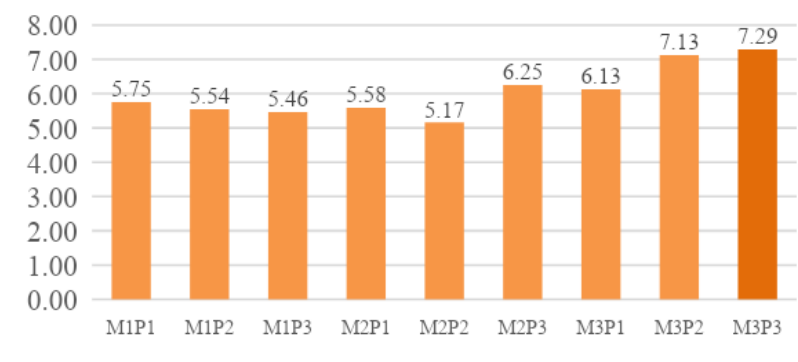

Figure 4. graph of average dry weight of title age 84 DAP

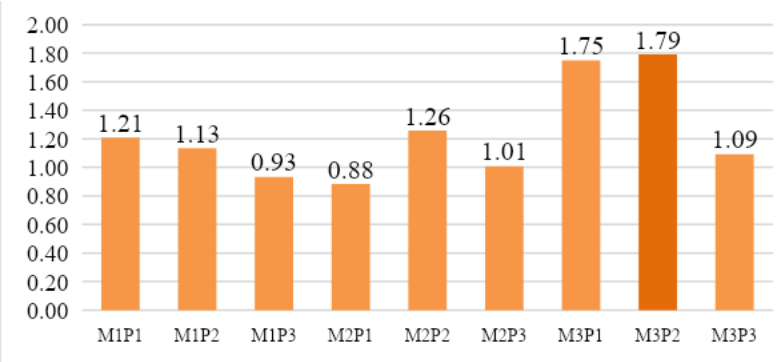

Figure 5. Average graph of root dry weight age 84 DAP

Table 7. Further test of dry weight of roots of cocoa plants aged 84 DAP on factor (M) using DMRT at 1% level

Treatment	Mean	Notation	DMRT 1%
M2	1.05	a	0.5444
M1	1.09	a	0.5677
M3	1.56	b	05831

Note: Values followed by the same letter are not significantly different in the Duncan Multiple Range Test (DMRT) at the 5% level.

Table 8. Further test of dry weight of roots of cocoa plants aged 84 DAP on factor (P) using DMRT at 1% level

Treatment	Mean	Notation	DMRT 1%
P3	3.03	a	0.5444
P1	3.84	b	0.5677
P2	4.18	b	0.5831

Note: Values followed by the same letter are not significantly different in the Duncan Multiple Range Test (DMRT) at the 5% level.

Based on Table 8, it shows that giving *Trichoderma* sp. in the P2 treatment, 10 ml/l, it was able to increase the dry weight of cocoa plant roots. *Trichoderma* sp. can reproduce quickly in the root area, is able to compete with other fungi but at the same time develops well in the roots, making the presence of this fungi able to act as a *biocontrol* and increasing plant growth (Amiruddin, 2021). Various investigations have found that the highest bacterial population, 24 x 10^{12} CFU/g, was obtained after treating 10 kg of compost with *Trichoderma* sp. These bacteria are prevalent and symbiotic in the roots of tangerine plants, and it is believed that they have an impact on the growth of tangerine plants (Siswadi et al., 2024).

In the M3 treatment, namely the use of chicken manure with a dose of 400 grams, it increased the dry weight of cocoa plant roots. This shows that plants can grow and develop well in the media mixture so that nutrient and water absorption is maximized. The use of higher and more balanced manure has the best effect on the dry weight of cocoa plant roots. Crumbly media allows roots to explore wider and deeper so that they form more tissue and will affect root weight. According to (Saputra et al., 2023), the friability of the media is a condition that determines how easy it is for roots to penetrate the planting medium.

The use of chicken manure showed the best treatment at a dose of 400 grams on the parameters of stem diameter, wet weight of the crown, wet weight of the roots, and dry weight of the cocoa plant roots. While the application of *Trichoderma* sp. showed the best treatment at a level of 10 ml/L affecting stem diameter, wet weight of the roots, and dry weight of the roots.

CONCLUSION

The results showed that giving chicken manure at a dose of 400 g had the best effect on stem diameter, canopy wet weight, root wet weight and root dry weight of cocoa plants. Meanwhile, when giving *Trichoderma* sp. showed that the best treatment at the level of 10 ml/L had an effect on stem diameter, root wet weight and root dry weight. The interaction shows the best treatment combination of chicken manure with a dose of 400 g and *Trichoderma* sp. with a level of 10 ml/L which influences the parameters of stem diameter and root fresh weight.

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AUTHORS CONTRIBUTIONS

DGC, KH and S considered and planned the experiment. GFD carried out the preparation of *Trichoderma* sp. IH performed analysis data. KH carried out the preparation of chicken manure. DG, S, IH and KH interpreting the data. DGC and GFD prepared the manuscript. The authors provided responses and comments on the research flow, data analysis, and interpretation as well as the shape of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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