

Growth of Cocoa Seedlings Affected by Application of Zeolite-Cocopeat-Manure Mixture as Soil Conditioner Enriched by *Trichoderma* sp.

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Abstract

Soil conditioners such as the combined of zeolite, cocopeat, and manure can help to enhance the quality of the soil and improve plant growth. *Trichoderma* sp., is a beneficial microbes and endophytic fungus has also been applied into planting media to promote plant growth in the production of cocoa seedling. The objective of this research was to study the effect of combining zeolite, cocopeat, and manure mixture formula in different dosage levels enriched by *Trichoderma* sp. on cocoa seedling growth. This research was conducted in a glass house in Kaliwining Experimental Station of Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember, East Java with 25–38 °C of daily temperature and 76–90% of relative humidity. The research was designed by using complete randomized design with seven treatments, i.e. no application of soil conditioner (control), the application of 200 g, 400 g, and 600 g polybag⁻¹ of soil conditioner, 200 g, 400 g, and 600 g polybag⁻¹ of soil conditioner that enriched by *Trichoderma* sp. with 5 replications and 5 subsamples. The result of the study demonstrated that *Trichoderma* sp. successfully colonized in leaf tissue of cocoa seedlings in all treatments, and the highest colonization was found in the soil conditioner that was enriched by *Trichoderma* sp. The result of the research was the application of zeolite-cocopeat-manure formula as soil conditioner and/or combined with *Trichoderma* sp. enhanced the growth and biomass of cocoa seedling. The addition of *Trichoderma* sp. into the soil conditioner in planting media positively increased the cocoa seedling growth.

Keywords: Soil conditioner, zeolite, cocopeat, manure, *Trichoderma* sp., cocoa growth

INTRODUCTION

Over the past decades, the concern of soil degradation has been an important issue for cocoa plantations. The continuous decreasing of soil fertility could impact the decreasing of plant production. According to a survey in cocoa plantation during 2014-2017 in some central cocoa plantation areas in Indonesia, the status of soil nutrient was mostly below the threshold for carbon and nitrogen for cocoa trees (Daymond *et al.*, 2017).

The alternative to increase the production due to soil degradation could be overcome by using soil conditioner compound. Decree of Ministry of Agriculture of Indonesia no. 01 year 2019 defined soil conditioner compound as organic or mineral, synthetic or natural in the form of solid or liquid that has the ability to improve physical, chemical or biological character of soil. Some examples of soil conditioner compounds are biochar, humic acid, cocopeat, zeolite, or plant residues. The application of a single or formulated soil

conditioner can be used to repair soil quality in cocoa plantation. The main objective of application of soil conditioning compounds is to create an environment that supports plant growth and production, development of beneficial soil microbes, and improve soil physics. The research of application of zeolite as soil conditioner in cocoa has been reported that it accelerates the growth of cocoa seedling Romadoni & Nursanti (2024).

Trichoderma is a fungus that colonizes plant roots as a symbiont and possesses the ability to promote plant growth and development. It has long been recognized as an agent for plant disease control and a promoter of plant growth and development. *Trichoderma* is a naturally occurring soil fungus that has been extensively commercialized. Its use as an organic matter enhancer is known to enhance the absorption of micronutrients for plants such as Cu, Zn, and Fe, and its role in phosphate solubilization in soil, making it available to plants. *Trichoderma* enhances overall plant health by creating a favorable environment for plant growth and releasing various types of secondary metabolites, including growth hormones, endochitinase, and proteolytic enzymes (Kamal *et al.*, 2018). Currently, various *Trichoderma* species have been widely utilized by farmers, including *T. harzianum*, *T. viride*, *T. reesei*, *T. polysporum*, *T. koningii*, and others. *T. harzianum* is known to improve plant tolerance to environmental stress and mineral uptake. Moreover, *Trichoderma* is also effective in controlling several pathogenic fungi responsible for diseases, including *Fusarium*, *Rhizoctonia*, *Pythium*, *Sclerotinia*, *Verticillium*, *Alternaria*, and *Phytophthora* (Kamal *et al.*, 2018).

Incorporating beneficial microorganisms like *Trichoderma* species has the potential to enhance both plant quality and environmental sustainability. *Trichoderma* sp. in cocoa plants has been widely used in cocoa plantations, from seedlings until mature trees. *Trichoderma*

martiale species is employed to manage cocoa pod rot disease caused by *Phytophthora* sp. (Hanada *et al.*, 2009). *Trichoderma asperellum* has the capability to decrease the severity of vascular streak dieback (VSD) disease and increase cocoa growth (Simamora *et al.*, 2021). Additionally, *Trichoderma* sp. that was combined with organic material plays a role in promoting plant growth and development of cocoa growth (Rafiuddin *et al.*, 2020). The objective of this research was to study the effect of soil conditioner in different dosage levels enriched by *Trichoderma* sp. on cocoa seedling growth and biomass.

MATERIALS AND METHODS

This research was conducted in glasshouse Kaliwining Experimental Station of the Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember, East Java. The daily temperature ranged was 25–38 °C and relative humidity was 76–90%. The research design arranged by using complete randomized design with one factor using seven treatments, i.e. no application of soil conditioner (control), the application of 200 g, 400 g, and 600 g of soil conditioner, the application of 200 g, 400 g, and 600 g of soil conditioner enriched by *Trichoderma* sp. The spore population of *Trichoderma* sp. 10^8 cfu mL⁻¹ was (Amaria *et al.*, 2016).

The research used 5 replications and five subsamples. Polybags (40 cm x 25 cm) were arranged on thick polythene sheets to prevent the penetration of roots into the ground and was filled with top soil. The top soil media was Inceptisol type, clay loam texture, with 0.47% of C-content, 0.11% of total N, and 14 mg kg⁻¹ of P₂O₅. Cocoa seedling was derived from half-sib cocoa pods from Sulawesi 01 clones. Cocoa seeds were germinated for 24 hours and transplanted to the polybags. Soil conditioner was made by mixing manure,

zeolite, and cocopeat (1 : 1 : 1 ratio). Soil conditioner was added to the top layer of planting media of cocoa seedling at one month after germination. The formula of zeolite, cocopeat, and manure mixture as a soil conditioner contained 22% of organic C, 0.77% of total N, 0.49% of P₂O₅, 2.53 of K₂O, and 6.91 of pH. The insecticide treatments of 2 mL L⁻¹ which consisted of lambda sihalotrin 106 g L⁻¹ + Tiametoksam 141 g L⁻¹ were applied every month to prevent the mealybugs and caterpillar attack.

Preparation for *Trichoderma* fungus cultivation involves the following steps. The cultivation of *Trichoderma* fungi was carried out at the Plant Protection Laboratory of the ICCRI. Each fungal isolate was grown on *Potato Dextrose Agar* (PDA) medium (containing 200 g potato, 20 g dextrose, 20 g agar, and 1,000 mL distilled water) for a period of 3 days. Fungal multiplication was conducted on a sterilized corn medium. One loopful of fungal isolate from the PDA medium was inoculated onto 200 g of sterilized medium, followed by an 8-day incubation period. The fungal isolates on the corn medium, when ready, were further processed to produce pure spore powder. The soil conditioner formulation is mixed with pure *Trichoderma* fungus spore powder at a spore density of 10⁸ colony-forming units per milliliter (cfu mL⁻¹), with each spore's viability exceeding 80%, as per the established formulation.

To detect the presence of *Trichoderma* in leaf tissues, leaf samples were collected from the seedlings. All material was surface-sterilized as follows: leaf pieces were washed twice in sterile tap water, followed by a 1-minute immersion in a 70% (v/v) ethanol solution, and then a 4-minute soak in a 5% (v/v) sodium hypochlorite solution, and then washed two times in sterile distilled water (SDW). After the surface sterilization process, the samples were transferred under aseptic conditions onto plates containing Potato Dextrose Agar

(PDA). These plates were sealed and placed in an incubator at room temperature (25±2 °C). An initial screening for *Trichoderma* isolates was conducted using macroscopic observation, focusing on the rapid growth rate and the typical green colony coloration associated with the *Trichoderma* genus. This preliminary identification was subsequently confirmed through microscopic examination, based on the characteristic conidiogenesis pattern of *Trichoderma*.

Plant growth was observed including plant height increment, stem diameter increment, number of leaves increment, and plant dry mass. Data on seedling morphological growth was subjected to analysis of variance (ANOVA) and Duncan test (at 95% confidence level) to compare their means using Statistical Package for the Social Sciences (SPSS).

RESULTS AND DISCUSSIONS

The existence of *Trichoderma* sp. as an endophytic fungus was confirmed through tissue analysis. PDA medium, the hyphae cover the entire petri dish from a leaf tissue (0.5 cm x 1 cm) in 3–4 days. The conidia grew, replicated, and aggregated at the tip of a conidiophore. Mature conidia developed as a sphere-like structure composed of numerous individual conidia after 7 days and displayed a green color (Figure 1). *Trichoderma* sp. colony existed in control, meanwhile the application soil conditioner treatments enhanced the *Trichoderma* sp. colony. The highest colony of *Trichoderma* existed in soil conditioner + *Trichoderma* (Table 1).

Trichoderma species are grouped as soil borne organisms that occur in nearly all soils and commonly found in plant rhizosphere (Sharma & Gothwal, 2017). The fungi are typically considered free-living organisms associated with plant roots and are considered for their potential to control plant disease because

of their close association with plants, with many aspects typical of endophytic associations. The report of *Trichoderma* sp. as endophyte and enter to the cocoa plant tissue was assessed by Nurlaila *et al.* (2020) and Nur'Aini *et al.* (2020). In the addition of soil organic matter and zeolite could increase the development of *Trichoderma* sp. colony. Saju *et al.* (2002) concluded the population of *Trichoderma harzianum* could increase when added the organic matter such as coir pith, farmyard manure, neemcake, and decomposed coffee pulp. Lopez-Busio *et al.* (2015) stated that *Trichoderma* sp. had a mutualistic relationship with plants, *Trichoderma* obtained benefit from a nutrient rich environment, and it penetrates the root system resulting in several beneficial effects to the plant. The application of ex-situ of *Trichoderma* sp. also increased the colony *Trichoderma* sp. on soil aggregate and leaves tissue.

Plant height, number of leaves, and stem diameter of cocoa seedling was presented in Table 2, 3 and 4. The treatments affected plant height and number of leaves after 3–4 weeks, meanwhile, the stem diameter was affected 6 weeks after treatments. On the early growth (3–4 weeks after treatments), the highest seedling and largest plant number was found on the application of 600 g of soil conditioner, meanwhile, the soil conditioner that was enriched by *Trichoderma* sp. the application of 400 g of soil conditioner increased the plant height and number of leaves.

The application of 600 g soil conditioner promoted the plant height, stem diameter, and number of leaves than control at 6 weeks after treatment. The addition of *Trichoderma* sp. gave the higher acceleration on plant growth in lower dosage, that 400 g of soil conditioner significantly affected plant height, number of leaves and stem diameter. The addition of *Trichoderma* sp. in to 200 g polybag⁻¹ of soil conditioner could accelerate plant growth in cocoa seedlings. This finding was similar to de Sousa *et al.* (2021) that adding *Trichoderma* sp. to the substrate media increased plant height of cocoa seedlings. The application of cocopeat as a soil conditioner to the top soil of planting media increased the plant growth of dipterocarps seedlings has been reported by Marjenah *et al.* (2016). However, the combination between cocopeat and *Trichoderma* sp. had been reported by Nasir *et al.* (2022) that the addition of cocopeat and *Trichoderma* sp. was not affected on the growth of ginseng plant.

The effect of treatments was significant on shoot fresh weight, total fresh weight, shoot dry weight, total dry weight, and shoot root-ratio (Table 5). The highest fresh weight and shoot dry weight was obtained in 600 g of application soil conditioner with and/or without *Trichoderma* sp. The highest total dry mass was obtained in the application of 400 and 600 g of soil conditioner enriched by *Trichoderma* sp.

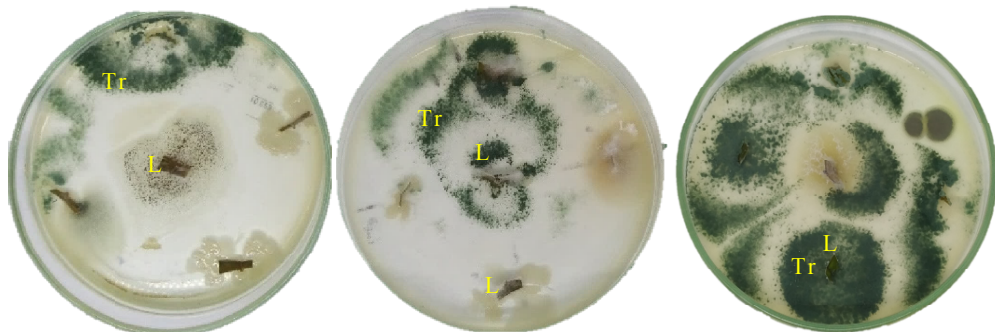


Figure 1. The existence of *Trichoderma* sp. on leaves tissue after seven days incubation in PDA media at six months after treatments; L = Leaves tissue; Tr = Colonization of *Trichoderma* sp.

Table 1. The existence of *Trichoderma* sp. on leaves tissue at six months after treatments

Treatments	Existence in leaves tissue
Control	+
Soil conditioner	++
Soil conditioner enriched with <i>Trichoderma</i> sp.	+++

Notes : - = not detected, + = fairly detected, ++ = moderately detected, +++ = strongly detected on leaf tissues.

Table 2. Plant height (cm) of cocoa seedlings with different treatment of soil conditioners

Treatment (g polybag ⁻¹)	Weeks after treatments					
	1	2	3	4	5	6
	Plant height, cm					
Control	39.9 ^a	44.6 ^a	48.6 ^c	53.2 ^c	57.1 ^c	58.6 ^c
Soil conditioner						
200	37.4 ^a	43.3 ^a	55.1 ^{abc}	61.7 ^{ab}	66.1 ^{bc}	64.5 ^{bc}
400	34.3 ^a	38.6 ^a	49.8 ^{bc}	56.2 ^{bc}	66.4 ^{bc}	68.1 ^{bc}
600	37.0 ^a	45.0 ^a	57.4 ^a	66.4 ^a	74.0 ^{ab}	75.7 ^{ab}
Soil conditioner						
enriched with						
200	39.7 ^a	45.7 ^a	55.2 ^{abc}	62.1 ^{ab}	70.1 ^{ab}	73.2 ^{ab}
400	38.6 ^a	44.9 ^a	59.4 ^a	65.5 ^{ab}	76.7 ^a	81.4 ^a
600	35.0 ^a	44.4 ^a	56.6 ^{ab}	64.9 ^a	78.7 ^a	84.5 ^a
Significance	ns	ns	ns	*	**	**

Note : Values on the same column in each variable followed by the same letter were not different based on the Duncan test at $\alpha = 5\%$. ns = not significantly different; * and ** = significantly different at 5 and 1% significance level.

Table 3. Number of leaves of cocoa seedlings with different treatment of soil conditioners

Treatment	Dosage (g polybag ⁻¹)	Weeks after treatments					
		1	2	3	4	5	6
		Number of leaves					
Control		15.1 ^a	16.7 ^a	18.6 ^b	19.4 ^c	21.2 ^b	20.2 ^c
Soil conditioner							
200	14.5 ^a	16.9 ^a	20.0 ^{ab}	21.1 ^{abc}	25.1 ^a	21.2 ^{bc}	
400	15.0 ^a	16.7 ^a	21.0 ^{ab}	23.6 ^{ab}	24.6 ^{ab}	23.3 ^{abc}	
600	16.3 ^a	17.6 ^a	22.2 ^a	25.1 ^a	25.3 ^a	24.2 ^{ab}	
Soil conditioner							
enriched with							
200	15.6 ^a	18.2 ^a	21.1 ^{ab}	21.8 ^{bc}	25.6 ^a	25.3 ^{ab}	
400	16.6 ^a	17.0 ^a	22.4 ^a	24.6 ^a	28.4 ^a	27.1 ^a	
600	15.7 ^a	18.1 ^a	21.4 ^a	24.5 ^a	27.2 ^a	27.3 ^a	
Significance		ns	ns	*	**	*	**

Notes: Values on the same column in each variable followed by the same letter were not different based on the Duncan test at $\alpha = 5\%$. ns = not significantly different; * and ** = significantly different at 5 and 1% significance level.

The application of soil conditioner enriched by *Trichoderma* increases the shoot growth. Shoot-root ratio of cocoa seedling tend to increase after application of the soil conditioner enriched by *Trichoderma* sp.

The application of *Trichoderma* sp. increased shoot and total dry mass of the plant is aligned with the result of Lee *et al.* (2016) in tomato and Tchameni *et al.* (2017) in cocoa plant. The organic matter that combined with *Trichoderma* sp. increased the biomass. However, in this research, the root fresh and dry

weight was not affected in addition of soil conditioner and its enrichment with *Trichoderma* sp. *Trichoderma* sp. improve the photosynthetic efficiency and capacity on plants (Harman *et al.*, 2021). The higher photosynthetic efficiency and capacity lead to improve the accumulation of biomass in plant.

According to contrast analysis (Table 6), the application soil conditioner and soil conditioner with *Trichoderma* sp. significantly affected the plant height, stem diameter, number of leaves, shoot fresh weight, total fresh weight,

Table 4. Stem diameter of cocoa seedlings with different treatment of soil conditioners

Treatment	Dosage (g polybag ⁻¹)	Weeks after treatments					
		1	2	3	4	5	6
		Stem diameter, mm					
Control		3.5 ^a	4.9 ^a	5.9 ^a	7.0 ^a	8.9 ^a	9.0 ^b
Soil conditioner	200	3.5 ^a	4.8 ^a	6.4 ^a	7.5 ^a	8.8 ^a	9.5 ^{ab}
	400	3.1 ^a	4.6 ^a	5.9 ^a	7.6 ^a	8.2 ^a	9.8 ^a
	600	3.3 ^a	4.9 ^a	6.3 ^a	7.8 ^a	8.6 ^a	10.1 ^a
Soil conditioner enriched with <i>Trichoderma</i> sp.	200	3.4 ^a	5.0 ^a	6.1 ^a	6.9 ^a	8.7 ^a	9.6 ^{ab}
	400	3.2 ^a	4.7 ^a	5.9 ^a	6.9 ^a	8.4 ^a	9.9 ^a
	600	3.2 ^a	5.0 ^a	6.4 ^a	7.6 ^a	8.9 ^a	10.3 ^a
Significance		ns	ns	ns	ns	ns	*

Notes : --Values on the same column in each variable followed by the same letter were not different based on the Duncan test at $\alpha = 5\%$. ns = not significantly different; * and ** = significantly different at 5 and 1% significance level.

Table 5. Shoot fresh weight, root fresh weight, total fresh weight, shoot dry weight, root dry weight, total dry weight, and shoot-root ratio of cocoa seedlings at six months after treatment

Treatment	Dosage g polybag ⁻¹	Shoot fresh weight, g	Root fresh weight, g	Total fresh weight, g	Shoot dry weight, g	Root dry weight, g	Total dry weight, g	Shoot root ratio
Control		55.4 ^d	27.8 ^a	83.2 ^c	19.3 ^c	8.01 ^a	27.3 ^c	2.40 ^b
Soil conditioner	200	76.1 ^{cd}	42.1 ^a	118.1 ^{ab}	27.3 ^b	9.50 ^a	36.8 ^{ab}	3.28 ^{ab}
	400	91.3 ^{bc}	37.6 ^a	128.9 ^{ab}	30.5 ^{ab}	9.69 ^a	40.2 ^{ab}	3.24 ^{ab}
	600	101.5 ^a	31.8 ^a	133.2 ^a	32.5 ^{ab}	8.71 ^a	41.2 ^{ab}	3.83 ^{ab}
Soil conditioner enriched with <i>Trichoderma</i> sp.	200	77.7 ^{cd}	23.2 ^a	100.9 ^{bc}	26.9 ^b	6.63 ^a	33.5 ^b	4.60 ^a
	400	94.7 ^{bc}	31.4 ^a	126.1 ^{ab}	34.2 ^a	8.80 ^a	43.0 ^a	4.16 ^a
	600	117.4 ^a	27.9 ^a	145.3 ^a	35.3 ^a	8.11 ^a	43.4 ^a	4.54 ^a
Significance		**	ns	**	**	ns	**	*

Note : -Values on the same column in each variable followed by the same letter were not different based on the Duncan test at $\alpha = 5\%$. ns = not significantly different; * and ** = significantly different at 5 and 1% significance level.

Table 6. Contrast analysis of plant height, stem diameter, number of leaves, fresh weight, dry weight, and shoot-root ratio among of the treatments

Contrast	PH	SD	NL	SFW	RFW	TFW	SDW	RDW	TDW	S/R ratio
Control vs Soil conditioner	**	**	**	**	ns	**	**	ns	**	*
Control vs Soil conditioner + <i>Trichoderma</i> sp.	**	**	**	**	ns	**	**	ns	**	**

Notes: PH = Plant height, SD = stem diameter, NL = number of leaves, SFW = shoot fresh weight, RFW = root fresh weight, TFW = total fresh weight, SDW = shoot dry weight, RDW = root dry weight, TDW = total dry weight, S/R = shoot root ratio.

shoot dry weight, total dry weight, and shoot/root ratio than control treatments. The relationship between dosage of soil conditioner with/without *Trichoderma* sp. and plant height had been demonstrated in Figure 2, 3, 4, and 5. The linear regression showed that, the increment of plant height, stem diameter, number of leaves, shoot dry weight in lined with the increment of dosage of soil conditioner. The addition of 100 g soil conditioner in to planting

media, increased 2.7 cm of plant height, 0.18 mm of stem diameter, 0.7 leaves. Meanwhile, the increment 100 g of soil conditioner that enriched with *Trichoderma* sp. increased the 4 cm of plant height, 0.2 mm of stem diameter, and 1.16 leaves of cocoa seedlings. This research finding was the addition of beneficial microbes such as *Trichoderma* sp. in to zeolite, cocopeat, and manure mixture could increase the plant growth.

Zeolite has positive effects on soil characteristics, such as improving soil moisture, and increasing yields in acidified soils; they are widely used as soil conditioners to improve soil physical and chemical properties. The using zeolites as soil amendment could increase the cation exchange capacity (CEC) and commonly it used with fertilizers can improve soil pH levels. The manure as the source of nutrient especially organic C and other nutrient, and

humus was a food source for cocoa seedlings. Cocopeat provided good physical properties for planting media, with many pores space and low bulk density (Marjenah *et al.*, 2016). The combination of zeolite, manure, and cocopeat may allow nutrient ions to flow from planting media to water, where plants can absorb the nutrient. Organic material such as cocopeat and compost has been reported by Marjenah *et al.* (2016) that could be used for stimulant in planting media.

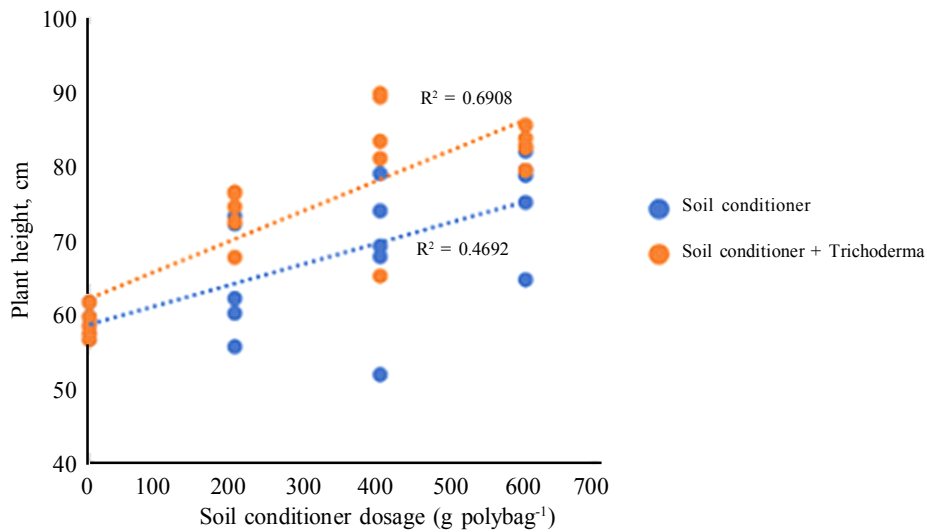


Figure 2. Relationship between dosage of soil conditioner and soil conditioner enriched by *Trichoderma* sp. on cocoa plant height at six months after transplanting

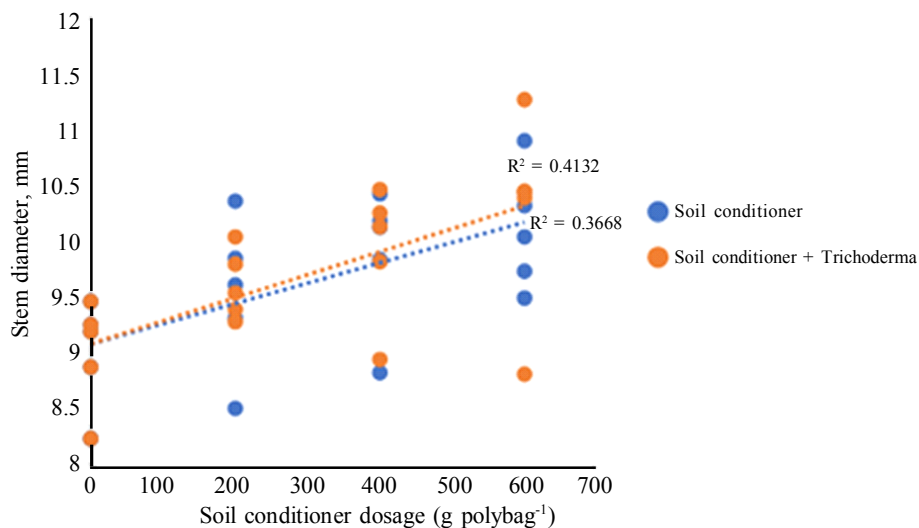


Figure 3. Relationship between dosage of soil conditioner and soil conditioner enriched by *Trichoderma* sp. on stem diameter

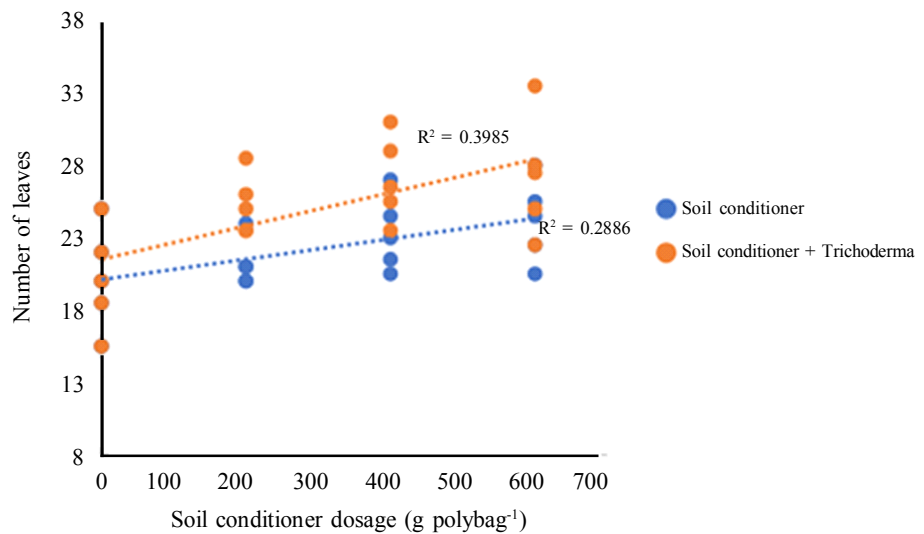


Figure 4. Relationship between dosage of soil conditioner and soil conditioner enriched by *Trichoderma* sp. on number of leaves

CONCLUSIONS

Trichoderma sp. successfully colonized in leaf tissue of cocoa seedlings in all treatments. The highest colonization was in the soil conditioner of zeolite, cocopeat, and manure mixture enriched by *Trichoderma* sp. The application of 600 g soil conditioner increased the plant growth, such as plant height, stem diameter, and number of leaves. The addition of *Trichoderma* sp. to the 400 g soil conditioner positively promoted the plant growth. According to the biomass, the application of 400–600 g of soil conditioner increased plant fresh weight and dry weight of cocoa seedling. Meanwhile, the addition of 400 g of soil conditioner with *Trichoderma* sp. increased the shoot fresh weight, shoot dry weight and total dry weight.

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