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Research Article Physiological and Agronomical Characteristics Evaluation of Soybean Grown under Oil Palm Stands Applied with Tri-po Enriched Compost

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Abstract

Background and Objective: Utilization of soybean plants as cover crops under oil palm trees is one of the solutions to maintain food security in Indonesia. Therefore, the objective of this research was to study the effect of organic matter derived from the Oil Palm Empty Fruit Bunches (OPEFB) and *Trichoderma harzianum + Pleurotus ostreatus* (Tri-Po) combination to the leaf physiological properties and productivity of soybean planted under 4 years old oil palm stands. **Materials and Methods:** A factorial design with Randomized Complete Block Design was employed as environmental design. The first factor was OPEFB compost consisting of three levels and the second factor is the Tri-Po combination which consists of four levels. The treatments were repeated three times resulted in 36 experimental units. Observations focused on some physiological characteristics and agronomical characters of the soybean. **Results:** The results showed that the use of OPEFB compost applied with *Trichoderma harzianum* and *Pleurotus ostreatus* improved the biological, physical and chemical characteristics of the soil, enhanced the physiological performance and productivity of soybean plants. The combination of 10 kg ha⁻¹ OPEFB and 4g:6g Tri-Po was the best formula to improve the physiological characters of soybean leaves grown under oil palm stands. **Conclusion:** The research can increase the land economic value of smallholder or palm oil industry and increased the soybean supply for domestic demand.

Key words: Compost, Trichoderma harzianum, palm oil, Pleurotus ostreatus, soybean, domestic demand

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soybean is one of the important strategic commodities as a staple food after rice and corn in Indonesia¹. In Indonesia, soybean is the most consumed as the ingredient of tempeh, tofu, milk and others. In addition, the soybean has an important value in the industrial development of Indonesia, especially in the food and feed industry². To date, the soybean availability as the main material in industry has not yet been fulfilled from domestic production. Crop management is one of the aspects that limit soybean productivity and development, hence its productivity is lower than rice and corn¹. In other hand, increased population and industrial development can add the problem to soybean availability³. Therefore, improvement of the soybean production is crucial in Indonesia.

Soybean production of Indonesia in the 2015 period only achieved 33.56 % of national soybean demand per year, hence the gap was covered by import⁴. In the past 5 years, the government has tried to implement programs in order to increase the national soybean production in Indonesia. One of the efforts is soybean extensification through the use of the available space under plantation crops such as oil palm up to the age of 4 years. Previous studies has shown that intercropping of soybean and oil palm could rise the oil palm growth⁵ and at the same time can retard the weed growth in the oil palm plantation⁶. In addition, according to Nchanji *et al.*⁷, the intercropping could give benefits in economic factor, especially on the smallholders. Based on that, the intercropping soybean-palm oil can be a solution in increasing soybean production.

The main problem with intercropping plants in plantation crop is the low light intensity obtained by the cover crop caused by the shade factor, especially under more than 4 years old oil palm stands. In general, the low light intensity due to shading will affect the growth and yield of plants, include soybean⁸. Stress caused by the 50% shading has an impact to decrease 10-40% of the soybean yield⁹. This is due to decreased photosynthesis rate caused by the heavy shade level. To overcome this limitation, it is necessary to improve the leaf physiology and productivity of soybean under the oil palm stands.

The improvement effort of soybean yield under shade stress can be done with the application of organic matter from Oil Palm Empty Fruit Bunches (OPEFB). It has been reported by Amalia *et al.*⁹, the OPEFB significantly influenced soybean growth in intercropping with oil palm at the juvenile phase. The OPEFB is a waste product from an palm oil factory that has potential to decompose as organic fertilizer¹⁰. Beside as fertilizer, OPEFB can increase the stock of soil organic matter in the agroecosystem. It had the potential to decrease atmospheric carbon through the increase of carbon absorption in the soil¹¹. However, The OPEFB has an abundant of lignin with a percentage of 17.1% of the total OPEFB component. The lignin is a barrier of enzyme in degrading celluloses and hemicelluloses of OPEFB, therefore become the main problem in making organic fertilizer from OPEFB¹². A catalyst is needed to speed up the decomposition process of OPEFB. One of its solutions is the use of microorganisms in degrading the OPEFB.

Trichoderma harzianum and Pleurotus ostreatus are the fungi mostly used to speed up the degradation of organic matter, especially the material contained high of lignin and cellulose such as the OPEFB¹³. The utilization of *Trichoderma* sp. as OPEFB bioconversion in the peatlands could increase the productivity of intercropping plants under the oil palmstands^{14,15}. Similarly, *Pleurotus ostreatus* can be used for the degradation of the lignin, cellulose and hemicellulose in organic matter to be CO₂ and H₂O. Therefore, the use of Trichoderma harzianum and Pleurotus ostreatus can be expected to increase the speed of the OPEFB degradation. Therefore, the objective of the research was to study the influence of organic matter derived of the OPEFB degradation with Trichoderma harzianum and Pleurotus ostreatus as a catalyst on the leaf physiological properties and productivity of soybean plant grown as cover crop under the 4 years old oil palm stands.

MATERIALS AND METHODS

Study area: The study was conducted from October, 2018 to April, 2019 at the South Sulawesi Provincial Plantation Office in Tulung Indah Village, Sukamaju District, North Luwu Regency.

Sample collection: The main materials used in this experiment were *Trichoderma harzianum* and *Pleurotus ostreatus* obtained from the Laboratory of Plant Pests and Diseases at the Faculty of Agriculture, Hasanuddin University. Meanwhile, the OPEFB as compost material was obtained from PTPN XIV's Palm Oil Mill in Burau, North Luwu Regency. The plant materials used were Soybean variety, namely Dena1. The soybean was planted on the available spaces under oil palm trees (4 years old) using an intercropping system. Growing condition for the Soybean under the stands, from vegetative to harvest, had an average temperature of 24.84°C.

Research methodology: The study was set using a factorial design with a Randomized Complete Block Design as

environmental design. The first factor was the application of OPEFB compost consisted of 3 levels, namely: Control (C₀), OPEFB compost 10 (C₁) and 20 t ha⁻¹ (C₂). The second factor was the combination of *Trichoderma harzianum+Pleurotus ostreatus* (Tri-Po) consisted of four levels namely, Tri-Po with a dose of 4+2 (P₁), 4+4 (P₂), 4+6 (P₃) and 4+8 g tree⁻¹ (P₃), respectively. The treatment was repeated 3 times so that there were 36 experimental units. Each experimental unit has a plot size of 4×3 m.

Preparation and application of the OPEFB compost and

Tri-Po: Preparation of the OPEFB compost was carried out by cutting the OPEFB raw materials into a small size and composted by previously added with Tri-Po with dose according to the treatment. After 3 months, the compost has matured with the criteria: brownish-black color, stained with soil, loose texture, C/N ratio 15-20, with pH 6.5. The compost was applied by spreading it on the soil surface before planting, then mixed with the soil during the soil tillage. Planting was done with a spacing of 20×20 cm. Each hole was planted with 2-3 soybean seeds. In the 1st week after planting, the thinning was conducted with 2 plants per hole remained. Besides, the Tri-Po applications also were conducted according to all combination treatments. The applications were carried out by digging a hole with a depth of 3 cm beside the plant. The soybean plants also were maintained by additional NPK fertilizer (100 kg ha⁻¹), pest and disease control and weed control management.

Observation of parameters: Observations focused on some physiological characteristics of plants such as light interception, stomatal density, width of stomatal openings, leaf water content, the relative humidity of leaves, assimilation rate, transpiration rate, leaf intercellular CO₂ and stomatal conductance, observations were made twice on the final vegetative and generative phases, respectively. Observations were carried out using a Li-Cor 6400 XT Portable for all physiological parameters, except the density and area of the stomata opening. Observation of the stomatal density and width of stomatal openings were performed on the epidermis of the lower leaves by the nail polish method (acetone) and then observed under a light microscope with a magnification of 400 times for the width of stomatal openings and 1000 times for the stomatal density, respectively. Other observations were made in the form of the dry weight of 100 seeds and yield per plot at 14% water content.

Data analysis: The observation data were analyzed by using analysis of variance (ANOVA). For treatments that show a significant effect, a further test was conducted using Tukey's

test at α level of 5%. In addition, Pearson correlation analysis was also conducted to determine the relationship between characters observed.

RESULTS

The results of the research show that application of Tri-Po and its combination with the OPEFB did not significantly affect the width of stomatal opening in the epidermis of the lower leaves, weight of 100 grains and yield per plot. On other hand, the OPEFB compost variance had a significant effect to the parameters observed except for the stomatal density. The Tukeys analysis at 5% level in Table 1 showed that the use of 20 t ha⁻¹ OPEFB compost resulted in higher responses in stomatal density, the width of stomatal opening and the weight of 100 grains parameters compared to the other dosages. In general, the compost treatment was significantly different with control or without compost treatment in terms of stomatal density, width of stomatal opening and weight of 100 grains.

The interaction between the compost treatment and Tri-Po had a significant influence on the leaves' water content, the relative humidity of leaves and stomatal conductance, internal CO₂ content, transpiration rate and assimilation rate (Table 2). In general result in Table 2, the given of some compost dosages did not significantly differ to leaf physiological parameters on P1, P2 and P3 combination of Tri-Po, except the light interception. In the P₄ combination, the given of compost levels has a dynamic pattern to the leaf physiological responses. As for, the application of some Tri-Po combination could increase the leaf physiological characters. For leaf water content, the P₂ and P₃ have a better response than P_1 and P_4 combination. Based on this character, a combination of 10 t ha⁻¹ OPEFB compost dosage (C₁) and Tri-Po 4g:6g (P₃) was the best combination (27.9%). For the relative humidity of leaves, Tri-Po P2 treatment has the greatest effect on this character. However, the effect did not significantly differ from P₃. The best combination between compost and Tri-Po dosage in the relative humidity of leaves was a combination of C_0 and P_2 (89.9%). For the stomatal

Table 1: Average of width of stomatal opening, weight of 100 grains and yield at 14% water content of Soybean on oil palm empty fruit bunches compost treatments

OPEFB compost	Width of stomatal	th of stomatal Weight of		
dosage (t ha ⁻¹)	opening (µm²)	100 grains (g)	Yield (g)	
0 (C ₀)	0.00135 ^b	13.49 ^b	633.32°	
10 (C ₁)	0.00163 ^{ab}	15.29ª	893.75 ^ь	
20 (C ₂)	0.00189ª	15.66ª	1223.71ª	
Tukey's α 0.05	0.00049	0.75	249.49	

Numbers followed by different letters in the columns are significantly different

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Table 2: Average of leave soybean physiological characters on the combination of OPEFB compost and Tri-Po

	OPEFB compost dosage (t ha ⁻¹)	Tri-Po	Tri-Po			
Parameters		 4 g:2 g (p1)	4 g:4 g (p2)	4 g:6 g (p3)	4 g:8 g (p4)	Tukey's compost at 5%
Leaf water content (%)	0 (C ₀)	18.6 _a q	24.8 _a ^p	24.5 ^a ^p	24.7 _a p	5.8
	10 (C ₁)	20.2 _a q	25.5 ^{apq}	27.9 _a p	20.9 ^{bq}	
	20 (C ₂)	19.0 _a q	26.3 _a p	23.9 ^{apq}	19.7 _b q	
Tukey's _{fungus} at 5%		5.03				
Relative humidity of leaves (%)	0 (C ₀)	72.0 _a r	89.9 _a p	85.2 _a pq	73.7 _a qr	
	10 (C ₁)	70.4 ^{,q}	86.2 _a p	85.8 _a p	69.5 _a q	11.99
	20 (C ₂)	68.0 _a q	87.8 _a ^p	77.5 ^{, pq}	68.0 _a q	
Tukey's _{fungus} at 5%		10.38				
Stomatal conductance (mol CO ₂ H ₂ O ⁻¹ m ⁻² s ⁻¹)	0 (C ₀)	3.08 ^a q	4.71 ^{,pq}	6.77 _a p	4.70 _a pq	3.77
	10 (C ₁)	3.08 ^{,q}	5.89 ^{, pq}	7.58 ^{, p}	4.68 ^{, pq}	
	20 (C ₂)	4.88 [°] a	5.65 ^{Pq}	7.25 ^{° pq}	5.01 ^{pq}	
Tukey's _{funaus} at 5%		2.82				
Intern CO ₂ contain (μ mol CO ₂ mol air ⁻¹)	0 (C ₀)	415.31 ^ª	441.70 _a pq	465.38 ^a ^p	441.47 _b pq	40.77
	10 (C ₁)	425.17 ^a 9	444.54 _a q	476.80 _a p	441.03 ^a 9	
	20 (C ₂)	420.96 ^a ^p	431.71 ^a	436.36 ^a ^p	418.49 ^{abp}	
Tukey's _{fungus} at 5%		30.71				
Light interception (lux)	0 (C ₀)	1038.22 _b q	1010.67 _b q	835.67 _b p	827.55 ^{ap}	199.73
	10 (C ₁)	808.67 ^ª	651.89 ^{apq}	519.33 _a p	563.22 ^{ap}	
	20 (C ₂)	870.70 ^{,q}	875.33 ^{bq}	592.44 _a P	722.23 ^{pq}	
Tukey's _{fungus} at 5%		264.33				
Transpiration rate (mol $H_2O m^{-2} s^{-1}$)	0 (C ₀)	14.90 _a q	19.14 _a p	23.53 _b r	20.03 ^p	3.6
	10 (C ₁)	17.71 [°]	19.70 [°]	27.82 [°]	18.60 [°] a	
	20 (C ₂)	17.07 ^{,q}	20.40 ^a	24.08 _b ^p	19.49 ^{,q}	
Tukey's _{fungus} at5%		3.12				
Assimilation rate (μ mol CO ₂ m ⁻² s ⁻¹)	0 (C ₀)	20.28 ^a q	23.16 ^{, p}	23.89 _a p	20.61 _b p	3.23
· -	10 (C ₁)	21.43 ^a q	24.13 ^{Pq}	25.86 ^a ^p	23.86 [°] q	
	20 (C ₂)	22.75 ^a q	24.26 _a p	25.38 ^{Pq}	22.72 ^{bq}	
Tukey's at 5%		2.42				

Numbers followed by different letters in columns (a, b) and in rows (p, q, r) for each parameter are significantly different based on the Tukey's test α 0.05



Fig. 1: Regression graph of correlation among width of stomatal opening with assimilation rate and stomatal conductance

conductance, the P₃ treatment was relative has the highest value in this character. However, this character did not significantly differ from P₂ and P₄ treatment. The best combination between compost and Tri-Po dosage in the stomatal conductance was a combination of C₁ and P₃ (7.58 mol CO₂ H₂O⁻¹ m⁻² s⁻¹). For the intern, CO₂ contains, Tri-Po P₃ treatment has the greatest effect on this character.

However, the effect did not significantly differ from P_2 . The best combination between compost and Tri-Po dosage in the intern CO_2 contain was a combination of C_1 and P_3 (476.8 µmol CO_2 mol air⁻¹). For light interception, Tri-Po P_1 treatment has the greatest effect on this character. However, the effect relative did not significant difference to P_2 . The best combination between compost and Tri-Po dosage in the light



Fig. 2(a-c): (a) Regression graphs of the relationship between leaf water content with relative humidity of leaves, transpiration rate, (b) Width of stomatal openings, stomatal conductance and leaf intercellular CO₂ and (c) The relationship between relative humidity of leaves with relative leaf water content, relative transport rates and assimilation

interception was a combination of C_0 and P_1 (1038.22 lux). For the transpiration rate, Tri-Po P_3 treatment has the greatest effect on this character. The best combination between compost and Tri-Po dosage in the transpiration rate was a combination of C_2 and P_3 (27.82 mol H_2O m⁻² s⁻¹). For the assimilation rate, the P3 treatment was relative has the highest value in these characters. However, these characters did not significantly differ from P_2 . The best combination between compost and Tri-Po dosage in the assimilation rate was a combination of C₁ and P₃ (25.86 mol CO₂ H₂O⁻¹m⁻² s⁻¹). Based on all results, the interaction of 10 t ha⁻¹ dosage with Tri-Po (4:6 g) per plant was relative has a higher response than the other treatment to these parameters.

The regression analysis show that the width of stomatal opening had positive correlation with the stomatal conductance, assimilation rate and transpiration rate of soybean (Fig. 1). The result of the experiment was obtained that the wider the stomatal opening the higher the stomatal



Fig. 3: Regression graph of the correlation among assimilation rate with weight 100 grains, grain dry weight/plot (yield) and light interception

conductance (y = 28.109x + 0.7119, r = 0.84**), assimilation rate (y = 34.078x + 17.662, r = 0.84**) and transpiration rate (y = 65.016x + 9.6533, r = 0.81**). The leaf water content had linear regression with relative humidity of leaves (y = 2.5459x + 19.289, r = 0.85**), transpiration rate (y = 0.844x + 0.8014, r = 0.76**) (Fig. 2a), width of stomatal opening (y = 4.6262x + 331.88, r = 0.79**), intercellular CO₂ (y = 0.3276x - 2.2583, r = 0.71**) and stomatal conductance (y = 0.0871x - 0.3804, r = 0.63*) (Fig. 2b). Whereas, relative humidity of leaves has linear regression to water contain of leaf (y = 0.2829x + 0.9738, r = 0.85**), transpiration rate (y = 0.1157x + 14.19, r = 0.62*) (Fig. 2c).

Based on the study, it was also obtained that assimilation rate had positive linear regression with the dry weight of 100 grains (y = 0.4372x + 4.6753, $r = 0.70^*$) and dry weight of grain per plot (y = 65.07x + 2284.7, $r = 0.68^*$). However, it had negative regression to light interception (y = 99.599x - 1393.1, $r = 0.64^*$) (Fig. 3). The negative correlation between assimilation rate and light interception indicated that the higher assimilation rate would increase the growth and development of leaf area. It caused the more amount of light absorbed by the leaves and the less light passes to the surface under the plant canopy.

DISCUSSION

The research showed that the OPEFB compost could improve some physiological and yield component characters of soybean grown under oil palm stands, except for the stomatal density (Table 1 and 2). The OPEFB compost could increase the organic matter and soil organic carbon¹⁶. These would improve soil physical characteristics, especially to the increase of soil water retention and water capture capacity, the stability of soil structure at various scales and the change of the soil thermal characteristic¹⁷. Besides that, the addition of soil organic matter could improve the cation exchange capacity, pH stability, the increase of the soil nutrition availability (especially N, P, K, S) and promoted the soil minerals to bind the organic matter^{18,19}. Therefore, higher dose of the OPEFB compost given could improve the soybean growth parameters when planted in the low light condition such as under the oil palm stands.

The addition of *Trichoderma* sp. and *Pleurotus* sp. (Tri-Po) improved the leaf physiological performance of soybean under oil palm stands. The Tri-Po fungus could speed the degradation of cellulose, hemicellulose and lignin in organic matter by enzyme production. The kinds of the enzyme produced by the Tri-Po were cellulase enzyme, laccase to degrade the hemicellulose and lignin, lignin Peroxidase (Li-P) and Mn peroxidase (Mn-P)²⁰⁻²². In addition, the decomposition process of organic residue from Tri-Po compost also increased the pH soil, released the human material dissolved and the dissolved aliphatic organic acid²³. Therefore, the addition of Tri-Po help the degradation of OPEFB, hence the plant nutritions were available faster.

Based on the study, the combination of 10 kg ha⁻¹ OPEFB and 4g:6 g Tri-Po was found to be the best formula to improve the physiological characters of soybean under palm oil stands. Improvement in these characters could be attributed to a better photosynthesis process. In general, the microorganism had been reported as biodegradation of organic matter^{24,25}. Each microorganism has a specific function in degrading organic matter. Therefore, the microbial community composition and its interaction among the microorganism have an important role to keep the performance of the composting process and effectiveness in the degradation of the organic matter²⁶. In addition, the source and count of organic matter also have the influence to determine the effectiveness of microorganism performances²⁷. Therefore, the combination was considered as the best combination in improving the growth and physiology character of soybean under oil palm stands.

The impact of the soil characteristics improvement could contribute to the better root capability in absorbing cations and water. Stable water absorption by the plant would increase the leaf water content and the dissolved cation in a leaf cell so that the guard cell turgidity increased. The increase of turgidity in guard cells could increase the stomatal opening and then it influenced some plant physiology regulated process depended on by the environment. The stomatal opening was measured as CO₂ conductance. It was continually various with environmental changes (light intensity, CO₂ concentration in the atmosphere, temperature and humidity, wind, period and plant water status)^{28,29}. Based on the result of the present study, the correlation between the width of stomatal opening and stomatal conductance caused the increase of diffusion rate and concentration of CO₂ into leaf intercellular spaces. The high water content and CO₂ concentration in intercellular space would increase the assimilation rate due to increased in Rubisco enzyme on the C₃ plants, like Soybean³⁰. Therefore, Tri-Po Compost has a positive impact on Soybean growth.

The plant growth and productivity were phenotypes from the combination of genetics, environment and the interaction of both aspect. If the good plant material had been optimized in cultivation system, then the environmental factors (macro and micro) were the main factor that determined the plant growth and production. Some variables could be relevant influencing assimilation activity and plant development and at the end, would determine the yield. One of the important abiotic factors in controlling carbon and water flow into the plant was soil humidity³¹. The OPEFB compost treatment was thought to increase organic matter and organic carbon in the soil. In the end, it could increase the soil water content. The soil water level determined how much water that has been extracted by plant root and arranged stomatal conductance. Eventually, it determined the plant water status, the yield rate of the primer biomass and the transpiration rate^{32,33}. Besides that, according to Palacio et al.34, the soil humidity could arrange plant growth through the allocation of the carbon exchange.

The negative correlation shown between the plant assimilation rate and light intercept and the positive correlation between the weight of 100 grains and the yield was due to the increase of water and plant nutrition supply. These increases were positive impacts from the combination of compost and Tri-Po treatment. The water supply could induct plant growth through new cell growth, especially xylem and phloem³⁰. Xylem was the transportation tissue which brought water and dissolved nutrition into a shoot through stem and branch. The water transport in xylem could be used to change the lost water due to transpiration so that it was connected with the photosynthesis process^{35,36}. In other hands, phloem was the transportation tissue brought carbohydrate from leaves as the source to meristematic and storage tissue as sink³⁷⁻³⁹. The xylem and phloem tissues each on both were interacted through osmotic pressure exchange and water potential, arranged water and carbohydrate transportation in the plant^{40,41} and turgidity pressure determination. Therefore, they were interacted to modify transpiration and primer productivity of plants⁴².

The treatment without OPEFB organic compost would be faster to undergo water limitation. The long period of soil water limitation could induce widespread hydraulic damage (cavitation)⁴³⁻⁴⁵ and decrease the immune to against the pathogen affecting to the low of the plant growth and yield. The soil humidity was also an important regulator in the heterotrophic respiration⁴⁶, which represented half of the total CO₂ emission of soil. The low soil humidity limited the heterotrophic respiration rate through the decline of dissolved compound transport which could induce microorganism dormition in extreme drought stress⁴⁶⁻⁴⁹.

Soil moisture conditions also regulate surface temperature because evaporation is a more effective cooling mechanism than heating⁵⁰. Thus changes in surface temperature will modify respiration and various biological processes: lower soil conditions and humidity⁵¹. The combination of various mechanisms such as those that have been described can ultimately improve the growth and productivity of soybean under 4-year-old oil palm stands.

CONCLUSION

Conclusively, the use of compost of the oil palm empty fruit bunches and the combination of the fungus *Trichoderma harzianum* and *Pleurotus ostreatus*, can improve the biological, physical and chemical properties of the soil, improve physiological performance and productivity of soybean plants grown under 4-years old oil palm stands. The combination of 10 kg ha⁻¹ OPEFB and 4g:6 g Tri-Po was the best formula to improve the physiological characters of soybean under oil palm stands. The result of this research is expected to increase the economic value of oil palm plantation in Indonesia.

SIGNIFICANCE STATEMENT

This study discovered the combination of oil palm empty fruit bunches (OPEFB) with *Trichoderma harzianum* and *Pleurotus ostreatus* in creating compost that can be beneficial for improving the physiological characters and yield component of soybean grown under oil palm stands. This study will help the researchers to uncover the critical areas in the intercropping system improvement in land use of palm oil plantation based on the combination of OPEFB with *Trichoderma harzianum* and *Pleurotus ostreatus* that many researchers were not able to explore. Thus, a new theory on the use of compost from a combination of OPEFB with *Trichoderma harzianum* and *Pleurotus ostreatus* in increasing the growth and yield of an intercropped plant in palm oil plantation may be arrived at.

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