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Research Article
Chemical Analysis and Microorganisms of Compost of Rice Harvest Residues Through Various Kinds of Decomposers

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Abstract
Background and Objective: Rice residues such as rice straw and rice husks are very abundant in Indonesia. However, until now the remaining rice harvest has not been utilized properly. Whereas, the rest of the rice harvest can be used as organic material that can increase agricultural productivity by making compost. Therefore, this study aims to determine the most effective decomposer to be used as an acceleration in composting the remaining rice harvest. **Materials and Methods:** The method used in this study was to compare 4 decomposers namely PGPR, EM 4, Semanggi and Garuda in composting rice straw and rice husks. After that, the compost was analyzed for its chemical properties and bacterial diversity. **Results:** The results of the bacterial analysis found 26 bacteria which 4 were gram-negative with genera *Pseudomonas* and 22 were gram-positive, with 9 genera of *Coryneform/Streptomyces* and 12 genera of *Clostridium*. From the chemical analysis, it was found that there were differences in decomposition activity during composting due to differences in compost materials, as indicated by the observed physical and chemical parameters. **Conclusion:** The highest production of C-organic was straw compost with Garuda decomposer at 39.47%, also in straw Garuda decomposer compost were found 4 different genera of bacteria which mean it has a good of diversity microorganism.

Key words: Compost decomposers, compost microorganisms, rice harvest residue, rice husk, rice straw, inorganic fertilizers, water retention

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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

The high Rice production in East Java has increased the rice harvest residues. Rice harvest residues can be determined by rice straw and rice husk. Rice straw is one of the major agricultural waste amounts and has not been fully utilized[1]. Rice straw has several advantages over the waste that is others, such as corn residue, sweet potato leaves, sugar cane leaves, peanut rending and soybean biomass. Thus, the rice straw is very potential to be used as a source of nutrients or organic fertilizer[2]. This organic matter is a buffer and serves to improve the physical, chemical and biological properties of the soil. Soil that is poor in organic matter will reduce the ability to support inorganic fertilizers so fertilization efficiency decreases because most of the fertilizer will be lost through washing, fixation or evaporation and as a result decreased productivity.

Soil physical, chemical and biological conditions are strongly influenced by soil organic matter, this is because organic matter consisting of several carbon compounds can maintain soil quality[3]. Soil organic matter also affects soil physical such as porosity, water content or water retention[4]. In the dry season, the problem of drought in the tropics is still a major problem that affects crop yields. This problem can be overcome with organic matter because it also has functions in modifying the dynamics of groundwater and increasing water holding capacity[5]. That's why organic matter can reduce the possibility of drought during the dry season. The difference between soils that are given organic matter and those that are not is physical, which has a lower bulk density and higher aggregate stability[6]. Organic matter from plant residues such as rice straw can improve soil quality.

In general, agricultural residue products are a source of organic matter potential. However, the high C/N ratio of organic matter is a major obstacle (C/N ratio of coffee husk 140, rice straw 80). As a result, the decomposition process will naturally run longer. One of the efforts to speed up the composting process is done by providing decomposers. Various types of decomposers such as *Trichoderma* spp., have been used frequently[7]. To accelerate the straw decomposition its needs a decomposer that can produce cellulase like fungi and bacteria[8].

In this study, 4 different decomposers were used, such as PGPR, EM 4, Semanggi and Garuda. Therefore, this study aims to determine the most effective decomposer for each type of organic material rice harvest residue that is decomposed.

MATERIALS AND METHODS

Research design: This research was conducted in Blitar and Malang from June to September, 2022. This research was conducted using a factorial randomized block design (RAKF) with two factors, the first factor was 4 types of decomposers and the second factor was 2 parts of the corn harvest residues with the total combination of treatments being 8. The first factor is the type of decomposer consisting of (1) Plant Growth Promoting Rhizobacter (PGPR), (2) Effective microorganisms (EM-4), (3) Semanggi and (4) Garuda. The second factor in the form of the remaining part of the corn harvest consists of (1) Rice straw and (2) Rice husk. The combination of treatments was shown in Table 1.

Composting methods: This research begins by making compost with different types of decomposers and different harvested residues. Green rice straw was chopped into 1-3 cm. The dry rice husks were moistened first. The remaining rice harvest was taken at 120 kg per treatment. The composting technique was carried out by reversing, composting is done by piling plant residues in a 20-25 cm thick compost box then sprinkling them with the required bio-decomposer, then stacking them again on top of organic material and so on then covered with black plastic to maintain moisture. Maintain the temperature of 40-50°C.

Compost observation: After the composting process is complete, the next step in this research is to make observations on several physical and chemical analyzes of compost, in the form of temperature, pH, water content, NPK content and weight loss. As well as analysis of compost microorganisms, in the form of gram staining, KOH test, oxidative-fermentative (OF) test, fluorescent pigment in media King's B and growth in YDC selective media. Weight loss value was calculated using the formula:

$$\text{Weight loss (\%)} = \frac{W_0 - W_1}{W_0} \times 100$$

Where:

W_1 = Weight after decomposition

W_0 = Weight before decomposition

Table 1: Treatments combination

Treatment	Rice straw	Rice husk
PGPR	Rice straw+PGPR	Rice husk+PGPR
EM-4	Rice straw+EM-4	Rice husk+EM-4
Semanggi	Rice straw+Semanggi	Rice husk+Semanggi
Garuda	Rice straw+Garuda	Rice husk+Garuda

Weight loss data obtained were analyzed using Analysis of Variance (ANOVA) at the 5% level, if the test results obtained a significant effect, then continued with a comparison test between treatments using least significance different (LSD) at the 5% level.

Compost chemical analysis: Analysis of the organic carbon content of fertilizers was carried out using the Walkley-Black method using a spectrophotometer technique[9]. The Walkley and Black method is a wet oxidation process for sample extraction. The analysis begins with preparing 0.05 g of compost sample which is put into a glass beaker. Then add 5 mL of 1 N $K_2Cr_2O_7$ reagent and 7 mL of H_2SO_2. Then the mixture is heated for 30 min. The mixture that has been heated is then added with distilled water up to 100 mL, then let the solution stands for 24 hrs. Then do the absorption of the standard glucose solution and also the mixture of sample solutions that have been prepared with a single UV-Visible spectrophotometer using a wavelength of 561 nm. The value of C-organic was then calculated by the equation:

$$\text{C-organic (\%)} = \text{Calculated (ppm)} \times \frac{100}{\text{Sample (mg)}} \times \frac{\text{Extract (mL)}}{\text{1000 mL}} \times \text{cf}$$

Compost microorganism analysis

Isolation of bacteria: Isolation of bacteria using multilevel dilution method according to Pepper *et al.*[10]. Each compost sample was analyzed for bacterial diversity, 1 g of the sample was diluted with 10^{-1}-10^{-9} dd H_2O and 100 µL of 10^{-7} and 10^{-9} dilution was spread on NA medium with spread methods. After incubation for 24 hrs in RT, the bacteria were purified. Purification was carried out on each bacterial colony that had a different colony morphology. Colony morphology observations included the shape, colour, elevation, surface and the edge of bacterial colonies. Each colony whose density has been calculated is taken using an Ose needle and then grown on a new NA medium. Bacterial colonies were incubated for 24-48 hrs at RT.

Bacterial characterization and identification: The stages of bacterial identification to the genus stage refer to Schaad *et al.*[11] and Bergey's manual of determinative bacteriology test methods in the identification process are as follows:

Gram staining: Gram staining is used to differentiate between Gram-positive and Gram-negative bacteria, which assists in the analysis and differentiation of different microorganisms. These bacteria were first treated with the primary stain known as crystal violet (5%). In the second step, it was treated with Gram's iodine. Then used safranin for the last step. Subsequent observations using a microscope with a magnification Gram staining indicate that if the bacteria are gram-positive, the colour is bluish purple if the bacteria are gram-negative then red.

Potassium hydroxide (KOH) test: The KOH test was carried out by taking 2 drops of KOH and then adding one dose of the bacterial isolate to the object glass. The bacterial suspension was stirred with an Ose needle rapidly and slightly removed several times. If when the Ose is removed there is mucus, it forms like a thread and is sticky, it is a Gram-negative bacteria. Meanwhile, if it is watery and not slimy then the bacteria are Gram-positive bacteria.

Oksidatif-fermentatif (OF) test: The OF test is a test to determine the ability of bacteria to decompose glucose. The media used in this test is based on the formula of Hugh and Leifson[12] using oxidative-fermentative media with a composition of 5 g per 500 mL.

Fluorescent pigment in media King's B: Bacterial isolates were grown on King's B media and incubated for 24-48 hrs at room temperature. Bacterial growth was observed under UV light. Bacteria that emit a green or bluish colour and fluoresce are *Pseudomonas* bacteria from the "fluorescent" group, otherwise, the results will be negative.

Growth in YDC selective media: Bacterial testing on YDC selective media was carried out to distinguish Erwinia bacteria from Pantoea. One Ose of the bacterial isolate was grown on YDC media. The bacteria were incubated for 48 hrs at 30°C and then observed. If the bacterial colony is yellow, it means the bacteria of the genus Pantoea, while if it is white, it means the bacteria Erwinia.

RESULTS AND DISCUSSION

This research used 4 different decomposers: PGPR, EM 4, Semanggi and Garuda. The second factor is rice residues: rice straw and rice husk. The total combination of treatments is 8 combinations. The temperature of the compost will increase at the beginning of composting and at the end of composting it will be stable at temperatures below 35°C as shown in Fig. 1. Compost pH data in Fig. 2 showed that compost made from straw tends to be more acidic at the four decomposers with a pH below 5, while husk compost tends to be close to neutral with the pH at the end of composting being at 6.6-6.73.

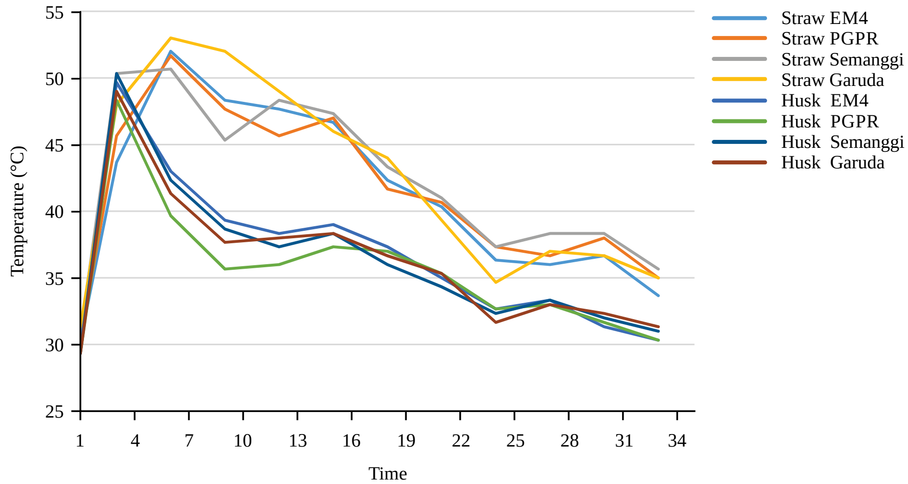

Fig. 1: Temperature of straw and rice husk compost at various decomposers

Fig. 2: pH of straw and rice husk compost at various decomposers

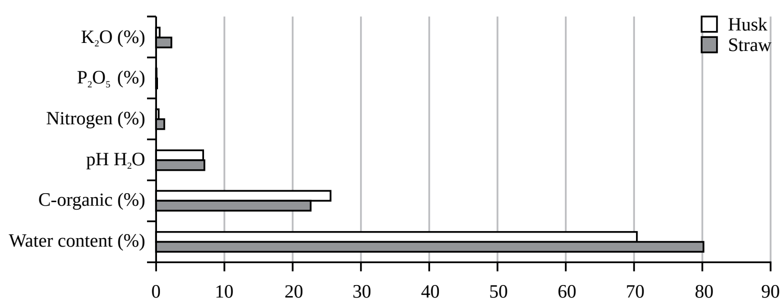

Fig. 3: Result of chemical content analysis before composting

X-axis: Value of compost material water content (%), C-Organic (%), pH, Nitrogen (%), P_2O_5 (%) and K_2O (%) before composting, Y-axis: Compost material chemical analysis before composting (compost water content (%), C-Organic (%), pH, Nitrogen (%), P_2O_5 and K_2O (%))

When compared to before composting (Fig. 3), the four decomposers were able to increase the chemical content of the compost. The results of the analysis of the chemical content of compost (Fig. 4), showed that the water content of compost made from straw was higher than that of husk material. The water content of PGPR compost straw compost

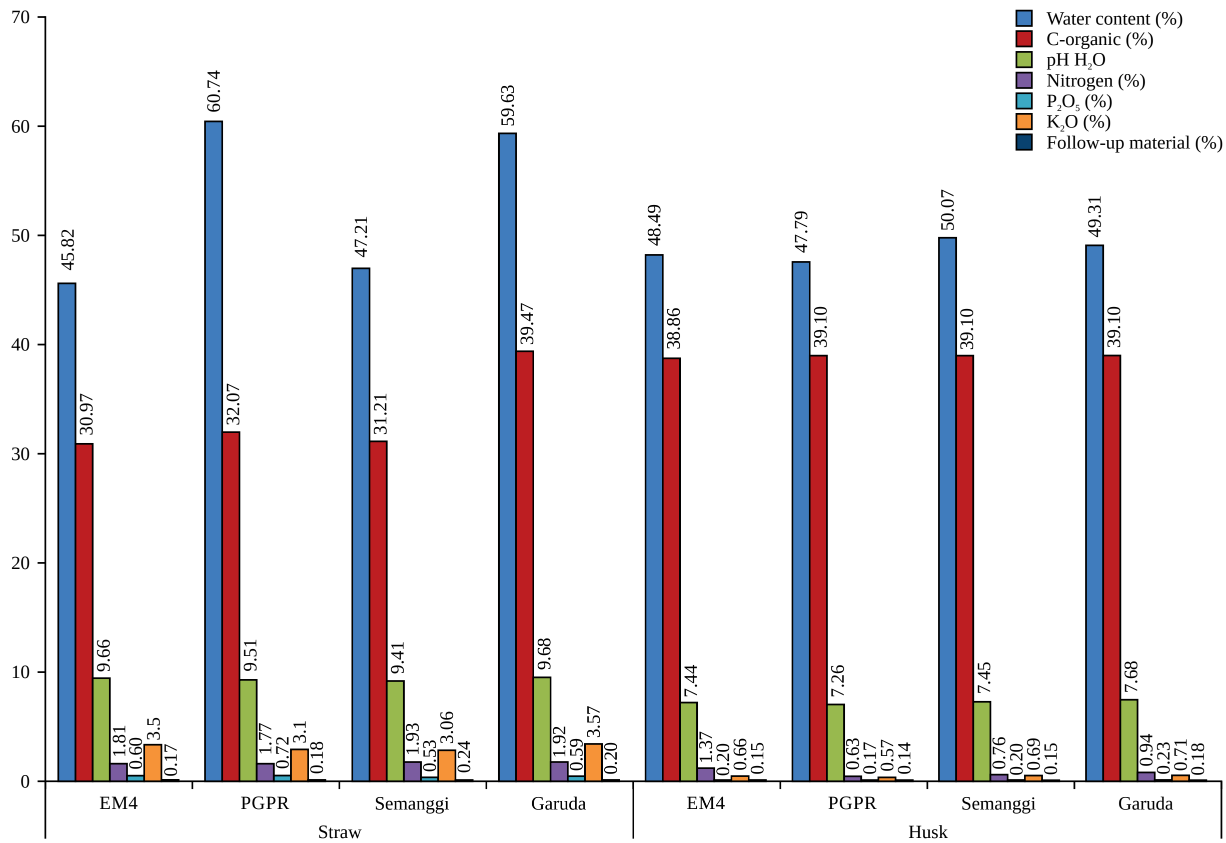

Fig. 4: Result of chemical content analysis after composting

X-axis: Types of compost and decomposer materials used, Y-axis: Value of compost water content (%), C-Organic (%), pH, nitrogen (%), $P_2O_5K_2O$ (%) and Follow-up material (%)

Table 2: Interaction of compost materials and decomposers on weight loss of compost

	Decomposer			
	EM4	PGPR	Semanggi	Garuda
Straw	28.11^{a}	36.83^{b}	38.02^{b}	39.36^{bc}
Husk	41.94^{bc}	43.36^{bc}	43.71^{bc}	45.96^{c}
LSD 5%	7.53			

Numbers accompanied by the same superscripted letter in the same column and row show that they are not significantly different based on the 5% LSD test

reaches 60.74%, in straw compost the most effective way to suppress the water content of compost using an EM4 decomposer is 45.82%. The C-organic content of straw compost is lower than that of husk compost. In straw compost, the Garuda decomposer produced 39.47 C-organic but in other decomposers, C-organic was less than 35%, while in husk compost the four C-organic decomposers were at a value of 38.86-39.10%. However, the total nitrogen and P_2O_5 content of straw compost have better content than the husk.

The weight loss of compost in straw with EM4 is the lowest, meaning that the use of EM4 in straw material is effective in suppressing the weight loss of compost as shown in Table 2. Meanwhile, in the husk material in the four decomposers, the weight loss reached more than 40%. From 8 combinations of compost, the result of microorganisms was shown in Table 1.

The results of the bacterial analysis in Table 3 found 26 bacteria which 4 were gram-negative (Fig. 5a) with genera *Pseudomonas* and 22 were gram-positive (Fig. 5b), with 9 genera of *Coryneform/Streptomyces*, 12 genera of *Clostridium*. It's widely known that there is more than one type of bacteria in the soil. Such as *Bacillus*, *Acetobacter*, *Clostridium*, *Azotobacter* and others. One bacteria has more specific functions than others. For example, *Clostridium pasteurianum* is a bacteria that can reshuffle organic matter and its role in nitrogen fixation. *Clostridium* spp., is a Gram-positive bacteria, that forms bacilli, capable of fermentation, negative catalase and negative oxidation and requires a medium enriched with yeast extract, vitamin K and haemin[13]. Many Coryneform bacteria are isolated from plants, soil and animals. Bacteria such as *Corynebacterium glutamicum* and *Corynebacterium ammoniagenes* are used in biotechnological production processes[14]. One of the microorganisms that have an important role in fertilizing the soil is bacteria.

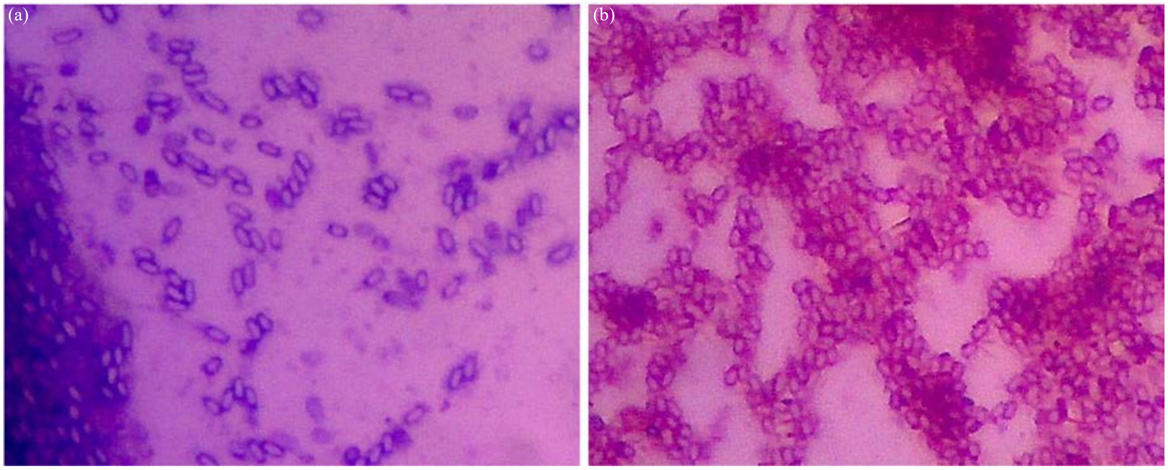

Fig. 5(a-b): Result of gram staining (a) Straw Garuda-2 as gram-positive bacteria (+) and (b) Husk Semanggi-3 as gram-negative bacteria

Table 3: Result of bacterial analysis

Isolate	Gram staining	KOH (33%)	Endospora	OF	YDC medium	King's B medium	Genus
Straw EM4 1	+	+	+	*	*	*	*Clostridium*
Straw EM4 2	+	+	+	*	*	*	*Clostridium*
Straw EM4 3	+	+	-	*	*	*	*Coryneform/Streptomyces*
Straw PGPR 1	+	+	+	*	*	*	*Clostridium*
Straw PGPR 2	+	+	-	*	*	*	*Coryneform/Streptomyces*
Straw PGPR 3	+	+	+	*	*	*	*Clostridium*
Straw Semanggi	+	+	-	*	*	*	*Coryneform/Streptomyces*
Straw Garuda 1	+	+	-	*	*	*	*Coryneform/Streptomyces*
Straw Garuda 2	+	+	+	*	*	*	*Clostridium*
Straw Garuda 3	+	+	-	*	*	*	*Coryneform/Streptomyces*
Straw Garuda 5	-	+	*	F	*	+	*Pseudomonas*
Husk EM4 1	+	+	-	*	*	*	*Coryneform/Streptomyces*
Husk EM4 2	+	+	+	*	*	*	*Clostridium*
Husk EM4 3	-	-	*	F	*	+	*Pseudomonas*
Husk EM4 4	+	+	-	*	*	*	*Coryneform/Streptomyces*
Husk PGPR 1	+	+	-	*	*	*	*Coryneform/Streptomyces*
Husk PGPR 3	+	+	+	*	*	*	*Clostridium*
Husk PGPR 4	+	-	+	*	*	*	*Clostridium*
Husk PGPR 6	+	+	-	*	*	*	*Coryneform/Streptomyces*
Husk Semanggi 1	+	+	-	*	*	*	*Coryneform/Streptomyces*
Husk Semanggi 2	-	-	*	F	*	+	*Pseudomonas*
Husk Semanggi 3	-	-	*	F	*	+	*Pseudomonas*
Husk Garuda 1	+	+	-	*	*	*	*Coryneform/Streptomyces*
Husk Garuda 2	+	+	-	*	*	*	*Coryneform/Streptomyces*
Husk Garuda 3	+	+	+	*	*	*	*Clostridium*
Husk Garuda 6	+	+	+	*	*	*	*Clostridium*

+: Positive reaction, -: Negative reaction, O: Oksidatif, F: Fermentatif and *Not tested

The quality and time of composting are influenced by environmental and biological factors, like temperature moisture and oxygen level[15]. The type of biomass can affect the complexity of the degraded material and the final product of the compost. Therefore, various agricultural by-products have been used to find the best raw materials for the growth of microorganisms. Composting is a decomposition process that involves microbes and the most biodegradable decomposers. de Bertoldi and Civilini[16] found that in the cooling and maturation phases of compost there was an increase in the diversity of both Gram-positive and Gram-negative bacteria.

CONCLUSION

This study aims to determine the best decomposer among PGPR, EM 4, Semanggi and Garuda in composting rice straw and rice husk. Of all the decomposers found 26 bacteria 4 were gram-negative with genera *Pseudomonas* and 22 were gram-positive), with 9 genera of *Coryneform/Streptomyces* and 12 genera of *Clostridium*. The result found that the highest production of C-organic was straw compost with Garuda decomposer at 39.47%, also in straw Garuda decomposer compost were found 4 bacteria which means it has a good diversity of microorganisms.

SIGNIFICANCE STATEMENT

One of the principles of zero waste and low external input is sustainable agriculture, one of which is the use of organic fertilizers from crop residues that are returned to the land for nutrient intake in the land. Rice plant waste is generally not used properly by the community and even a lot of it is removed from the land. Identification of the quality of the compost from the remaining rice harvest is very important to calculate the nutrient requirements of the plant. This study focuses on the types of decomposers and analysis of the quality of the composting results with the 4 types of decomposers available.

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