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THE ABILITY OF *TRICHODERMA* SP AND *PLEUROTUS* SP FOR THE DECOMPOSITION OF OIL PALM EMPTY BUNCHES

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ABSTRACT

The empty bunches of palm can-not directly decompose into compost because it is still in the form of complex elements, so it must be degraded first, and the process of degradation naturally takes a very long time, for which the fungus used to accelerate the process of degradation. The fungus has a ligninolytic ability, namely the ability to dissipate enzymes that can degrade lignin. Materials used in this study include empty fruit bunches (EFB), bran, and dolomite lime. The ingredients were evenly mixed and then inoculated with the fungus in four treatments, which were without mushroom fungus, *Pleurotus* sp, *Tramella* sp, and *Trichoderma* sp. This study shows several attributes related to the quality of compost such as temperature, pH, macronutrient and C: N ratio. The treatment of *Trichoderma* sponge inoculation gives the best quality compared to others. The C / N ratio after 8 weeks of decomposition is 22.09. It can be concluded that the *Trichoderma* spray inoculation treatment can be a potential biodecomposer for EFB.

Keywords: EFB, fungus, *Pleurotus* sp, *Trichoderma* sp, *Tramella* sp.

INTRODUCTION

It has been widely acknowledged that in palm oil processing industry, the main wastes are empty fruit bunches (EFB). In the processing, for every-one ton of fresh fruit bunches as much as 0.21 ton 21% of crude palm oil (CPO) and 0.05 ton 5% of palm kernel oil (PKO) were produced, as well as the remaining waste 23% of the EFB, 13.5% of fiber, and 5.5% of seed shells, (Kavitha *et al*., 2013). EFB contains various macro and micro nutrients that are respectively very important for plant growth, among others: 0.8% N; 0.22% P_2O_5; 2.9% K_2O; 42.8% C; 0.30% MgO, 23 ppm Cu, and 51 ppm Zn (Singh *et al*., 1989).

Indonesia's palm oil production in 2010 amounted to 13,468,966 tons of 4,391,624 ha of land. This number increased 29.67% compared to the year 2014 which amounted to 17,464,905 tons for the same land area. Today, the increase of the oil palm plantation area reached 27.31% and every year the area and the production are also increasing (BPS 2016). Thus, EFB waste is also increasing. The waste if not processed will cause serious problems for the environment. Currently, there have been several introduced solutions for utilization of EFB such as: materials for steam generator at the plant (Ma *et al*., 1993), EFB compost (Shafawati and Siddiquee, 2013), and mulches in plantations (Mohammad, 2012). However, these solutions are considered ineffective and costly.

One problem of composting the EFB in traditional method is that it takes too long (months or years). This is due to the high C:N ratio and existence of polymers such as cellulose and lignin which act as natural barrier that hamper the natural biodegradation. However, according to Gaind and Nain (2007), addition of specific microorganisms can cut the composting time of EFB. Goyal *et al*., (2005) stated that the fungus helps in organic matter decomposition by actively decomposing cellulose, hemicellulose, and lignin. Due to the ability in producing enzymes for decreasing cellulose, hemicellulose and lignin (Shafawati *et al*., 2014; Gaind *et al*., 2006), inoculation of cellulolytic fungi such as *Aspergillus*, *Pleurotus* and *Trichoderma* can accelerate composting process by one month (Biswas and Narayanasamy, 2002; Amira *et al*., 2012), *Trichoderma* species have been shown to effectively degrade organic materials, as biological control agents, and produce toxins against phytopathogenic fungi that can increase plant growth (Zainuddin and Faridah, 2008; Pandya and Saraf, 2010).

Other researches confirmed the role of *Trichoderma* for the issue in hand. The *Trichoderma reesei* was capable in improving soil biochemical properties (Gaind and Nain, 2007), whereas. Pandya and Saraf (2010) reported this *Trichoderma* genus are important biocontrol agent against some phytopathogenic fungi. More specifically, Amira et al., (2012) added *Trichoderma virens* to the

composting of empty bunches and palm oil mill waste which because of the higher levels of enzymatic activity, this application resulted in the reduce of composting period. The EFB in this study was composted with addition of three potential fungal plane strains, with the aim was to examine physio-chemical changes (temperature, pH, and macro nutrient content) during the process of the composting that had been inoculated by the three types of fungus.

MATERIALS AND METHODS

Materials: Materials used in this study include EFB, bran, and dolomite lime with a composition of 5: 1: 0.05. EFB is derived from Luwu Unit 1 Palm Oil Plant, Lagego Village, Burau District, East Luwu Regency, Indonesia. The bile was collected from a rice mill in Pattimang Village, Malangke. Lime dolomite bought from a farm shop. EFB was chopped into small pieces of about 5-10 cm in size to accelerate decomposition.

Implementation of the study: The material was mixed evenly and then inoculated with the moldy fungus in four treatments. Treatment 1 (K0): No Fungus (Control), Treatment 2 (K1): *Tramella* sp Fungus, Treatment 3 (K2): *Pleurotus* sp Fungus, and Treatment 4 (K3): *Trichoderma* sp. The fungus is derived from the collection of Puslitbag Agricultural Biotechnology, Hasanuddin University. Composting is done on a 200 x 100 x 80 cm tub with aeration and water discharge holes which located on the sides and bottom of the tub. Compost is covered with tarpaulins and reversed every 2 weeks and placed in the shade for up to 8 weeks to speed up decomposition.

Observation Temperature and pH: Temperature of composting was observed every day and the humidity was set to always around 60%, then left until composting was complete. Water content, carbon, nitrogen, phosphorus, potassium, calcium, and magnesium in the compost were measured and in order to determine the C: N ratio. Weekly temperature measurement was performed during the composting process as in Erwan *et al.* (2012). Three different places were taken for this temperature measurement i.e. the top, middle and bottom of the compost heap where the thermometer was inserted and left for five minutes prior for each reading. Compost pH was measured every week during the composting period by reading the pH of solution which were obtained by the procedure, about 10 g of compost were dissolved into a 500 mL glass of water then added with 50 ml of sterile water, this solution were then shaken for about 30 minutes with a shaker (Jeong and Kim, 2001).

Measurement of NPK and C: N; Each 15 g sample was taken and put into the furnace for 5 hours. The first one- hour temperature was 300 °C and the next 4 hours was 500 °C. Then the sample was rested overnight before the ashes were weighed. Erwan *et al*. (2012) suggested the formula for calculating the percentage of carbon content formulas follows:

$$\%\ of\ Carbon = \frac{(Sample\ of\ dry\ weight) - Weight\ of\ ash}{Sample\ dry\ weight}\ x\ 100\%$$

As for the Nitrogen content determination, the digestive method was employed with the following procedures. At a temperature of 60 °C, each sample was dried for 72 hours, it was then filtered using 20 mm mesh. As much as 0.25 g of the sample is mixed with 5 ml of concentrated sulfuric acid (H_2SO_4) into the gut flask and for 30 minutes were digested at 200 °C. The temperature was then raised to 360°C for 1 hour after which 10 mL of hydrogen peroxide (H_2O_2) is added until the reaction is complete. The final solution is produced by adding 100 mL of distilled water. Nitrogen is determined by using Auto-analyser (System 4, Chemlab). The formula for calculating percentage of nitrogen content was suggested by Erwan *et al*. (2012):

$$\%\ Nitrogen = \frac{(ppm\ x\ 400\ x\ dilution)}{10^6}\ x\ 100$$

Procedures for obtaining C:N ratio calculation was started by adding about 0.50 g of finely ground samples with 3 mL of 37% HCl and 1.0 mL of 68% HNO_3 all were put into 50 mL of Kjedahl flask and then heated at 110 °C in the gastrointestinal block. This process was intended to obtain 1 mL of fixed sample solution. It is necessary to add 3 mL HCl and 1 mL HNO_3 in case that the sample color does not turn white. After cooling down, 10 mL NO_3 1.2% (v / v) was added to the sample and heated for 30 min at 80 °C. Distilled water is used for cooling down the sample and maintaining it at 20 mL volume. After cooling, and ensuring the distilled water is added to the final volume of 20 mL, the solution was then taken for analysis by using an Atomic Absorption Spectrophotometer to determine the macro nutrient values of P and K. The C: N ratio on the other hand is calculated with the following formula (Erwan *et al*., 2012):

$$C:N\ ratio = \frac{Total\ carbon}{Total\ nitrogen}$$

RESULT AND DISCUSSION

Changes in pH and Temperature: Figure 1 illustrates different temperatures recorded during composting process. All treatments had a range of starting temperature from 25 to 36°C, while the ambient temperature was 30°C. Compost temperature increased sharply during the first three weeks, then gradually decreased until the final stages of composting. The highest recorded temperatures were 50 °C of K0 (Without Fungus) treatment, 55ºC of K1 treatment (*Pleurotus* sp), 53 °C of K2 treatment (*Tramella* sp), and 59ºC of K3 treatment (*Trichoderma* sp). The pH value of the compost is also presented in Figure 1. The pH values range from 5.4 to 7.8 at the beginning of composting. All treatments had fluctuating pH values and tend to be more acidic. At the end of the composting process, the pH values of each treatment are K0 (5,3), K1 (6,1), K2 (6,4) and K3 (6,9).

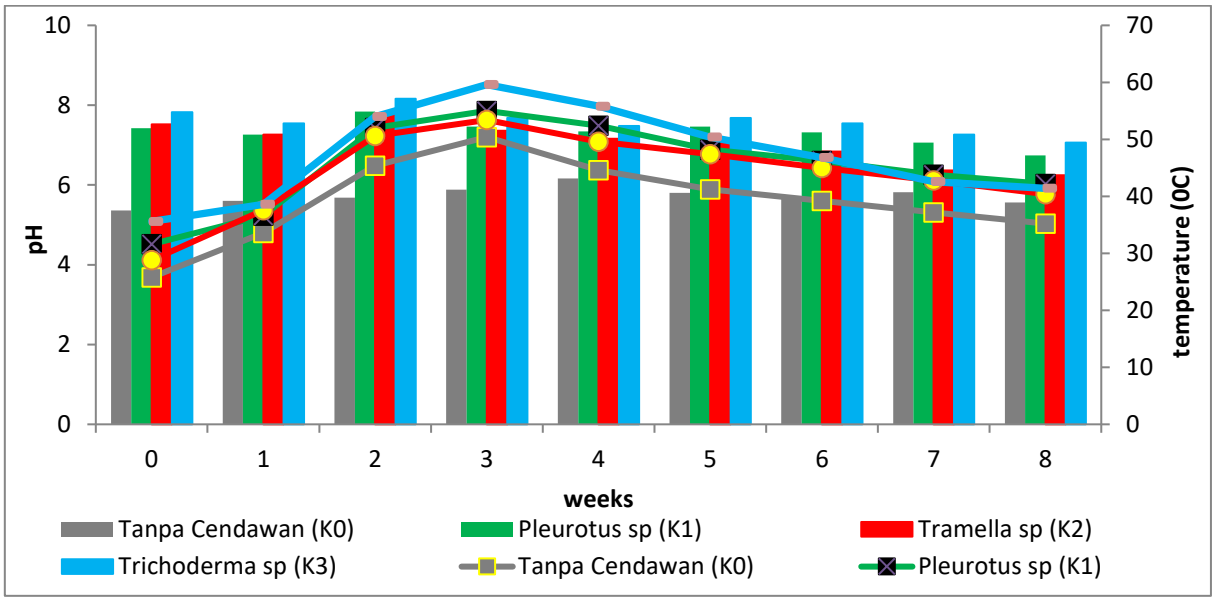

Figure 1: Changes in temperature and pH of EFB compost in treatment without fungus (K0), Pleurotus sp (K1), Tramella sp (K2) and Trichoderma sp (K3).

One important parameter in assessing composting process is temperature. The temperature rises and fall during the process which could be the result of organisms' dynamic related to their type, metabolism and growth rates in the compost (Tiquia and Tam, 2002). The results showed that the temperature values were in the thermophilic range from 40 to 65 °C (Bernal *et al*., 2009). In this study, all treatments except K0 (without fungus inoculation) reached the 55 °C, this temperature is required to kill pathogenic microorganisms (Kala *et al*., 2009). The temperature rise was due to metabolic heat from the activity of the high decomposition and high cellulose (EFB) compost. In general, the temperature at all treatments varies. After three weeks, the temperature started fluctuating, though still above the ambient temperature. This fluctuation was achieved in the first 21 days of composting due to EFB biodegradation by the fungus. After eight weeks, the compost temperature approaches ambient temperature; maybe the EFB biodegradation is almost complete and the process is stabilizing. This occurs because environmental conditions support the occurrence of biodegradation by the molding fungus.

Changes in pH due to acid formation during the EFB decomposition process by the fungus. In the first week, all fungal inoculation treatments showed a small decrease in pH. Thereafter, the pH of all fungal inoculation treatments increased during the second week. This increase was the result of decomposition of organic matter, the degradation of acidic compounds, such as carboxylic and phenol, and mineralization of other organic compounds such as proteins, amino acids, ammonia and peptides (Ishak *et al*., 2014; Rana and Dahot, 2017). Compost pH at the end of composting is more acidic than early composting. This is due to the nitrification that decreases the pH of the compost. The pH decrease was significant when the compost was mixed with *T. Reesei* (Gaind and Nain, 2007). Optimum pH for compost is 6.5-8 (Said-Pullicino *et al*., 2007). During composting process, microorganisms digest organic matter which result in decrease in pH value in the first week. Thereafter, the pH value significantly incre-

ased at the decomposition stage. The increase is due to increased ammonia (Gajalakshmi and Abbasi, 2008; Petric *et al*., 2009).

Ingredients of macronutrient elements: Intrubulated EFB composite *Trichoderma* sp had the highest value for the three main macro nutrients (Nitrogen), phosphorus and potassium. The N, P, K, values respectively were 1.22; 2.61; 8.12 observed at week 8, and these are better compared to the inoculated compound of *Pleurotus* sp, *Tramella* sp, and control (Figures 2a, 2b, and 3a). The amount of macronutrients found increased from week 4 to week 8. Erwan *et al*. (2012) suggested that C and N influence the content of macronutrient in the compost. Chabbey (1993) stated that the high macronutrient value is a result of decomposition of organic content during the composting process. The high nitrogen content of the compost inoculated by the fungus was compared with the controls due to the increased decomposition of organic materials by *Trichoderma* sp (Pramanik *et al*., 2007). This is in line with the Gaind and Nain (2007) study on the effect of inoculant fungal mixtures, it was found that nitrogen content increased significantly in the mixed fungal inoculants compared to that with single inoculation. Moreover, Wong and Saddler (1992) added that the high nitrogen content of compost was also determined by the initial nitrogen content of the used raw materials.

High phosphorus content in the three composts inoculated by the fungus (Fig. 2). This is because the phosphorus was not lost during the composting process due to evaporation or lixiviation. Instead, the concentration of phosphorus increased as a result of composting (Wong and Saddler (1992). Gaind and Nain (2007) suggested that the inclusion of fungi may enhance phosphatase activity.Correspondingly, the potassium content of the three composts was higher than that without inoculation. The release of PO_4^- ions from the colloidal humor into the system become the cause of this (Pramanik *et al*., 2007). Overall, Amira *et al*., (2012) therefore suggested that the presence of fungal strains plays an important role in increasing phosphorus and potassium content during the composting process.

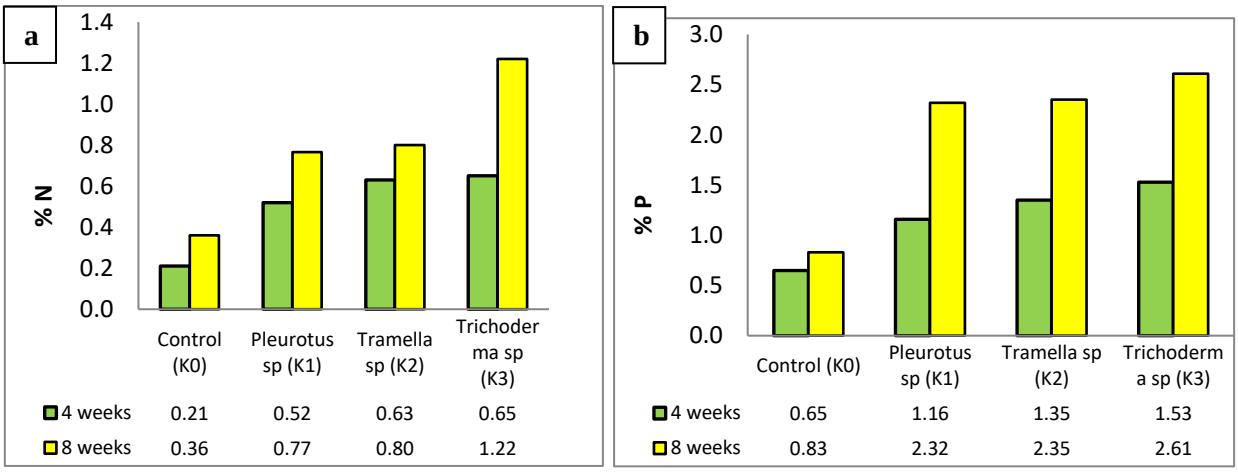

Figure 2: Nitrogen (a) and phosphorus (b) analysis during field trial

Ratio of C: N: The change in C:N ratio of EFB composts of all treatments is shown in Figure 3b. The highest C: N ratio was obtained in the 36.58 fungus control compound; followed by compost treatment of *Tramella* sp 26,68; compost treatment *Pleurotus* sp 24,38 and compost treatment *Trichoderma* sp 22,09. The C: N ratio describes the portion of C per N unit required by microorganisms. The C: N ratio is a significant parameter when compost is about to be applied as fertilizer on the soil. With high C: N ratio, it can inhibit the decomposition and availability of nitrogen in the soil. During composting process, the C: N ratio decreased due to the conversion of organic C to CO_2, and the loss of nitrogen in the form of NH_3 (Ishak *et al*., 2014).

This study identified C: N ratio decrease for all treatments during the composting process (Figure 3b). As above, the C: N ratio decreased due to the mineralization of organic materials by microorganisms (Kala *et al*., 2009). According to Shilev *et al*., (2007), mature compost has a C: N ratio of less than or equal to 25. Nagasaki *et al*., (1992) suggested that the ratio of C: N to compost should be in the range of 16 to 25. The results showed that the inoculation treatment of *Pleurotus* sp and *Trichoderma* sp fungi was in the range of C: N optimum ratio (≤25) and ready for use as organic fertilizer.

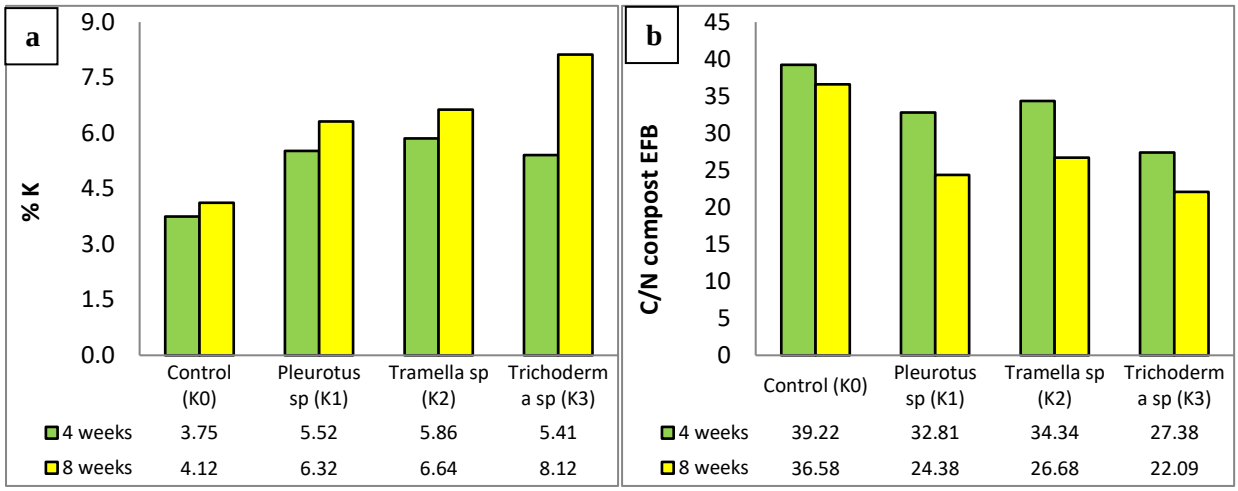

Figure 3. Potassium analysis (a) and C: N (b) ratio during field trial

CONCLUSION

Inoculation of the fungus accelerates the composting process, improves the physical and chemical properties as well as contributes to the nutrient contents of the compost. This study identified the quality of compost under different parameters such as temperature, pH, macro nutrient and C: N ratio. The treatment with fungal inoculation of *Trichoderma* sp and *Pleurotus* sp provides better quality compost than other treatments with C: N ratios of 22.09 and 24.38 respectively, indicating that they are ready for use as organic fertilizer.

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