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# THE UTILIZATION OF MUSHROOM WASTE SUBSTRATE IN PRODUCING VERMICOMPOST: THE DECOMPOSER CAPACITY OF *LUMBRICUS RUBELLUS*, *EISENIA FETIDA* AND *EUDRILUS EUGENIAE*

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The objective of this study was to determine the capacity of *Lumbricus rubellus*, *Eisenia fetida* and *Eudrilus eugeniae* earthworms in vermicompost production utilizing mushroom waste substrate based on weight; number and weight loss of earthworms; temperature; pH; moisture content of media; and C/N ratio. The results showed that, by using 42 g of *E. eugeniae*, *E. fetida* and *L. rubellus* earthworms, there was an increase in weight of earthworms and vermicompost by more than 300% and 75%, respectively. In general, these three species of earthworms were able to produce vermicompost in compliance with quality standards, showing C/N ratio lower than 20.

**Keywords:** earthworms; mushroom waste substrate; vermicompost

Demand for mushrooms as a protein source is steadily increasing (Mukherjee and Nandi, 2004). Over the past 35 years, mushroom production increased 25-fold, from approx. 1 billion kg in 1978 to 27 billion kg in 2012 (Royse, 2014). Increased mushroom production results in an increase in the amount of waste substrate (Hanafi et al., 2018) – approx. 5 kg per mushroom substrate (Zisopoulos et al., 2016). The major components of mushroom waste substrate are sawdust; banana leaves; peanut shells; corn leaves and husk; sugarcane leaves; wheat straw; cotton waste; or paper waste (Jordan et al., 2008; Sendi et al., 2013), which are degraded during the process of mushroom production (Roy et al., 2015).

Mushroom waste substrate can be converted into valuable material by composting (Garg et al., 2006). This process is simple, economically valuable and safe for human health and environment as a waste management technology (Kovačić et al., 2019). Composting can be performed utilizing e.g. earthworms – this is known as vermicomposting (Suthar and Singh, 2008). Earthworms break down and degrade the waste by ingesting the organic materials, i.e. plant litter, soil organic matter and mineral soil particles (Bath et al., 2017; Vidal et al., 2019). Contents of plant nutrients, hormones, beneficial enzymes and microbes in vermicompost is higher in contrast to conventional compost (Bajal et al., 2019). However, the process of vermicomposting is very dependent on the type of substrate and species of earthworm used (Sinha, 2009; A'ali et al., 2017). Usually, mushroom waste substrate with sawdust is treated using certain species of earthworm, such as *Lumbricus rubellus*, *Lumbricus terrestris*, *Eisenia fetida*, *Eisenia andrei* and *Eudrilus eugeniae* (Tajbakhsh et al., 2008; Izyan et al., 2009; Suparno et al., 2013).

Numerous researchers have dealt with vermicomposting of various types of substrate using different earthworm species. Chaulagin et al. (2017) reported that a mixture of cow manure and sawdust in vermicomposting increase the weight and number of worms of *Eisenia foetida* species. Use of *Lumbricus rubellus* in composting of mushroom waste substrate mixed with goat manure resulted in vermicompost with a high content of N, P, K (Bakar et al., 2012; Jamaludin et al., 2012) and low C/N ratio (Jamaludin et al., 2012).

Addition of nutrient solution containing protein, fat, cellulose, phosphorus and microbes to mushroom waste substrate can increase both the earthworm growth and vermicompost (Tran, 2016). Based on the existing studies, earthworms are often used as decomposer in vermicomposting process (Safdar and Kor, 2014). However, the description of earthworms in production of vermicompost on the basis of the mushroom waste substrate has not yet been thoroughly investigated. Therefore, the aim is to observe the capacity of *Lumbricus rubellus*, *Eisenia fetida* and *Eudrilus eugeniae* as decomposers in vermicompost production using mushroom waste substrate.

## Material and methods

The study was conducted in Karangsoka village, Kembaran subdistrict, Banyumas district, central Java, Indonesia in February to April 2018. Randomized complete block design (RCBD) with four different substrate treatments by 42 g, 84 g, 126 g, and 168 g of earthworms of *Lumbricus rubellus*, *Eisenia fetida* and *Eudrilus eugeniae* species was tested with four replications. Mushroom waste substrate was obtained

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from oyster mushroom farmers "Agro Jamur Pabuaran" in Baturraden subdistrict, Banyumas district, Central Java, Indonesia. Mushroom waste as compost material was stored for 7 days after harvest. The earthworms with age of approx. 2.5 months were obtained from Sumbang subdistrict, Banyumas district, Central Java, Indonesia.

### Preparation for cultivation of earthworms

Firstly, mushroom waste substrate for cultivating earthworms was taken from the plastic bag, crushed to a smaller size and then filtered using a sieve with a diameter of 50 mm. A total of 42 kg of mushroom waste substrate (with ±70% moisture content and C/N ratio of 48.54) was put into a plastic container for the purposes of vermi bed. Subsequently, the acclimatization process was carried out for each vermi bed and nitrogen nutrition of approx. 67.5 g was added to each. It was then evenly mixed and kept to meet the requirements for composting, i.e. temperature <30 °C (Manaf et al., 2009) and C/N ratio approx. 20–30 (FAO, 2015). After completion of acclimatization process, six 40 cm long pipes with a diameter of 3.81 cm were put in each container and 28 holes with a diameter of 5 mm each were made on sides in order to facilitate air circulation. Pipes were put vertically to the bottom of the container. In addition, thermometer was placed in the middle of the vermi bed in a vertical position below the surface (±20 cm) and the container was kept closed.

### Cultivation of earthworms

Acclimatization of earthworms was carried out after reaching the standard vermi bed conditions, i.e. temperature of 29 °C; pH of 7; and C/N ratio of 24 as a result of prior preparations. Finally, ten earthworms were put on the vermi bed surface within 48 hours for acclimatization process to observe the earthworms response. The vermi bed proved to be appropriate, as the earthworms stayed inside. Therefore, the process of cultivation could begin, and 42–168 g of earthworms of *Lumbricus rubellus*, *Eisenia fetida*, and *Eudrilus eugeniae* species were put in vermi bed, depending on the treatment.

During the vermicomposting process, the vermi bed moisture content was maintained at approx. 70% by spraying water using a hand sprayer and manual reversing was performed every two weeks in order to ensure good degradation of material at both the top and bottom. Earthworms were taken from compost after the compost C/N ratio was relatively constant by removing the vermi bed and spreading it to separate and collect the earthworms.

### Analysis method

Temperature, pH and moisture content of the media were monitored using an alcohol thermometer, pH meter and hygrometer, respectively, on a daily basis from the very start of the experiment until the last day of experiment. The weight of earthworms was counted before their application to vermi bed and at the end of the experiment using a Mini Pesa digital scale TB-01 model and their number was manually counted in the same manner. Vermicompost weight was measured at the end of the experiment utilizing Excellent ACS-A Portable digital scale. Total organic carbon content was determined using partial oxidation method; total nitrogen was determined by means of a Kjeldahl method. Total organic carbon and nitrogen contents, as well as C/N ratio, were calculated every 15 days for 75 days of the experiment.

### Statistical analysis

Variance analysis ($\alpha = 0.05$) was employed to examine the differences in observed variables of different treatments. If the observed differences were significant, the results were processed using Duncan's Multiple Range Test (DMRT) with $\alpha = 0.05$.

## Results and discussion

### Weight and number of earthworms

The number and weight of *L. rubellus*, *E. fetida* and *E. eugeniae* earthworms in mushroom waste substrate showed significant changes at the end of composting (Table 1). The initial weight of 168 g of earthworms resulted in high weight, but low enhancement percentage. Low initial weight of 42 g of earthworms had the opposite effect (Table 1). The biggest earthworm population at the end of composting was obtained by cultivating *E. fetida* – it increased by 73.26%. The highest enhancement percentage was obtained by *E. eugeniae* – initial population of 42 earthworms increased by 345.82%, ending with 187 earthworms.

Higher earthworm population in culture media resulted in the lower earthworm weight percentage. Safdar and Kor (2014) reported that, under ideal conditions, earthworms are able to consume the amount exceeding their body weight each day; however, they commonly consume the amount equal to approx. half of their body weight per day. However, the amount of food supplied to earthworms must be controlled to avoid worm mortality (Vodounnou et al., 2016; Musyoka et al., 2019). Getachew et al. (2018) reported that 50 kg of earthworms is able to consume 375 kg of feed within one month during the breeding process. Therefore, food must be added before it is completely consumed during the earthworm cultivation process in order to gain the maximum population growth. If no food is added to the container, earthworm population growth is inhibited.

### Weight of vermicompost

The vermicompost weight produced in each treatment ranged from 27.8 to 30.6 g with weight loss percentage of 27.2–33.8% (Table 2). Vermicompost production is very dependent on the earthworm species used (Kostecka et al., 2018) and the type of substrate used for feeding of earthworms (Saikrithika et al., 2015). *E. eugeniae*, *E. fetida* and *L. rubellus* earthworm species are epigeic or litter dweller group earthworms and play a critical role in the decomposition of organic matter into vermicompost (Gomez-Brandon et al., 2012; Chatelain and Mathieu, 2017; Ramesh et al., 2018). Therefore, the earthworms will produce the same amount of vermicompost in weight as is the original weight of mushroom waste substrate in the pre-treatment. The increasing earthworm population resulted in reduction of the vermicompost weight due to the fact that the substrate was not only converted into vermicompost but

**Table 1** Weight and number of *E. eugeniae*, *E. fetida* and *L. rubellus* earthworms in vermicomposting

| Treatment | Weight of earthworms | | | Number of earthworms | | |
|---|---|---|---|---|---|---|
| | at initial (g) | at the end (g) | increase (%) | at initial | at the end | increase (%) |
| **42 g** *E. eugeniae* | 42 | 183.5 a | 336.90 e | 42 | 187 a | 345.82 f |
| **84 g** *E. eugeniae* | 84 | 269.9 b | 221.28 bcd | 84 | 275 cd | 227.84 d |
| **126 g** *E. eugeniae* | 126 | 362.0 c | 187.30 abc | 126 | 369 f | 193.16 bc |
| **168 g** *E. eugeniae* | 168 | 457.8 d | 172.47 a | 168 | 467 gh | 178.03bc |
| **42 g** *E. fetida* | 42 | 184.3 a | 338.69 e | 88 | 236 bc | 168.43bc |
| **84 g** *E. fetida* | 84 | 272.8 b | 224.70 cd | 175 | 350 ef | 99.82 a |
| **126 g** *E. fetida* | 126 | 368.5 c | 192.46 abcd | 263 | 472 h | 79.63 a |
| **168 g** *E. fetida* | 168 | 473.0 d | 181.55 ab | 350 | 606 j | 73.26 a |
| **42 g** *L. rubellus* | 42 | 187.5 a | 346.43 e | 53 | 213 ab | 302.02 e |
| **84 g** *L. rubellus* | 84 | 277.8 b | 230.65 d | 105 | 316 de | 200.60 cd |
| **126 g** *L. rubellus* | 126 | 377.5 c | 199.60 abcd | 158 | 429 g | 171.50 bc |
| **168 g** *L. rubellus* | 168 | 490.0 d | 191.64 abcd | 210 | 557 i | 165.13 b |

In the same column, the number followed by the same letter indicates no significant difference according to the DMRT at $\alpha = 0.05$ and each letter indicates the enhancement: the first letter of the alphabet suggests the smaller increase and the last of the alphabet letter suggests the greater increase

also served for the growth and development of earthworms. In order to multiply the vermicompost production, substrate addition must be performed in line with the earthworm population increase. High rate of earthworm growth and reproduction must be supported by optimal feeding (Siddique et al., 2005; Klok, 2007; Chauhan and Singh, 2013).

During composting, the temperature, pH and moisture in vermi bed were in ranges 27–28 °C, 6.4–6.7 and 68.5–70.3%, respectively (Table 3). It was a suitable living environment for observed earthworms. The favourable conditions are: 15–30 °C for temperature (Manaf et al., 2009); 4.3–8.3 for pH (Singh et al., 2005); and 59.5–77.9% for moisture content (Parvaresh et al., 2004; Palsania et al., 2008).

## Total organic carbon (TOC)

Different initial weight of earthworms used showed the same pattern of decrease in TOC by approx. 40–44% in all treatments (Fig. 1). Gougoulias et al. (2014) reported that certain amount of carbon is used as a microbial energy source in the decomposition process and is released in the form of $CO_2$, while the rest of it is assimilated to a microbial biomass form. Decreasing level of TOC is caused by the activity of respiration and assimilation of microorganisms and earthworms that can convert organic C into $CO_2$ (Suthar and Gairola, 2014). The addition of *E. eugeniae* showed that decrease in TOC was more rapid in contrast to treatments with *L. rubellus* and *E. fetida*; this is due to the fact that

**Table 2** Development of mushroom waste substrate weight treated using *E. eugeniae*, *E. fetida* and *L. rubellus* earthworms

| Treatment | Weight of vermicompost (× 1,000 g) | Loss of weight (%) |
|---|---|---|
| **42 g** *E. eugeniae* | 30.6 a | 27.2 a |
| **84 g** *E. eugeniae* | 29.7 a | 29.2 a |
| **126 g** *E. eugeniae* | 28.9 a | 31.1 a |
| **168 g** *E. eugeniae* | 28.3 a | 32.5 a |
| **42 g** *E. fetida* | 29.3 a | 30.2 a |
| **84 g** *E. fetida* | 28.9 a | 31.3 a |
| **126 g** *E. fetida* | 28.3 a | 32.5 a |
| **168 g** *E. fetida* | 27.8 a | 33.8 a |
| **42 g** *L. rubellus* | 29.2 a | 30.5 a |
| **84 g** *L. rubellus* | 28.9 a | 31.3 a |
| **126 g** *L. rubellus* | 27.9 a | 33.5 a |
| **168 g** *L. rubellus* | 28.1 a | 33.1 a |

In the same column, the number followed by the same letter indicates no significant difference in weight reduction according to the DMRT at $\alpha = 0.05$. The initial weight of mushroom waste substrate was 42 kg

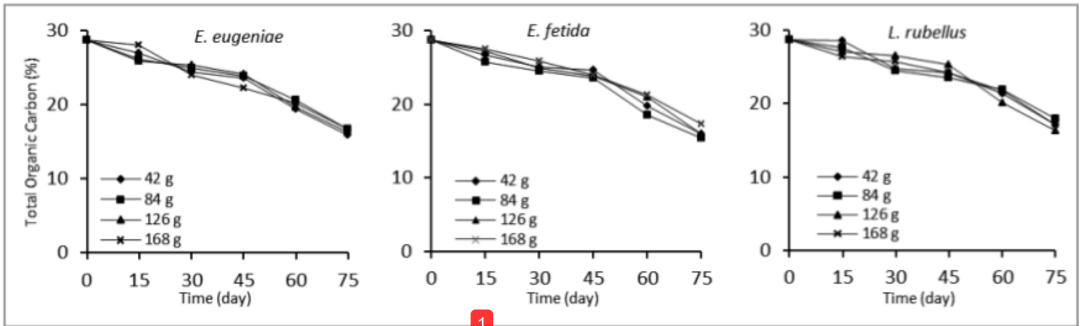


**Fig. 1** Total organic carbon in substrates treated using *E. eugeniae, E. fetida* and *L. rubellus* earthworms

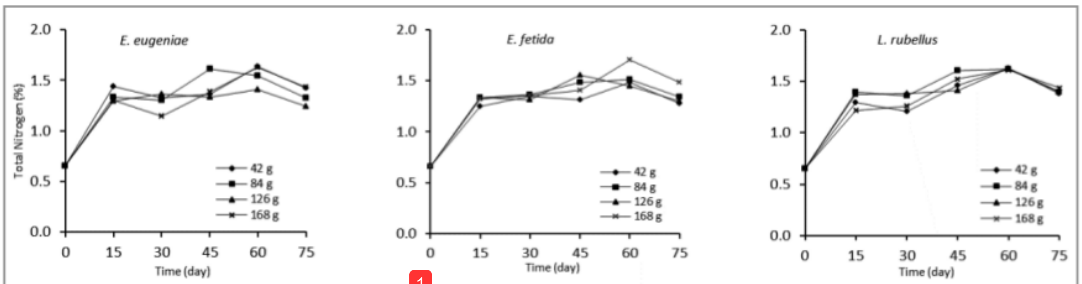


**Fig. 2** Total nitrogen in substrate treated using *E. eugeniae, E. fetida* and *L. rubellus* earthworms

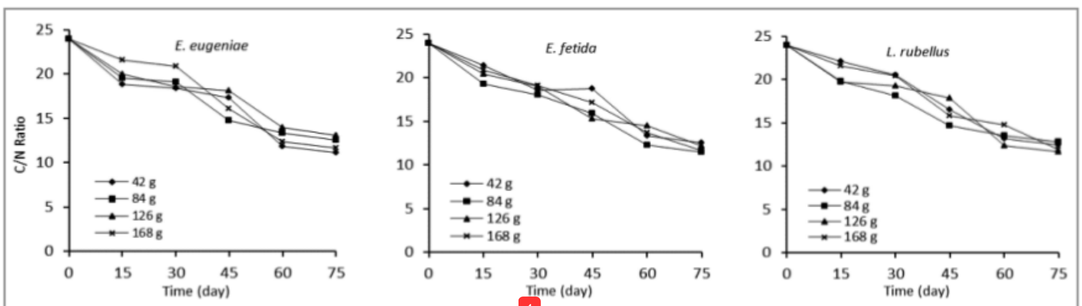


**Fig. 3** C/N ratio in vermicomposts treated using *E. eugeniae*, *E. fetida* and *L. rubellus* earthworms

*E. eugeniae* earthworm species is larger, and grows and multiplies more rapidly. Earthworm species with larger body are able to decompose larger amounts of organic matter in vermicomposting (Prabha et al., 2015).

### Total nitrogen (TN)

The TN content in vermicompost showed increasing tendency with fluctuations (Fig. 2). The fluctuation of TN content occurs due to varying activity of microorganisms and earthworms. The increase in TN can be in form of mucus, enzymes, or excretion of nitrogen-containing material; on the contrary, reduction in TN occurs due to consumption of microorganisms and earthworms themselves (Lim et al., 2012). In addition, earthworm mortality can also result in increase in TN, because the earthworm body consists of 45–65% of protein, which is a source of organic nitrogen (Lourdumary and Uma, 2012).

### C/N ratio

The C/N ratio is one of vermicompost maturity indicator. The C/N ratio under 20 indicates that the compost is mature. If the C/N ratio is equal to or lower than 15, it indicates that compost is of a high agronomic value (Suthar, 2009). The C/N ratio of all treated specimens decreased at the end of composting due to decrements in TOC content and increments in TN content. The highest reduction of C/N ratio occurred in treatment using *E. fetida* – 11.97; the final C/N ratio in composts treated using *E. eugeniae* and *L. rubellus* was 12.13 and 12.22, respectively.

Vermicomposts produced by treating the substrates by means of *E. eugeniae*, *E. fetida* and *L. rubellus* earthworm

**Table 3** Temperature, pH and humidity of media during vermicomposting of mushroom waste substrate treated using *E. eugeniae*, *E. fetida* and *L. rubellus* earthworms

| Treatment | Temperature (°C) | pH | Humidity (%) |
|---|---|---|---|
| **42 g** *E. eugeniae* | 27 | 6.5 | 68.7 |
| **84 g** *E. eugeniae* | 27 | 6.5 | 68.5 |
| **126 g** *E. eugeniae* | 27 | 6.5 | 69.3 |
| **168 g** *E. eugeniae* | 28 | 6.5 | 68.1 |
| **42 g** *E. fetida* | 27 | 6.4 | 70.0 |
| **84 g** *E. fetida* | 28 | 6.7 | 70.1 |
| **126 g** *E. fetida* | 28 | 6.7 | 70.3 |
| **168 g** *E. fetida* | 27 | 6.4 | 70.2 |
| **42 g** *L. rubellus* | 28 | 6.5 | 69.2 |
| **84 g** *L. rubellus* | 27 | 6.5 | 69.5 |
| **126 g** *L. rubellus* | 27 | 6.5 | 68.7 |
| **168 g** *L. rubellus* | 27 | 6.4 | 69.0 |

species were in compliance with mature or stable compost criteria given by the FAO (2015), indicating that observed earthworm species have the potential to serve as decomposers in the production of mushroom waste substrate vermicompost.

## Conclusion

The process of mushroom waste substrate vermicomposting using *E. eugeniae*, *E. fetida* and *L. rubellus* showed that earthworm species *E. eugeniae*, *E. fetida* and *L. rubellus* with original weight of 42 g were able to increase their weight percentage by 336.90, 338.69 and 346.4, respectively, in the observed substrate. The *E. eugeniae* earthworm population showed the highest increase – by 345.82%. Considering the criteria for vermicompost quality standards determined by the FAO, all the three earthworm species were able to produce appropriate vermicompost with *E. fetida* showing the best results (11.97).


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