



Unlocking data-driven innovation for improving productivity and data sharing in mushroom value chain

D3.4: Report on valorisation of spent mushroom substrate

DELIVERABLE NUMBER	D3.4
DELIVERABLE TITLE	D3.4: Report on valorisation of spent mushroom substrate (M34)
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Executive Agency for Higher Education, Research, Development and Innovation Funding (Romania)



Ministry of Environment and Food, Danish AgriFish Agency (Denmark)



National Research, Development and Innovation Office (Hungary) Department of Agriculture, Food and the Marine (Ireland)



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grand agreement no 862665 ICT-AGRI-FOOD.

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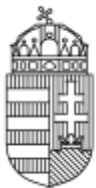
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ACRONYMS LIST

SMS	Spent Mushroom Substrate
BSFL	Black Soldier Fly
MSW	Municipal Solid Waste
T	Total number of Days
WRR	Waste Reduction Rate
WRI	Waste Reduction Indice
CR	Conversion Rate
RGR	Relative Growth Rate

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1 ABSTRACT

The linear production model is inadequate for addressing rising global food insecurity due to a growing population and diminishing production resources. A circular bioeconomy model focusses on recycling organic waste and closing nutrient loops. Spent mushroom substrate (SMS) was long considered a waste stream. However, it can be used as substrate or as feedstock for production of diversity of bioproducts.

In our current study, earthworms and black soldier fly larvae (BSFL) were used for the composting of spent mushroom substrate of *Pleurotus ostreatus* in a climate-controlled chamber at UCPH, Denmark in Taastrup campus. Organic wastes such as SMS, coco-coir, shredded papers, coffee grounds were used as feedstock for feeding BSF larvae and composting worms for 17 and 54 days respectively. The BSF larvae composting recorded higher waste reduction rate of 94.20 per cent and higher waste reduction index of 5.54 g.day⁻¹ compared to the vermicomposting (63.41 per cent and 1.17 g.day⁻¹). The study found that the conversion rate of organic waste to valuable compost was 14.98 per cent during 54 days of vermicomposting, a process that uses worms to break down the waste, while it was 2.97 per cent during 17 days of BSFL composting.. These results suggested that BSF larvae are more efficient in converting spent mushroom substrate (organic material) than vermicomposting. Our results also showed that spent mushroom substrates, viz. *Pleurotus ostreatus* SMS, can potentially be used as feedstock for BSFL and vermicomposting for production of insect protein and compost to encourage circular economy in more sustainable manner.

Keywords: Vermicompost, Black Soldier Fly, Earthworms, Spent Mushroom Substrate, Coco-coir.

2 INTRODUCTION

In recent decades, the increase in environmental pollution from municipal solid waste (MSW) has created a need for more sustainable waste management systems. Biodegradable municipal and food waste is an environmental and social problem with long-term consequences. Approximately 17 per cent of global food production is wasted, and food waste accounts for about 6 per cent of the world's total emissions (Hannah, 2020). Municipal Solid Waste (MSW) management has become a matter of global concern due to its environmental implications and the high costs associated with waste management (Marshall and Farahbakhsh, 2013). Innovation is crucial not only for reducing waste generation at production sites but also for developing new ways of waste valorization towards renewable energy production, as part of Sustainable Development Goal 7 (Affordable and clean energy). Various strategies, such as organic waste collection services, composting, and food waste prevention programs, are being implemented to address this issue and reduce its impact on the planet.

Vermicomposting is a fast and cost-effective way to turn organic waste into high-quality compost using earthworms. It produces nutrient-rich fertilizer that can improve soil structure and water retention, making it a valuable tool for sustainable agriculture (Ramnarain et al. 2019, Das et al. 2021, Katiyar et al. 2023). The waste from the coir yarn industry, known as coir pith, accumulates in large quantities, making it difficult to dispose off. This waste is used as a soil conditioner, but its disposal poses environmental challenges, such as pollution and contamination of groundwater (Christopher et al. 2007). The combustion of coir pith leads to the release of high levels of carbon dioxide and smoke due to its poor combustion properties, resulting in environmental issues such as the deposition of carbon deposits and atmospheric warming (Ghosh et al. 2007, Seal et al. 2015). Using earthworms *Eudrilus eugeniae* for coco-coir waste decomposition reduced composting time and increased the amount of compost compared to microbial composting alone (Motha et al. 2008). Hence co-composting coir compost with earthworms enhances its microbial load and nutritive value, making it suitable for commercial scale implementation.

Agricultural residues used for composting by *E. fetida* species of earthworm could produce enriched soil which can boost the yield of crops, e.g. chili and brinjal (Katiyar et al. 2023) Suthar (2008) demonstrated the use of the earthworm *Eudrilus eugeniae* to convert crop residues and cattle manure into valuable products, showcasing the potential for bioconversion to enhance product value. A study on pineapple plants demonstrated that the use of vermicompost significantly increased both the macro- and micronutrient content of the plants compared to a control application (Mahmud et al. 2020). The application of vermicompost increased tomato yield by 22.12 per cent compared to the control over two years, and significantly enhanced the nutrient content of the tomato plants ($p < 0.05$) (Metin et al. 2023). Vermicompost has better nutrients and microbial properties compared to compost, according to scientific study by Patra et al. 2022.

The lack of certain enzymes and the tough nature of plant materials called lignocellulose make it hard for these materials to break down (Kong et al., 2018). Black soldier fly larvae (BSFL) can help solve this problem by breaking down the tough plant materials. The black soldier fly (BSF), *Hermetia illucens*, is a common fly found in tropical, subtropical, and warmer regions of America, belonging to the stratiomyidae family. BSF larvae can break down a wide range of organic waste, including food waste, agri-industry co-products, animal waste, and meat-based products, making them a promising tool for waste management and animal feed production (Schmitt et al. 2019, Surendra et al. 2020). The BSF larvae reduce organic waste by half and help control house flies. The waste reduction of various substrates using BSF ranges from 39 per cent dry weight when pig manure was used (Newton et al., 2005) and 90.22 per cent when fruit pulp was used in study conducted by Khaekratoke et al. 2022. About 3346 kg with compost of C/N ratio of 20.94 and 300 kg of dried larvae was produced from composting of 10 tonnes of FW using BSF (Salomone et al., 2017).

SMS is the leftover residue from mushroom harvesting, containing about 25 per cent edible fungal residue and 75-85 per cent of the unused nutrients in the mushroom substrate (Moon et al., 2012). The 2013 global mushroom industry was worth around US\$63 billion, producing approximately 34 billion kg of mushrooms (Royse et al., 2017). Every kilogram of edible fungi harvested produces 3.25 kilograms of spent mushroom substrate (SMS) Zhao et al., 2012. SMS, is a significant source of nutritious organic waste that holds promise for recycling, particularly in the context of sustainable agriculture and waste management. Cai et al. (2017) found that feeding BSFL with a mixed diet containing *F. velutipes* root waste, wheat bran, or kitchen waste increased the conversion rate by 31.2-172.7 per cent. Another study, by using *Eisenia fetida* suggested that 65 per cent spent mushroom substrate (SMS) + 35 per cent cattle dung (CD) combination was most suitable for vermicomposting, resulting in the highest vermicompost production (57 per cent) and earthworm biomass increment (268 per cent). SMS can be combined with other organic wastes, including food waste, to serve as a food source for rearing earthworms and black soldier fly larvae (BSFL) for composting and the resulting compost can be used as organic fertilizer for crop production.

Spent coffee grounds (SCGs) are a major by-product of the coffee industry, generating a large amount of waste. Recycling this waste through biorefineries to produce fuels and value-added products is a promising solution to the challenges and heavy costs associated with waste disposal in many countries. SCGs are rich in organic compounds, including proteins, carbohydrates, tannins, fibers, and cellulose (Ballesteros et al. 2015; Blinova et al. 2017). High-quality substrates with 21% (w/w) protein and 21% (w/w) carbohydrate can result in black soldier fly larvae developing faster, to a larger size, and with a higher survival rate (Laganaro et al. 2021, Leni et al. 2021). SCGs have recently gained attention as a low-cost dietary supplement for black soldier fly (BSF) composting, which could help reduce waste and promote sustainable development.

Further research needs to be carried out to identify the best ratio of combination of organic waste material for vermicomposting by *Eisenia fetida*, *Dendrobaena hortensison* and black soldier fly to support agricultural sustainability and further reduce environmental pollution. Food waste valorization recycles food waste to

create valuable products. The current study focused on analyzing insect, like earthworm and BSF, performance for composting organic material using specific parameters for the composting process.

3 OBJECTIVES

The objective of the current study was to assess the ability of black soldier fly and composting worms to degrade spent mushroom substrate to produce value added products (insect protein, vermicompost and insect frass):

1. Assessment of spent mushroom substrate composting by *E. fetida* and *D. hortensison* (composting worms) to work out composting indices (waste reduction rate, waste reduction index, conversion rate and relative growth rate).
2. The study aimed to evaluate black soldier fly composting of spent mushroom substrate to work out composting indices (waste reduction rate, waste reduction index, conversion rate and relative growth rate).

4 MATERIALS AND METHODS

4.1 Vermi-composting

Composting earthworm species of two types *Eisenia fetida* and *Dendrobaena hortensison* were fed on spent mushroom substrate on 3 August 2023 in a climate chamber at Taastrup campus of University of Copenhagen, Denmark. Earthworms were obtained from commercial worm production Ormeposten (<https://ormeposten.dk/>), Denmark. *Eisenia fetida* was selected because of its high speed of bioconversion (Khwairakpam and Bhargava 2009), wide range of temperature tolerance, high reproduction rate, etc. The spent mushroom substrate (*Pleurotus ostreatus*) was obtained from a private mushroom producer viz. FungaFarm, Denmark for vermicomposting experiment. 4 handful samples, from the procured earthworm bag, was taken and the earthworms were counted and weighed (Fig. 1). These four different samples from the bag gave an estimate for the earthworm population (~ 211) and total earthworm weight in the box (211.89 grams). For vermicomposting, Vermi-Hut Plus was set up using bedding material (bedding is crucial for maintaining a natural environment for earthworms in vermicomposting) and the spent mushroom substrate was fed on 3rd August, 2023, in the afternoon. Bedding material consisted of shredded paper (01 per cent), untreated-soil (06 per cent), peat (12 per cent), coco-coir (19 per cent), and news-paper (17 per cent) and the remaining 45 per cent was SMS (Fig.2). This entire Vermi-Hut Plus setup (Fig. 3) was placed in a climate chamber with temperature between 16 – 20 degree Celsius and humidity between 60 – 70 per cent (Reinecke and Venter, 1987) throughout the experiment period. Care was taken to maintain a humidity level of 60-70 per cent in the vermicomposting process by adding tap water on the composting material using spray bottle. This was done to ensure optimal conditions for the worms and to enhance the ability to break down the organic matter effectively. The temperature of the material in the Vermi-Hut Plus tray was monitored by placing a thermometer at five different points daily. The temperature monitoring data helped to decide whether to add water to the material or not, ensuring optimal conditions for the worms and the decomposition process. This setup was protected from ants with the help of four ant-trappers which were filled with water to avoid ants getting into the set-up. At the end of the vermicomposting process, a number of parameters were recorded viz. total quantity of waste, residue left after bioconversion, weight of the worms at the beginning and at the end of the composting period. The full list of parameters and data can be found in Table 1 and these parameters are used to calculate the composting indices.



Fig 1: Sampling for vermicomposting study using the VermiHut plus tray

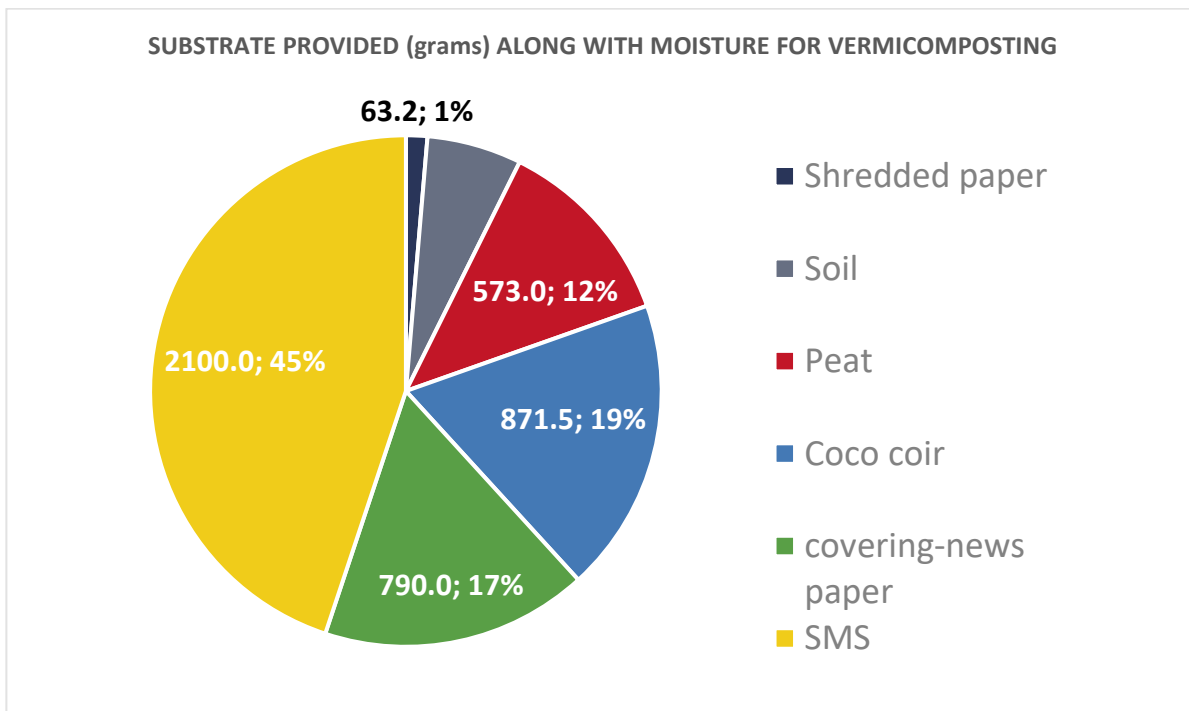


Fig 2: Quantity of bedding material and spent mushroom substrate (SMS) in grams and per cent supplied throughout the experiment period for vermicomposting.



Fig 3: Vermicomposting setup using VermiHut plus tray for composting organic material

Table 1. Parameters recorded for vermicomposting of spent mushroom substrate

S is the total quantity of substrate (grams)	R is the residue left after bioconversion (grams)	T is the bioconversion time (days)	W' is the weight of earthworm at the end (total wt. grams)	W² is the earthworm weight at the start (total wt. grams)
7952.26	2910.00	54	1403.05	211.89

During the composting process, sampling of composting worms and the amount of composted and uncomposted materials was conducted twice, as depicted in Fig 4, making sure that the tray was properly set up with even earthworm population distribution in the entire tray. T Each sampling was conducted within a 10 x 10 cm area in the Vermi-Hut Plus tray and four replications were recorded. The sampled quantity was divided into composted, un-composted, and earthworms, which were then measured using a calibrated weighing balance. The dark black, finely powdered material was considered as the composted material at the end of the experiment. The earthworms were separated from the vermicompost by hand sorting, counted, and weighed after washing with water and drying with tissue paper. At the end of the experiment, after 54 days, the hand sorting of the material was done to separate into composted, un-composted, and earthworms. The fresh weight of materials were used to calculate various vermicomposting parameters/ indices viz. waste reduction rate, reduction rate index, conversion rate and relative growth rate. These

parameters were essential for understanding the efficiency and effectiveness of the vermicomposting process in waste management.

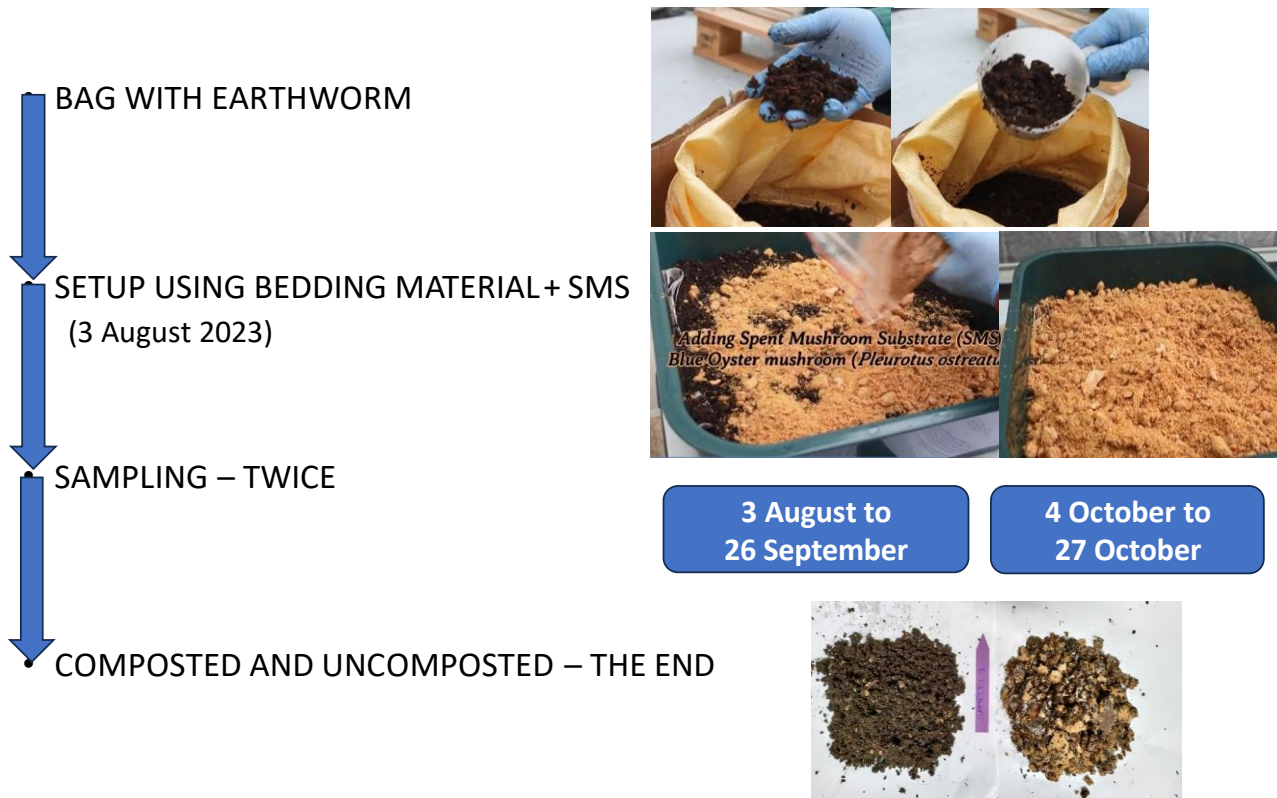


Fig 4: Work flow showing vermicomposting process

4.2 Black Soldier Fly composting:

Biopod Plus was obtained from Youngsville, North Carolina, United States to, Taastrup - 2630, UCPH, Denmark location for the study. Both the trays, nursery and rearing trays were setup inside a walk-in growth chamber under controlled temperature and humidity conditions in order to maintain and regulate humidity between 60 – 70 per cent and temperature not more than 25 degree Celsius (Harnden and Tomberlin, 2016). Eggs of black soldier fly were obtained from German based company Hermetia GmbH for the BSFL-composting study at Taastrup, UCPH, Denmark campus. Nursery tray was prepared for eggs to hatch on protein-rich chicken feed at 70 – 75 per cent moisture content (Banks, 2014). To maintain moisture level, regular warm water was sprayed using spray bottle. Small quantity of powdered wheat straw was placed at the border of tray to prevent the escape of larvae from tray. Entire nursery tray was setup for 11 days (Fig. 5) where majority of larvae reached to 4 – 5 instar stage. On the 11th day of the study, a handful of larvae samples from different points in the tray were weighed and counted to assess their abundance before the start of the composting study. These larvae which were 11 day old were used for composting experiment.

Received BSFL in gel



• Nursery tray (11 D)
(26 September to 6 October)



• Rearing tray (17 D)
(6 October to 23 October)



• Sampling twice

12 October

23 October



Fig 5: Work flow showing BSF composting process

The material composition of the bedding material was as follows: coffee-ground (8%), chicken feed (27%) and coco-coir (34%) and *Pleurotus ostreatus* species of spent mushroom substrate (SMS) accounted for 31 per cent of the total waste feed used for composting (Fig. 6). Moisture level of the bedding material was maintained between 60 – 70 per cent throughout the 17 days BSFL-composting experiment, by spraying tap water using spray bottle. A thermometer was used to measure the temperature of the Biopod Plus rearing tray. Ten black soldier fly larvae at the 5th 6th, and 7th instar stages were collected (Fig. 7) from four different sampling points during the experiment. The larvae were weighed using a calibrated weighing balance to assess differences in growth. An additional four sampling was conducted, using a 10 x 10 cm area to hand-sort and weigh composted material, uncomposted material and larvae (Table 1b). At the end on 18th October sampling of larvae was conducted from the collection bucket and fresh weight and dry weight was taken. At the end of the 17-day experiment, the larvae were separated from the composted material using a sieve (Fig. 8), and their weight was recorded. The fresh weight of both the un-composted and composted material was also measured for study. The different parameters recorded to work out the composting indices are provided in Table 2.

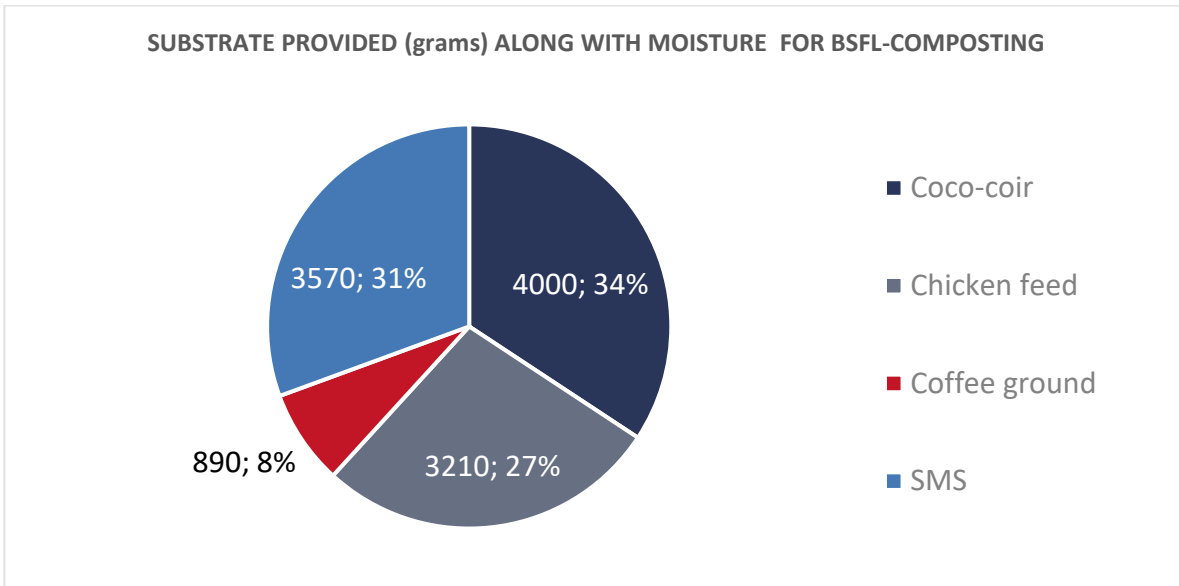


Fig 6. Quantity of bedding material and spent mushroom substrate (SMS) in grams and per cent supplied throughout the experiment period for BSFL-composting.



Fig. 7: Sampling of BSF larvae for weighing and population count



Fig. 8: sampling procedure of BSFL in the composting experiment

Table 2. Parameters recorded for BSFL composting of spent mushroom substrate

S is the total quantity of substrate (grams)	R is the residue left after bioconversion (grams)	T is the bioconversion time (days)	W¹ is the weight of BSFL at the end (total wt. grams)	W² is the BSFL weight at the start (total wt. grams)
22172.00	1286.00	17	6365.00	5707.55

5 Results

For the current study, parameters studied for vermicomposting and BSFL-composting indices were: waste reduction rate (WRR), waste rate index (WRI), conversion rate (CR) and relative growth rate (RGR). Based on measurements provided in Table 1 above on vermicomposting, we calculated the four composting indices provided in Table 3a. In similarity, the 4 composting indices calculated for BSFL composting are provided in Table 3b.

Table 3a. Composting indices for vermicomposting using *Eisenia fetida* and *Dendrobaena hortensison* species of earthworm

Indices	Formula	Unit	Value
WRR	$WRR = (S-R)/S * 100$	%	63.41
WRI	$WRI = [(S-R)/S]/T * 100$	g . day ⁻¹	1.17
CR	$CR = (W_1-W_2)/S * 100$	%	14.98
RGR	$RGR = (W_1 - W_2)/T$	g . day ⁻¹	22.06

Where: WRR (waste reduction rate), WRI (waste reduction index), CR (conversion rate) and RGR (relative growth rate).

Table 3b. Composting indices for BSFL composting

Indices	Formula	Unit	Value
WRR	$WRR = (S-R)/S * 100$	%	94.20
WRI	$WRI = [(S-R)/S]/T * 100$	g . day ⁻¹	5.54
CR	$CR = (W_1-W_2)/S * 100$	%	2.97
RGR	$RGR = (W_1-W_2)/T$	g . day ⁻¹	0.39

Where: WRR (waste reduction rate), WRI (waste reduction index), CR (conversion rate) and RGR (relative growth rate).

While calculating the indices for vermicomposting the waste reduction rate was found to be 63.41 per cent as compared to BSFL-composting which was found to be 94.20 per cent. The observation is supported by other studies where, BSFL - bioconversion of organic material was found to be 93.7 – 94.5 per cent (Aziz et al. 2023), 86.7 – 100 per cent (Nairuti et al. 2021), and 85 – 97 per cent (Nirmala et al. 2020). Similar to the current results, for vermicomposting, Shi et al. also conducted experiment using spent mushroom (*Lentinula*) substrate with different treatments for 28 to 56 days and observed composting between 48.37 to 77.05 percent. Waste reduction rate in current study of vermicomposting was comparable to the reported levels (54.86–63%) (Deka et al. 2011, Boruah et al. 2019). In the current study, the waste reduction index was higher in BSFL-composting (5.54 g.day⁻¹) compared to vermicomposting (1.17 g.day⁻¹). These two indices

demonstrated that BSFL composting was faster than vermicomposting for the quantity of SMS supplied in experiment.

Conversion rate of vermicomposting was found to be more (14.98 per cent) than the BSFL-composting (2.97 per cent). This was due to the differences in the days for both experiments. Study for BSFL-composting was conducted for 17 days and vermicomposting was carried out for 54 days. Documented conversion rate in BSFL shows that, the range of 1.0 – 1.1 for the conversion rate when the organic material was used for composting (Nairuti et al. 2021). In our study the relative growth rate was 0.39 g. day⁻¹ for BSFL population and 22.06 g.day⁻¹ for earthworm population. Grow rate of 1.41 ± 0.17 mg/day was observed when BSF larvae was fed on spent coffee ground waste in 16 day experiment (Permana et al. 2018), which is higher than our findings. It was observed in study by Finbarr et al. 2023 that 0.07 - 0.85 mg larva⁻¹ day⁻¹ was the relative growth rate of BSFL when used for the seven day decomposition of apple pomace waste. In our study, we found that the rapid degradation of waste material by BSF larvae was significantly more efficient in decomposition than earthworms.

6 CONCLUSIONS

In the current study, BSF larvae composting of waste material was carried out for 17 days and vermicomposting was for 54 day. Both composting experiments were studied for the organic waste using spent mushroom substrate, coco-coir and spent coffee ground. No adverse effect of organic material on the growth of insects was observed during the study period. Our result suggest that waste reduction rate of BSF larvae was found to be much faster than vermicomposting. Based on our results, under optimum condition, BSF larvae could convert organic waste material into compost with higher conversion rate than composting by *Eisenia fetida* and *Dendrobaena hortensison* earthworm species together. The current study can have relevance for valorization of different kinds of organic waste. Several other factors like the density of insect, nutritional quality of waste (Ash content, C:N ratio, fiber content) affects the bioconversion rate of organic waste which needs to be further studied.

BSF larvae and earthworms, can help farmers and entrepreneurs in low- and middle-income countries generate extra income by processing organic waste. This low-tech waste treatment method offers economic and environmental benefits, even with irregular waste input. This study provides the quantification of composting processes in black soldier fly composting and earthworm-composting system, which can be useful information for informed decision making for valorization of waste

7 FUNDING

MUSHNOMICS is part of the ERA-NET Cofund ICT-AGRI-FOOD, financially supported by national bodies in Denmark (Green Development and Demonstration Program under The Ministry of Food, Agriculture and Fisheries of Denmark within the framework of MUSHNOMICS project, journal number: 34009-20-1815), Hungary (National Research, Development and Innovation Office), Ireland (Department of Agriculture, Food and the Marine (DAFM)), and Romania (Romanian National Authority for Scientific Research and Innovation Funding), and co-funded by the European Union’s Horizon 2020 research and innovation program, Grant Agreement number 862665.

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