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Bioconversion of biomass residue from the cultivation of pea sprouts on spent *Pleurotus sajor-caju* compost employing *Lumbricus rubellus*

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Abstract: Vermicomposting is a green technology for the purpose of nutrient enrichment from a variety of organic waste products. In this study, saw dust-based spent mushroom compost (SMC), an organic waste and biomass residue, was used as a medium for the cultivation of pea sprouts. After harvesting the pea sprouts, the growth medium was reused to culture earthworms, *Lumbricus rubellus*. The culturing activity was conducted for 50 days without any pre-composting or thermocomposting. Thus duration of vermicomposting process was shortened as opposed to previous work on vermicomposting of saw dust-based SMC (no amendment) for 70 days. The culturing treatments were conducted in triplicate, including one treatment without earthworms as the control. The analysis showed that concentrations of macronutrients in vermicompost were higher compared to controls, in which N = 4.12%, P = 2.07% and K = 1.56%. The C:N ratio was 11.77, which indicates a stabilisation and maturity of the organic waste compost, compared with the C:N ratio for the control, which was 59.34. At the end of the experiment, increment of total biomass and number of earthworms were observed and no mortality was recorded. The results suggested that vermicomposting could be used as an environmentally valuable technology to convert saw dust used for mushroom and pea sprouts cultivation into vermicompost or bio-fertiliser by employing *L. rubellus*.

Keywords: bio-fertiliser, epigeic earthworms, spent mushroom compost, vermicomposting, waste management

INTRODUCTION

The current trend of utilising waste products as resources forms important part of green technology policies in many countries. To achieve sustainable development in any food-based industry, several novel technologies have been introduced in recent years with the aim of recycling the waste generated. Mushrooms has become an increasingly important industrial crop in Malaysia. Hence the amounts of organic waste generated from mushroom farming have multiplied accordingly. Spent mushroom compost (SMC) was generated when fruiting bodies were no longer produced, which typically occurs after six months of mushroom cultivation [1]. SMC is commonly discarded whereby more than 4000 tonnes per year were sent to landfills or openly burnt at mushroom farms. A proactive profit-earning solution to reduce the dumping of organic waste has been put forward such as thermo-composting, co-composting, vermicomposting, ruminant feed production, biofuel formation and biomass briquetting.

Vermicomposting of SMC has given the waste a value instead of having to be discarded. Vermicomposting has recently been tested with many types of organic waste [Table 1] with the aim of producing a high-quality end product in terms of nutrient content or of remediating pollutants. However, data from studies on the epigeic *Lumbricus rubellus* are limited. Vermicomposting is technically affordable and environmentally safe, compared to conventional industrial methods that require chemicals and expensive machinery. It is an eco-biotechnological process in which earthworms and associated microflora convert the organic waste into organic fertiliser utilising available forms of nutrients [2]. Moreover, vermicompost is fragmented and microbiologically active due to humification [3] and contains important plant nutrients (N, P, K and Ca) in the forms that are soluble and more easily available to plants than ordinary compost [4].

Therefore, the aims of this study are to investigate the potential of *L. rubellus* to convert SMC after cultivation of high-priced vegetables, i.e. pea sprout seeds, into a stabilised and nutrient-rich organic fertiliser, as well as to study the multiplication in number and biomass of worms during the process of vermicomposting and to measure the nutrient elements in the end product (vermicompost) compared to control (compost).

Table 1. Recent vermicomposting studies on different types of waste

Type of waste	Amendment	Earthworm species	Vermicomposting duration	Findings	Reference
Sewage sludge	Spent mushroom compost	<i>L. rubellus</i>	91 days	No foul smell and fine texture of vermicompost. Heavy metal content in vermicompost was higher compared to its initial levels due to breakdown of organic matter (explained by heavy metal and mass balance), but the content is below US and EU biosolid compost limits. It is safe to use as bio-fertiliser or soil conditioner.	[1]

Table 1. (Continued).

Type of waste	Amendment	Earthworm species	Vermicomposting duration	Findings	Reference
Human faeces	Soil and vermicompost	<i>Eisenia fetida</i>	1 year and 6 months (545 days)	Complete inactivation of total coliform	[27]
Beverage sludge	Cattle dung	<i>E. fetida</i>	4 months (120 days)	Degradation of 50:50 mixture (bio-sludge:cattle dung) achieved in 75 days when worms were inoculated at 25 g/kg feed mixture, but the best quality product was obtained after 105–110 days with 7.5 g worms/kg feed mixture.	[28]
Agro-industry sludge	Cow dung, biogas plant slurry and wheat straw	<i>E. fetida</i>	15 weeks (105 days)	40:60 (industrial sludge:cow dung) and 40:60 (industrial sludge:biogas plant slurry) showed highest mineralisation rate and earthworm growth pattern.	[10]
Municipal sewage sludge	Oyster shell	<i>E. andrei</i>	25 days	Powdered oyster shell sludge blend provided stable pH due to its buffering capacity because of the effects on the release of Ca^{2+} and OH^- .	[29]
Vegetable-market solid waste	Wheat straw, cow dung and biogas plant slurry	<i>E. fetida</i>	15 weeks (105 days)	Waste mineralisation and humification rate were higher in bedding of those containing easily digestible bulky agents, i.e. biogas slurry and cow dung.	[30]
Non-recyclable paper waste	Cow dung	<i>E. fetida</i>	91 days	FT-IR spectroscopy of vermicompost showed reduction in aliphatic compounds during vermicomposting process.	[31]
Coffee grounds and kitchen waste	Cow dung	<i>L. rubellus</i>	70 days	Coffee grounds can be decomposed through vermicomposting and help to enhance the quality of vermicompost produced compared to sole use of kitchen waste in vermicomposting.	[32]

MATERIALS AND METHODS

Cultivation of Pea Sprouts on Spent *Pleurotus Sajor-Caju* Compost (SMC)

Spent *P. sajor-caju* (grey oyster mushroom) compost was removed from plastic bags and broken up. A layer of the compost was filled into a tray to 5-cm height and moistened by spraying with tap water. Pea sprout seeds were soaked in tap water and then arranged on the moistened compost

at 1 cm apart. The trays were incubated at room temperature with regular spraying of tap water. Pea sprouts produced were harvested after 7-10 days. Two harvests were obtained before the compost was subjected to vermicomposting.

Vermicomposting Experiment

Clitellated earthworms (*L. rubellus*) were selected from a stock culture in the Earthworm Reservoir, Institute of Biological Sciences, University of Malaya. The treatment was carried out in an artificially designed microcosm (360 mm × 280 mm × 200 mm) with a net (250 mm × 100 mm) covering the centre of the lid to allow for aerobic exchange, to prevent any form of interruption and to ensure that the microclimate was maintained. Experiments were conducted in triplicate (T_A, T_B and T_C) with one control. The control received an identical treatment with no earthworms.

Thirty clitellated earthworms of approximately the same size were introduced into each bin containing 300 g of SMC and in the fourth week, another 300 g of the same substrate was added. The pH and temperature of the SMC were measured and an optimum level of pH 7±1 and a temperature of 27±1°C were achieved. Due to the optimum and stabilised pH and temperature at the initial period of vermicomposting, no pre-composting period was required. A pre-composting period is normally included to avoid the exposure of earthworms to high temperature during the initial thermophilic stage of microbial decomposition [5].

Vermicomposting lasted for 50 days. During this process, the moisture content of the feed materials was maintained at 60–70% by constantly spraying distilled water onto the surface, in combination with a manual turning of the feed material over once every few days to remove any stagnant water. At the end of the study period, the upper layer of the vermicompost produced in the plastic bin was sampled (~100 g with moisture content, i.e. 60%) for analysis of nutrient elements before all the earthworms were removed [6]. The upper layer was sampled because this was the first layer converted into vermicompost, which was classified by its fine, odourless texture. The number of living earthworms was determined after hand sorting and removal of all extraneous material. The biomass gain of earthworms was calculated as:

$$\frac{(\text{Biomass on day 50} - \text{Biomass on day 0})}{\text{Biomass on day 0}} \times 100$$

Nutrient Element Analysis

The production of organic C in the vermicompost was determined using the partial-oxidation method [7]. Kjeldahl digestion with concentrated H₂SO₄ (1:20, w/v) followed by distillation was used to estimate N content [8]. P was detected by a colorimetric method using ammonium molybdate in HCl [9]. K, Mg and Ca were measured by the ignition method using a Perkin-Elmer model 3110 double beam atomic absorption spectrophotometer [5]. The maturity of the vermicompost was calculated from the C:N ratio.

Statistical Analysis

Statistical analysis was carried out using SPSS v. 16.0. A paired samples t-test was performed to analyse the significance in the difference between the earthworms' biomass and number in percentage during vermicomposting at 0.05% level of significance.

RESULTS AND DISCUSSION

Multiplication of earthworms is an important indicator in determining the vermicomposting performance. The biomass and number of earthworms increased noticeably from day 0 to day 50 of the experiment (Table 2). The average of the triplicates revealed that the highest gain in biomass and number was in T_A with 178.28% and 143.33% respectively. The paired-samples t-test showed a significant difference ($df=2$, $t=4.86$, $P<0.05$) between the earthworms biomass and number. The rate of biomass gain of the earthworms ranged between 94.52 and 180.38 mg day⁻¹. No loss of biomass or numbers and no mortalities of earthworms were recorded at the end of experiment. The results clearly suggested that the multiplication of earthworms was directly related to the quality of feed materials and nutrients available in the SMC after cultivation of pea sprouts. This is supported by Suthar [10] who studied the recycling of agro-industrial sludge with organic bulky agents as amendment by vermicomposting.

Furthermore, the residue from the pea sprouts harvest, e.g. roots and the prevalent mycelium were additional food supplement for the earthworms and indirectly affected the earthworms' palatability. Due to the homogenous and non-foul-smelling feed materials, no pests were introduced into the earthworms' bin and this accelerated the vermicomposting process. In the natural environment, plant residues are quickly colonised by microorganisms. These microorganisms are common constituent of the earthworms' food resources, in particular protozoa and fungi that formed a substantial part of their diet [11-13]. However, maintaining high and stable moisture content is an important factor for the earthworms' mobility, ensuring successful feeding and copulation. The increase in the earthworms' growth may also be attributed to the low C:N ratio of the pre-decomposed substrate, i.e. the SMC used in this study [14].

Table 2. Earthworms multiplication in biomass and number

Treatment (T)	Biomass of earthworms (mg)		Earthworms biomass gain/loss (%)	Biomass gain rate (mg day ⁻¹)
	Initial	Final		
T _A	5059.0	14078.0	+178.28	180.38
T _B	5761.0	14054.0	+143.95	165.86
T _C	4649.0	9375.0	+101.66	94.52
	Number of earthworms		Earthworms number gain/loss (%)	Mortality rate (%)
	Initial	Final		
T _A	30	73	+143.33	Nil
T _B	30	55	+83.33	Nil
T _C	30	38	+26.67	Nil

The nutrient elements of the vermicompost are presented in Table 3 after 50 days of vermicomposting. The three salient macronutrients of the vermicompost, i.e. N, P and K, were relatively higher compared to control and its initial contents. The N content in the vermicompost was ~3% higher compared to that in the control. This might originate from the addition of N by the

earthworms itself in the form of mucus, nitrogenous excretory substances, growth-stimulating hormones and enzymes [15]. Nitrogen fixed by free-living N-fixing bacteria can also result in increased N content in the vermicompost [16] and the level depends on the initial N content present in the feed materials and on the degree of decomposition [17]. Furthermore, an updated perspective attributed the contribution of N in the vermicompost not only to the status of the feed mixture, excretory products, mucus, body fluid and enzymes, but also from the decaying tissue of the dead earthworms [35].

Table 3. Nutrient elements in saw dust, SMC, vermicompost and control (compost)

Nutrient element	Saw dust (%)	SMC ^a (%)	SMC ^b (%)	Vermicompost ^c (%)	Control ^d (%)
Nitrogen (N)	0.33	0.58	2.35	4.12 ± 0.248	1.10
Phosphorous (P)	0.02	0.27	1.23	2.07 ± 0.485	1.34
Potassium (K)	0.17	0.25	0.98	1.56 ± 0.155	1.24
Calcium (Ca)	0.01	0.01	0.11	0.08 ± 0.013	0.10
Magnesium (Mg)	0.01	0.01	0.01	0.01 ± 0.003	0.03
Organic Carbon (C)	32.52	34.40	56.30	47.89 ± 5.882	65.27
C:N ratio	107.41	83.30	23.96	11.79 ± 1.955	59.34

^a SMC prior to cultivation of pea sprouts

^b SMC after harvesting of pea sprouts

^c Vermicompost from SMC after 50 days of bioconversion. Values are mean and standard error (mean ± S.E.M.; $n = 3$)

^d SMC after harvesting of pea sprouts after 50 days

The P and K contents in the vermicompost were higher than those in the compost. They were also higher than those in saw dust, SMC prior to and after pea sprouts cultivation (Table 3). These were probably due to the direct action of the earthworms gut enzymes and indirectly to the stimulation of the microflora [18]. Barois and Lavelle [19] reported that earthworms produced a huge amount of intestinal mucus, a mixture of glycoproteins and small glucidic and proteic molecules which is rapidly incorporated into the microbial biomass in the gut. The higher K content in the vermicompost was due to a higher mineralisation rate as a result of enhanced microbial and enzymic activities in the earthworms gut [15]. Nevertheless, according to a recent report by Deka et al. [33], there were many contradictory reports regarding the reduction in K content in vermicompost obtained from different feedstock. It was difficult to find any conclusive explanations for this decrease. The considerable increase in P was ascribed to changes in sorption complexes induced by competition for sorbing sites between orthophosphates and carboxyl groups of glycoproteins within the mucus produced in the earthworms gut [20]. Referring to Edwards and Lofty [21], the rise in P during vermicomposting is probably due to P mineralisation and mobilisation because of the bacterial and faecal phosphate activity of earthworms. Meanwhile, Suthar [30] suggested that the P content in the final product may vary depending on the earthworms' metabolism and available P is contributed partly by earthworm gut and partly by further release of P through P-solubilising microorganisms present in the worm cast.

The Ca and Mg contents seemed to differ only slightly although their concentrations were low, these exchangeable nutrients contribute significantly to the sustainability of agro-ecosystem in food source cultivation. According to West et al. [22], *L. rubellus* accumulates Ca in their anterior alimentary canal in order to maintain their body Ca concentration and in some cases, the Ca metabolism in the earthworms' gut enzymes and bacterial communities in vermicast result in an increase of Ca. The Mg content made up the least amount of the nutrients tested and it is categorised as

a trace nutrient. However, this plant nutrient potentially increases agriculture-ready materials. No direct contribution of earthworms to the Mg metabolism is known. It is hypothesised that fungi and microalgae which easily colonise freshly deposited worm casts contribute to the trace level of Mg in ready vermicompost [10].

The organic C content from saw dust (32.52%) increased after cultivation of mushroom (34.40%) and pea sprouts (56.30%) but decreased after vermicomposting by *L. rubellus* ($47.89 \pm 5.882\%$). According to Suthar [23], earthworms promote microclimatic conditions in vermireactors that increase the loss of organic C from the substrates through microbial respiration. Moreover, loss of organic C due to mineralisation of organic matter during vermicomposting may be the reason for the increased N in the end product [34]. The C:N ratio is used as an index for the maturity of organic wastes. In this study, the C:N ratio for the vermicompost was less than 20 (Table 3). According to Senesi [24], a C:N ratio of less than 20 indicates an advanced degree of organic matter stabilisation and reflects a satisfactory degree of maturity of organic waste. A high C:N ratio, as in the control, reflects a reduction in biological activity and consequently a slow degradation [25]. Compared to a previous study by Sailila et al. [26], this study has shown stabilisation of the C:N ratio compared to the previous forms of organic waste or biomass residue (saw dust and SMC).

CONCLUSIONS

Reuse and recycle saw dust-based SMC need to be comprehensive and practical yet environmentally sound. The amounts of nutrients available in the saw dust-based SMC after harvest enable the cultivation of pea sprouts and subsequent conversion of the waste generated postharvest of pea sprouts into a valuable product i.e. vermicompost. Hence, the saw dust-based SMC can be reused to cultivate other vegetation i.e. pea sprouts and yet recycled into a bio-fertiliser via vermicomposting. In this study, the pre-composting period was excluded and there was no amendment of the substrate with other bulky organic waste. Thus, the process can be shortened and inclusively utilise the same substrate while still yielding material that is rich in nutrient elements. Vermicomposting using *L. rubellus* has succeeded to convert a reusable biomass residue into a nutrient-rich end product for sustainable agricultural farming as an alternative to synthetic chemical fertilisers.

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