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Research Article

Biotransformation of Soybean Residuals into Vermicompost by Red Earthworms (*Eisenia fetida*)

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Abstract

The effective management of organic solid wastes presents a growing challenge due to population growth, intensified agricultural practices, and industrialization. Vermicomposting, a cost-effective method utilizing earthworms, has emerged as a viable solution for the disposal of organic waste. This study investigated the potential of different mixtures of soybean residuals (SR) and cow dung (CD) on vermicompost (VC) production. A completely randomized design with four replicates was used to allocate six treatments i.e; T_1- 100% SR, T_2- 80% SR + 20% CD, T_3- 60% SR+ 40% CD, T_4- 40% SR + 60% CD, T_5- 20% SR + 80% CD, T_6- 100% CD. The results showed significant differences in the production of live biomass of earthworms, weight of the harvested VC, pH, salinity and total dissolved solids (TDS), except for electrical conductivity among the treatments. The study demonstrated the potential of bioconversion of SR using Eisenia fetida, when combined with different proportions of CD. However, the current vermicomposting process of SR must be optimized as pH and electrical conductivity values were not among the SLS recommended range for compost in some treatments.

***Keywords**: Live biomass of earthworms, organic solid waste, red earthworm, vermicomposting*

1. Introduction

Waste management is currently defined as the regulation of waste-related activities to safeguard human and ecosystem health while encouraging available resource conservation (Pongrácz and Pohjola, 2004). The task of effectively handling and controlling organic solid wastes has become more challenging due to the rapid growth of the population, intensified agricultural practices and industrialization (Jalil, 2010). Further, the per capita waste generation rise is directly linked to economic growth and swift urban development (Venkiteela, 2020). Waste materials are generated after the manufacture or formulation of the primary product created by human activities. Depending on the treatment ability, waste materials are either usable or unusable goods whereas waste can be classified as organic and inorganic. Among them, agricultural waste is one of the organic waste types and the agricultural industry is making a significant contribution to waste generation through activities conducted both on and off the field (Maji *et al.*, 2020).

Biotransformation refers to biological processes involving specific organisms that convert organic materials that have plant or animal origin, into valuable products or energy sources. These processes extend beyond the conversion of crops and plant waste and encompass the generation of usable products from organic waste and unconventional feedstock (Munroe, 2007). There has been a notable surge in the adoption of organic farming practices, accompanied by an increased emphasis on recycling organic waste. This has drawn attention to the necessity of assessing the management of food production wastes. As a cost-effective way of treating organic wastes, vermicomposting harnesses the capacity of certain earthworm species to mechanically break down waste residues by their grinding gizzards (Edwards and Neuhauser, 1988). Vermicomposting has emerged as a viable alternative for the secure, sanitary, and economical disposal of organic solid wastes (Nagar *et al.*, 2017). Earthworms can breakdown waste very effectively and efficiently alternating the physical and chemical composition of the organic matter (Orozco *et al.*, 1996). According to Albanell *et al.* (1988), Vermicompost (VC) has low solubility, increased salt content, increased cation exchange capacity and content of humic materials.

VC encompasses biologically active compounds, including plant growth regulators, which can improve plant growth rates in both controlled and open-field experiments. It has been shown to improve plant development whether used as a soil supplement or as a replacement for soilless growing media. It has been confirmed by the studies of Krishnamoorthy and Vajranabhaiah (1986). The compost exhibited lower microbial populations (including bacteria, fungi, actinomycetes, coliforms, proteolytic and cellulolytic bacteria) compared to VC. Conversely, earthworm compost had higher levels of plant hormones (such as kinetin, gibberellic acid, and indole-3-acetic acid) compared to compost. Additionally, VC showed higher levels of microbiological biomass carbon than compost (Usmani *et al.*, 2019).

VC is produced using a specific species of earthworm called *Eisenia fetida*, commonly known as red worms (Ramnarain *et al.*, 2019). Vermiculture and vermicomposting are two similar yet distinguishable procedures (Munroe, 2007). Organic wastes have been subjected to the vermicomposting process, resulting in their transformation into alternative fertilizers (Manyuchi *et al.*, 2017). The objective of the present study was to identify the potential of bioconversion of soybean residues into VC by mixing with cow dung in different ratios.

2. Materials and Methods

The current study was conducted at the Department of Crop Science, Faculty of Agriculture, University of Ruhuna, from June to September 2022. Soybean residues (SR), which is the byproduct of soya sauce production, was gathered and subjected to four to five rounds of washing to eliminate salt content. Subsequently, SR were left to air-dry approximately one week. Similarly, the CD was collected from the cattle shed and air-dried for one week. *Eisenia fetida*, a specific kind of red earthworm, was collected near the faculty farm's cow shed. Before initiating the experiment, the required quantity of *Eisenia fetida* was multiplied in plastic containers with a capacity of 5 liters. In this multiplication process, CD was utilized as a culture medium, following the methodology outlined by Devi and Khwairakpam (2020). Additionally, finely chopped fruit and vegetable peels were incorporated to promote the growth and reproductive capacity of the earthworms. Water was added as needed during the multiplication process, and drainage holes were created in each container to remove excess water. The containers were covered with nets to protect from external damage and kept under the shade house for a duration of two months for multiplication of earthworms. The ambient temperature inside the shade house ranged between 25 to 29 °C.

The experimental design consisted of six distinct treatments denoted as T_1, T_2, T_3, T_4, T_5, and T_6. T_1- use of 100% soybean residuals (SR) alone, T_2- a combination of 80% SR and 20% cow dung (CD), T_3 - a combination of 60% SR and 40% CD, T_4-

a combination of 40% SR and 60% CD, T_5 - a combination of 20% SR and 80% CD and; T_6 - use of 100% CD alone.

The experimental setup followed a Completely Randomized Design (CRD) with four replicates, where 24 experimental units assigned randomly. The combination of SR and CD in four different proportions and CD alone and SR alone, totaling 250 g, was added to all the reactors. Each reactor was treated with 25 g of earthworms with a bedding material weighing about 200 g at the bottom. Nets were used to cover the reactors from outside invaders. Water was sprayed as required, regularly. Throughout the process, the ambient temperature was ranged from 25°C to 32°C.

2.1. Assessments

The VC was harvested 75 days after the establishment of the experiment. The harvested VC samples from 24 reactors were passed through a 2 mm sieve and subsequently air-dried before taking the weight. Finally, as quantitative parameters, the live biomass of earthworms in each reactor, air-dried weight of the VC harvested, pH, electrical conductivity (EC), total dissolved solids (TDS) and salinity of the aqueous solution were measured from each treatment. The air-dried samples (5 g) of VC from each reactor were dissolved in 25 ml of distilled water to produce an aqueous solution and let the samples settle for 10 minutes. The EXTECH pH meter was used to measure the pH of each sample and the WalkLAB Conductivity, TDS, and Salinity meter was used to get values of EC, TDS, and salinity. As the qualitative parameters, the colour, odor (Wijeysingha and Fernando, 2021), texture and appearance were evaluated based on the below scale (Table 1), before sieving each sample.

Table 1: Scale for the evaluation of colour, smell, texture and appearance of VC samples

Scale	Colour	Smell	Texture	Appearance
1	Light brown	Extremely bad odor	Very rough	Very bad
2	Brown	Moderately bad odor	Moderately rough	Bad
3	Dark brown	Bad odor	Rough	Neutral
4	Grayish black	Odorless	Fine	Good
5	Black	Earthy smell	Extremely fine	Very good

The quantitative data were subjected to statistical analysis at 5% significance level using SAS software, employing one-way ANOVA. To compare means, the Duncan Multiple Range Test (DMRT) was applied with a 5% significance level. The qualitative data analysis involved the utilization of the Kruskal-Wallis test using SAS software. Descriptive data were visually presented through the use of charts and graphs.

3. Results and Discussion

In the present study, live biomass productions of earthworms were significantly different among treatments ($p<0.0001$). The highest live biomass production was reported in T_4, T_5 and T_6 while the lowest values were reported in T_1 and T_2 treatments (Figure 1A). The results clearly indicate that CD has provided conducive conditions to grow earthworms, as CD has supplied nutrients to boost earthworms' growth and reproduction. The form of bulking material, such as CD, which has been partially decomposed by microorganisms can regulates earthworm development and reproduction (Sharma and Garg, 2018). Earthworms' increased capacity for reproduction may be facilitated by consumption of nutrient-rich substrates, which may account for their increased biomass. When there is adequate food, well-aerated bedding with 70-90 percent moisture, a temperature between 15 and 30 °C, and initial stocking densities of 2.5 to 5 kg m^{-2}, and the compost worm population expected to be doubled by every 60 to 90 days (Munroe, 2007). The vermicomposting experiments totally rely on earthworm generation, which is an important and integral part of the vermicomposting process (Ananthavalli *et al.*, 2019). The efficiency of vermicomposting is governed by a range of organic components, some of which have already been investigated for earthworm growth and reproduction (Nagavallemma *et al.,* 2004). In 100% soybean treatment, the growth rate of the earthworms is low (Najar and Khan, 2010). According to Devi and Khwairakpam (2020), *Eisenia fetida* can adapt well to a variety of organic feeds and a variety of abiotic environmental conditions. Earthworms' population is controlled by the amount of food they consume, and greater nitrogen ratios stimulate fast development and cocoon formation (Gajalakshmi and Abbasi, 2004).

There was a significant difference among treatments for the percentage change of live biomass production of earthworms ($p<0.0001$) at the end of the experiments (Figure 1B). A positive change was observed in T_4, T_5 and T_6 treatments, while a negative change or production loss of earthworm live biomass was observed in T_1, T_2 and T_3 treatments. It was examined that when increasing the proportion of CD in the substrate, the live biomass production of earthworms increased significantly. This may be attributed to the weight gain of earthworms as well as to their multiplication.

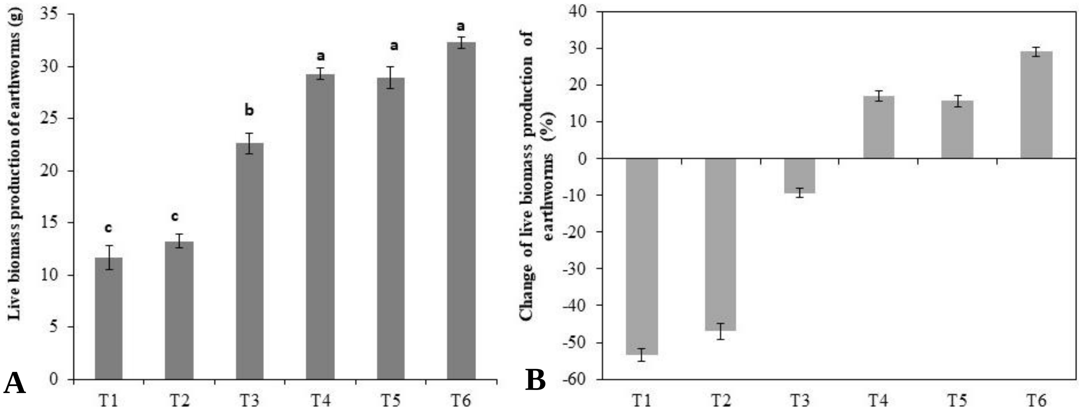


Figure 1: (A) Live biomass production of earthworms (B) Change of live biomass production of earthworms in different treatments at the end of the experiment (T_1- 100% soybean residuals (SR), T_2- 80% SR + 20% cow dung (CD), T_3- 60% SR + 40% CD, T_4- 40% SR + 60% CD, T_5- 20% SR + 80% CD, T_6- 100% CD). Error bars represent standard error of means. Treatments with the same letters are not significantly different at a 5% probability level of significance

The initial earthworm population, the kinds of earthworms, their reproductive and metabolic activities, their physicochemical makeup, the quantity and nutrient composition of the substrates, and the time duration of the vermicomposting process lasts have an impact on the production of VC (Sharma and Garg, 2018). Sinha *et al.* (2002) discovered that CD-like partially disintegrated materials were predominantly destroyed by microorganisms, are ideal food for earthworms. Therefore, adding CD to the process of making VC is advantageous. In addition, the higher the proportion of CD usually faster the substrate bioconversion.

Moreover, according to the Figure 2, a similar trend in the production of VC was shown. Treatments had a significant influence on VC production ($p<0.001$). However, there was no significant difference between the mean weights of T_4, T_5, and T_6. Further, the study revealed that there is no significant difference between the mean weights of T_1 and T_2 as well.

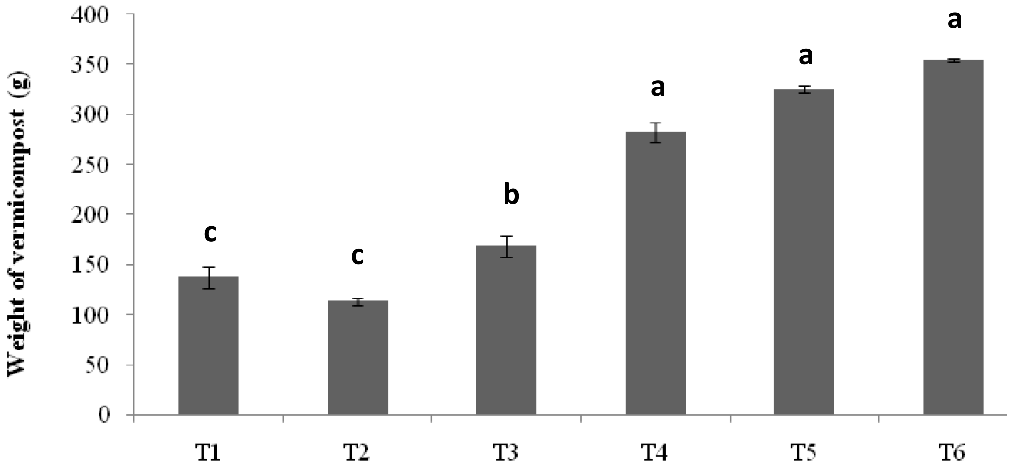


Figure 2: Weight of the VC (≤ 2 mm particle size) produced in different treatments at the end of the experiment (T_1- 100% SR, T_2- 80% SR + 20% CD, T_3- 60% SR + 40% CD, T_4- 40% SR + 60% CD, T_5- 20% SR + 80% CD, T_6- 100% CD) Error bars represent standard error of means. Treatments with the same letters are not significantly different at a 5% probability level of significance

The pH of the aqueous solution made from harvested VC was varied significantly among treatments ($p<0.001$) and ranged from 5.65 to 6.65 (Table 2). The pH value of T_3, T_4 and T_5 treatments was within the SLS standard pH values (6.5-8.5) for compost (Sri Lanka Standards, 2019). The pH of the VC is determined by the acidity of the substrate (Hanc and Chadimova, 2014) along with the availability of various intermediate substances. Therefore, the pH of VC may vary according to the substrate used for the vermicomposting. Some studies have revealed that many species of earthworms prefer a pH of about 7.0 (Suthar, 2008). According to Khare *et al.*, (2005), substrates with a high initial acidic pH were shown to be less appropriate for vermicomposting. Furthermore, the pH of the VC was near neutral at the end of the vermicomposting process and did not differ significantly across treatments.

In the present study, EC ranged from 5.58 to 7.16 (mS cm^{-1}) and there was no significant difference among treatments (Table 2). However, EC values of all treatments exceeded the SLS value of EC (4 mS cm^{-1}) for compost (Sri Lanka Standards, 2019). The high value of EC may be associated with the industrial ingredients of SR. Previous studies suggested that EC decreases as a result of earthworm activity and organic matter degradation (Amouei *et al.*, 2017). This can be linked to the biological buildup of certain minerals in the earthworms' bodies, resulting in a decrease in the amount of minerals in the soil. The creation of soluble salts during the breakdown process of organic matter indicates the liberation of calcium, total phosphorus, and potassium, which are known as exchangeable minerals, into vermi-reactors, hence increasing the EC value of VC (Yadav and Garg, 2019). According to Devi and Khwairakpam (2020), VC reactors with high concentrations of minerals such as calcium, phosphorus, and potassium have a direct relationship with proportionate increases in EC in the VC. Furthermore, EC is affected by CD concentration, and as the concentration of CD grew, the EC values steadily declined. As a result, it is possible that the EC value of VC is determined by both criteria included in the study. According to Majlessi *et al.* (2012), simple parameters like pH and EC may be useful indications of compost stability.

The salinity of VC varied substantially among treatments ($p<0.01$) (Table 2). According to prior research, lowering the reactor's CD proportion lowered the salinity of the end product. Salinity denotes the quantity of salts dissolved in a solution (Choudhary *et al,* 2019). However, according to the present study, there is no proportional relationship between salinity and EC of treatments.

Total dissolved solids ranged from 2.24 to 3.74 (ppt) in the present study and was significantly different among treatments ($p<0.01$). As a result, treatments with a high proportion of SR may be responsible for the maximum availability of organic and inorganic VC components as it recorded the highest values (Table 2). TDS reflects the total organic and inorganic materials available in a suspension (Thirumalini and Joseph, 2009) of VC.

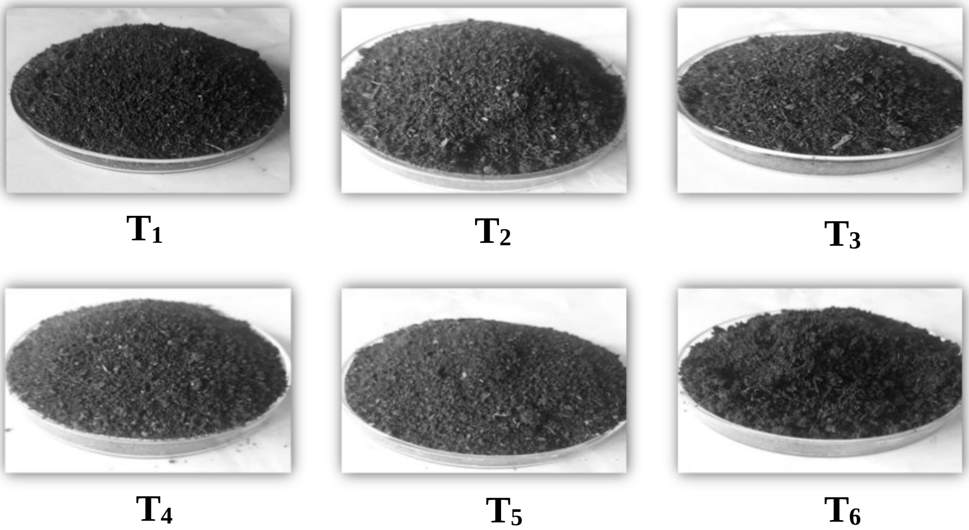
Table 2: Mean pH, electrical conductivity (EC), salinity and total dissolved solids (TDS) values of different treatments with standard error of the means

Treatment	pH	EC (mS cm$^{-1)}$	Salinity (ppt)	TDS (ppt)
T_1	5.98^{cd}±0.24	6.91^{a}±0.33	4.70^{ab}±0.58	3.68^{a}± 0.21
T_2	6.18^{abc}±0.17	7.16^{a}±0.46	4.00^{b}±0.47	3.74^{a}± 0.31
T_3	6.52^{ab}±0.04	7.05^{a}±0.17	4.87^{ab}±0.39	3.61^{a}± 0.43
T_4	6.47^{ab}±0.08	6.96^{a}±0.36	4.05^{b}±0.44	2.42^{c}± 0.57
T_5	6.65^{ab}±0.18	6.74^{a}±0.46	4.32^{b}±0.31	3.42^{ab}± 0.72

T_6	$6.02^{bcd}\pm0.07$	$5.58^{a}\pm0.35$	$5.97^{a}\pm0.04$	$3.11^{ab}\pm 0.22$
P	<0.001	*NS*	<0.01	<0.01

Mean values followed by the same letters are not significantly different at α =0.05 according to DMRT. *NS* = not significant

The color, appearance, texture and odour of VC were not significantly different among treatments (Figure 3). All of VC produced in different treatments was black in colour without bad odour but having rough in texture. Overall appearance of the end product of all treatments was neutral.



T_1 **T_2** **T_3**

T_4 **T_5** **T_6**

Figure 3: Overall appearance of VC produced in different treatments (T_1- 100% SR, T_2- 80% SR + 20% CD, T_3- 60% SR + 40% CD, T_4- 40% SR + 60% CD, T_5- 20% SR + 80% CD, T_6- 100% CD)

4. Conclusions

Some remarkable variations were observed among the treatments in the live biomass production of earthworms, changing pattern of live biomass during the experiment, the weight of vermicompost having $\leq$ 2 mm particle size, pH, salinity and total dissolved solids. When increased the proportion of cow dung in the mixture of substrate a greater live biomass production of earth worms followed by a higher weight of vermicompost was achieved. At the same time, an increased proportion of cow dung positively affected the changes in live earthworm biomass. However, pH and EC values were not in the range of recommended values for compost in many treatments. The study demonstrates the feasibility of bioconverting soybean residuals using *Eisenia fetida*, when combined with cow dung. However, further studies are needed to optimize the process with special reference to pH and EC values.

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