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A Study on Yield and Gut Flora Diversity on Vermicomposting of Bio-Waste Using *Eisenia fetida*

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ABSTRACT

Background and Objective: Management of accumulated refuse materials can be made effective through vermicomposting. The study aims to find the yield of compost from different bio-wastes and biochemical characterization of microbes present in the gut region. **Materials and Methods:** Collected and dried biowastes were spread over concrete tanks for composting. Addition of cow dung slurry for pre-treating the biowastes. *Eisenia fetida* was inoculated into the tanks in a ratio of 10:1 and left for vermicomposting. About one gram of earthworm tissue was dissected and taken for analysis. Microbiological methods were employed to isolate, identify and characterize the vermin bacteria. **Results:** Degradation of hard fibrous sugarcane bagasse resulted relatively in a higher vermicompost production (55.79 and 70.34%) and an increased percentage (29%) of earthworm when compared to other wastes used in the study. As 70.34% recovery during the second study period was obtained because of the longer pre-composting period. Eight different bacterial isolates identified from the gut region were found to be present in the soil, which has plant growth-regulating functions. **Conclusion:** The results of the work can be applicable in multi-faceted fields, including natural resources conservation, organic farming, recycling and reuse of solid and agro-wastes.

KEYWORDS

Vermicomposting, biowaste recycling, sugarcane bagasse, African red earthworm, *Eisenia fetida*, earthworm gut flora

INTRODUCTION

Waste management through the vermicomposting process is a suitable way to produce organic manure[1]. On considering the importance of soil health and the production of quality agro produces and industrial products, the organic farming system spans widely in the recent past[2]. Vermicomposting can produce better quality compost in a cost-effective manner when compared with other composting methods. Quality vermicompost production is essentially required to attain a sustainable environment and effective soil-use management practices. Bio-waste materials viz, plant debris, vegetable and kitchen waste, fruit

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skin, pulp waste, crushed cane waste and flower petals are easily bio-degradable wastes, which can be reused by recycling them as bio-composts. These compost products enhance the soil nutrient status, water holding capacity and soil microflora and their activities altogether are very useful in agriculture, horticulture, gardens and plantation crops.

Earthworms act as a catalyst and therefore speed up the process of decomposition. The excreted fecal wastes are called "worm castings"[3]. The utilization of earthworms for waste management has been practised for many years to improve soil ecology and its functioning towards crop improvement. The conversion of nutrients into soluble and available form with high amounts of major nutrients like nitrogen, potassium, calcium, magnesium and phosphorous and minor nutrients like iron, molybdenum, zinc and copper[4]. The selection of appropriate species of earthworm is very much important for the composting process. According to previous studies, native earthworms like *Lampito mauritii* haveless have potential in the rate of reproduction and cocoon formation when compared with *Eisenia fetida*[5]. *Eisenia fetida* is an epigeic earthworm with a high rate of proliferation followed by growth and they are suitable to Indian conditions for agro-practices[6]. The quality and composition of the substrate used for vermicomposting technique also influence the efficiency of earthworms, as well as the vermicompost quality.

The gut region of earthworms abodes with the symbiotic association of microfungi, bacteria, protozoa, etc. The nature and role of microbiota present in the earthworm gut comprise mucus, organic and mineral matter and enzymes. The earthworm casts will have highly colonizing bacteria which indicates that the earthworm gut forms into an ideal condition for the development of microorganisms, benefiting the soil quality. The bacterial diversity in the gut and casts has been reduced by earthworms[7]. Some of the varieties of vermin bacteria thriving in the intestine of earthworms were studied previously and identified as *Klebsiella*, *Bacillus*, *Azotobacter*, *Pseudomonas*, *Aeromonas*, *Serratia* and *Enterobacter*[8,9]. The present study focuses on the microbial population thriving in the gut region of *Eisenia fetida* and the biochemical characterization of isolated microorganisms. The quantity of vermicomposting produced and the rate of earthworm reproduced from sugarcane bagasse waste, vegetable waste and flower waste for one complete cycle were also studied.

MATERIALS AND METHODS

The degradation of solid waste materials through vermicomposting activity was done in the compost yard, Incubation Centre at Thiagarajar College, Madurai. The vegetable waste (7 kg) and flower waste (7 kg) materials were collected from the Central Market, Mattuthavanai, Madurai and sugarcane waste (7 kg) was obtained from a street cane juice vending shop, near Thiagarajar College. The study was conducted between December, 2019 and March, 2020, October, 2022 and February, 2023. Collected wastes were shade dried and shredded into small bits. The initial weight of sugarcane waste after drying was recorded and mixed with cow dung slurry. African red earthworm-*Eisenia fetida* was chosen, as it has proven with a high multiplication rate, thereby has the faster means of vermicompost production. The moisture level was maintained by sprinkling water over the bed and aeration was facilitated by gentle mixing until the fair decomposition through heat stability and colour transformation of the composting materials into brownish-black colour occurred. An appropriate quantity of *Eisenia fetida* 10:1 ratio (W/W) was introduced to the partly decomposed material kept in the vermin bed. Cow dung slurry was applied initially after the worm's introduction and thereafter water was sprinkled to keep the appropriate moisture level. After daily monitoring of the composting bed, the complete formation of composting was started within 15-20 days.

Harvesting of earthworms and vermicompost: Vermicompost was collected by separating the multiplied earthworms from the chamber. The collected compost was air-dried for a day and then weighed. Similarly, the multiplied earthworms collected after the harvest were also weighed:

$$\text{Vermicompost yield (\%)} = \frac{\text{Harvested vermicompost}}{\text{Total amount of substrate}} \times 100$$

Earthworm gut flora analysis: Ten earthworms from each of the composting beds, on the tenth day after introduction into the bed were collected for microbial analysis. The worms were washed with sterile water and then placed on a Petri plate, covered with moistened filter paper for 24 hrs. After 24 hrs, before dissection, the surface of the earthworm was cleaned with 70% ethanol and stored in a clean plastic bottle kept in a cool condition for further analysis.

One gram of tissue was collected from the stored specimen of earthworm gut region and using a sterile surgeon razor the gut region tissue was cut open. The tissue was homogenized for 5 min using a vortex mixture (CM 101, Remi Equipment's Ltd.) by adding 0.85% of NaCl solution.

The bacteria present in the gut region of earthworms were isolated through serial dilution. One-gram gut region of earthworm was excised and suspended in 10 mL of 0.85% NaCl solution, serially diluted (10^{-1} to 10^{-7}). From the serially diluted tubes, 0.1 mL of the solution was taken from each tube and spread over the Petri plate-containing nutrient agar medium. The culture plates were incubated at room temperature for 24 hrs, to observe the growth of bacterial community over the spread plate.

Morphological and biochemical characterization of bacteria: The morphology of the colonies was identified using Gram staining. Further biochemical characterization of vermi-bacteria through catalase Test (addition of hydrogen peroxide), Indole production test (addition of Kovac's reagent), methyl red (MR) Test (addition of methyl red), Voges-Proskauer (VP) Test (addition of Barrit A and B), Citrate utilization test (positive if the medium colour changes to green from blue), production of H_2S (appearance of deep black colour) was performed to identify vermi-bacteria.

RESULTS AND DISCUSSION

Vermicompost production: The yield of vermicompost was higher in sugarcane bagasse waste material (Table 1), as this bio-waste material contains 30-40% of pith fibre vermicomposting is generally done using several agro-waste materials like sugarcane bagasse and rice straw[2], cow manure and kitchen waste[5] and paper waste[10], bagasse[11], cow dung and domestic sludge[12] etc. Pre-composting of bagasse, using cow dung slurry was found to be improved the growth of earthworm *Eisenia fetida*[11] to the composting condition. The weight of sugarcane bagasse partially decomposed was noted and a further vermicomposting process was initiated by introducing earthworms *Eisenia fetida*. The ideal ratio suitable for the growth and proliferation of *E. fetida* was 50:50, as reported by Hu *et al.*[12]. However, 50% recovery was achieved in flower waste material in a shorter period of time.

Earthworm production: An appreciable rate of earthworm production was noticed with the use of pre-composted bagasse with a 29% increase in earthworm weight, during the period of vermicompost collection (Table 2). The growth and reproduction of earthworms throughout the cycle were found influenced by the type of feeding material and this report is in agreement with the previous report[13]. While using cow dung and sawdust as substrates, significantly higher production of earthworms and cocoons was observed in the study implying cow dung is essential for earthworms which contain easily digestible organic matter[14]. The mixture of cow dung and sugarcane bagasse in different compositions resulted in an increase in the number of earthworms from the 60th day of study and reached the maximum of 105th days[12]. An increase in the fresh weight of total and individual worms was noted in the second study period of sugarcane bagasse waste which confirms the fact that the enhanced production of earthworms could be achieved through the suitable selection of feedstock material, provided to the earthworms as described by Chaudhuri and Bhattacharjee[15] and Mupambwa *et al.*[16].

Table 1: Initial quantity and yield of vermicompost from flower waste, vegetable waste, sugarcane waste in two different study periods

Parameters	Flower waste	Vegetable waste	Sugarcane waste (2019-2020)	Sugarcane waste (2022-2023)
Dry weight (kg)	4.150	6.790	4.800	4.910
Cow dung applied (kg/per time)	2.180 (2 times at 5 days interval)	3.39 (2 times at 5 days interval)	2.500 (4 times at 5 days interval)	3.000 (for pre-composting) 1.500 (for vermicomposting)
Earthworm introduced (kg)	0.250	0.250	0.250	0.180
Compost Yield (kg)	2.111	2.100	2.678	3.741
Yield (%)	50.86	30.92	55.79	70.34
Number of days required	33	51	61	44 (for pre-composting) 77 (for vermicomposting)

Table 2: Weight gaining of *Eisenia fetida* earthworms during the vermicomposting of flower, vegetable and sugarcane waste decomposition

Substrate	Initial weight of earthworms (g)	Final weight of earthworms (g)	Increase (%)
Flower waste	250	151.44	Negative
Vegetable waste	250	-	-
Sugarcane waste (2019-2020)	250	254.28	1.71
Sugarcane waste (2022-2023)	180	233.50	29.72

Table 3: Biochemical characterization of bacterial colony isolated from the gut region of *Eisenia fetida*

Bacteria number	Shape of bacteria	Gram staining	Motility test	Catalase test	Indole test	Methyl red test	VP test*	Citrate test	H_2S production	Bacteria
B1	Rod	-	-	+	+	+	-	-	-	*Pseudomonas* sp.
B2	Rod	-	+	+	+	+	-	-	-	*E. coli*
B3	Rod	-	-	+	-	-	+	-	-	*Klebsiella* sp.
B4	Cocci	+	-	-	-	-	+	-	-	*Entrococcus* sp.
B6	Rod	-	-	+	+	+	+	+	-	*Pseudomonas* sp.
B7	Cocci	+	-	+	+	-	+	+	-	*Staphylococcus* sp.
B8	Rod	-	+	-	-	+	-	+	+	*Proteus* sp.
B10	Cocci	+	+	-	+	+	-	+	+	*Pediococcus* sp.

*VP test: Voges-Proskauer Test

The occurrence of fungi on the feeding material during vermicomposting adds additional food to the worms which could be one of the reasons for the increment in the weight of worms[17]. This is being achieved through the various activities of earthworms such as ingestion, breaking down and digestion of the biodegradable wastes, as feed materials, engulfed by the earthworms, which converts into finer humified microbially active material. A higher yield of the compost was obtained in cow dung as a substrate since it is easily metabolized by earthworms[14].

Gut microbial analysis: The gut region of earthworms contains numerous microbes like bacteria, fungi, protozoa and yeast. Ten different bacterial isolates were screened using serial dilution. Among them, only eight isolates were able to sub-culture (Table 3). The reason behind the absence of two isolates was not studied. Rod (B1, B2, B3, B6, B8) and cocci shaped (B4, B7, B10) bacteria were identified. The identified bacteria might be from the gut region of earthworm, cow dung and soil agreed with the findings. *Klebsiella* was found in different substrates like loamy soil, cow dung and press mud. *Escherichia coli* present in the gut region of different earthworms grown using cow dung as substrate, absent in cast. *Enterobacter* sp., thrives on the gut region of all the earthworms employed[18].

Biochemical characterization of bacteria: In the catalase test bubble production of bacterial colonies when placed on the glass slide containing hydrogen peroxide was noted positive (B1, B2, B3, B6 and B7) due to the production of enzyme catalase[19]. And the colony does not produce bubbles that remained negative. The development of stable red color on the surface of the broth indicates sufficient acid production in the methyl red test to lower pH to 4.4 and constitutes a positive. The culture B1, B2, B6, B8 and B10 was observed positive. The microbiological test commonly used for acetoin production is Voges-Proskauer Test (VP test). Positive reaction as emphasized[20], as represented by the development of red colour in the experiment, indicating the presence of diacetyl, an oxidation product of acetoin. Cultures of B3, B4, B6 and B7 were found positive and B1, B2 and B8 remain negative to the VP test. The citrate test identifies the use of citrate as the sole carbon source since there is no other nutrient in the medium. The basic end products cause the bromothymol blue indicator in the medium to change into bright blue colour from green colour. The cultures established as B6, B7, B8 and B10 in the experiment were found to be positive for the citrate test. The production of H_2S was observed in the test tubes, indicated by the colour change. The positive result for bacteria was observed by the development of deep black colour (B8 and B10).

While adding Kovac reagent to the tubes containing two days old stab culture the appearance of a pink colour layer indicated the positive result for the indole production test (B1, B2, B6, B7 and B10) and green colour indicated the negative result (B3, B4 and B8). The bacterial culture tested positive for indole test is plant growth promoting bacteria agreed with the earlier reports[21], showed the avoidance of root and stem rots disease in plants and also enhancing the seed germination and further growth and development of plants.

Based on the results of the biochemical tests for bacteria the genus of the isolated bacteria was given as *Pseudomonas* sp., *E. coli*, *Klebsiella* sp., *Entrococcus* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Proteus* sp. and *Pediococcus* sp. The microbial diversity and enzyme activity of earthworms are enhanced by the initial feeding substrate[22]. Vermi-bacteria isolated from earthworm gut region contains gram-negative bacteria which are sensitive to crystal violet containing medium[23]. The identified seven bacterial species of *Bacillus* present in the intestine of *Onychochaeta borincana*, Valle-Molinares *et al.*[24] reported as a general soil bacterium, further evidencing the influential role of microflora diversity dwelling in the gut region of earthworms. The process of vermicomposting decreases the bacterial activity of earthworm and increase bacterial diversity[25]. The digestive tract of the gut region of earthworms showed the abundant presence of *Pseudomonas* and *Bacillus* might be them exist in the applied cow dung during the composting process[12]. Diverse methods and techniques have been incorporated in the identification of bacterial species of the genus like *Bacillus*, *Pseudomonas*, *Klebsiella*, *Azotobacter*, *Serratia*, *Aeromonas* and *Enterobacter* in the intestinal tracts of earthworms (Singleton). Phosphate solubilizing bacteria like *Bacillus* and *Pseudomonas* are very useful in agriculture and crop production.

The usefulness of this experiment includes the proper management of bio-wastes through recycling and the utility of the compost serves to achieve the concepts of organic farming and natural resources management and land pollution reduction, Certain limitations of the present experiment are also recommended by not using the onion peel, tamarind leaves as waste materials could cause the death of earthworms after introduction to the decompose bio-wastes from those materials. The study recommends that the produced vermicompost can be used as a suitable bio-fertilizer for agriculture, horticulture and plantations[26]. Organic waste materials as crop resides, left over after food preparation, paper scrapping and industrial by-products are organic and can be recycled using worms-mediated composting, which can be applied to soil fertility and to protect crops from disease and pest attacks[27].

The study further recommends that the sugarcane bagasse can be easily decomposed using *Eisenia fetida* through vermicomposting process which is agreed with previous reports. The nature of the substrate and also the composting process determines the quality of vermicompost, hence these results have a wider scope with different kinds of organic solid wastes and the methods to adopt in the composting. Further, this experimental result suggests that vermicomposting becomes alternative organic manure with high mineralization function improving plant growth and yield[28]. However, certain limitations were found in the study in which inoculation of earthworms to the partially degraded vegetable waste causes the demise of earthworms, which might have been due to the presence of compounds which have hindered the earthworm activity and their vermicomposting process and the choice of composting substrate selection forms the key requirement[29].

The study implies that the production of good quality compost from refuse materials will be beneficial in improving soil health. The vermicompost prepared out of bio-waste can be used for crops and gardens for promoting plant growth through soil quality improvement, with moisture retention. The wasteland soil thus can be ameliorated using the application of soil mix using vermicompost. There are ample scopes for using various kind of bio-wastes and their decomposition through different composting technologies to obtain quality vermicompost, to effectively performs to achieve the circular economy through the proper bio-wastes recycling and further reuse mechanisms.

CONCLUSION

For the conversion of bagasse waste into nutrient-rich manure vermicomposting process was found facilitated by the gut flora diversity of *Eisenia fetida*. This would be an efficient technology for converting bagasse into a useful compost product, through which an efficient recycling process. The present study will be found useful in the production of bio-fertilizer through a vermicomposting process which helps to increase soil fertility to attain sustainable soil management and organic farming system and further a circular economy is developed. Future studies on enzymatic analysis can help us to understand better microflora diversity and its specific role in reducing the substrate.

SIGNIFICANCE STATEMENT

Environmental problems like land degradation, soil pollution and contamination by the waste generated from human activities and industries through improper management of waste. The novelty of the present work investigated the cost-effective and environment-friendly manner to recycle the sugarcane bagasse into an enriched organic compost, using the vermicomposting method. Since the hard fibres of sugarcane bagasse take a longer period for decomposition and this experiment finds a method, that substantially reduces the period. Further, the gut microflora enumeration provides insights into the biodegradation of organic waste. Vermicompost production, an essential bio-fertilizer enhances soil fertility and also attains sustainable soil management through solid waste recycling, thereby the proper environmental resources management system is applied.

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