



Efficacy of Vermicomposting for Recycling *Tectona grandis* and *Casuarina* Leaf Litter for Organic Fertilizer Production

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ABSTRACT

The aim of this work was to study the effect of earthworm on two different types of leaf materials namely *Tectona grandis* and *Casuarina* spp mixed with cow dung for preparation of vermicompost. For this experiment two different species of earthworms *Eisenia fetida* and *Eudrillus eugenia* were used. The vermicompost was maintained in laboratory condition for 6 weeks. The vermicompost caused a reduction in organic carbon (total organic carbon) and total organic matter while an increase in total nitrogen (total Kjeldahl nitrogen), available phosphorus (total phosphorus), electrical conductivity content and pH was noticed. The nutrient-rich vermicompost with acceptable C:N ratio ranges (<20:1) indicates its agronomic potential. Waste mixtures also supported the earthworm growth and reproduction rates in vermibeds. The Microbial study of the vermicompost revealed the presence of *Bacillus thuringiensis*, *Bacillus anthracis*, *Bacillus funiculus*, *Bacillus cerus*, *Lactobacillus patheries* which might have helped in degradation process. Further the Fourier transform infrared spectroscopy analysis clearly revealed the degradation of organic matter using different earthworm species. The result suggests that vermicomposting could be an efficient technology to convert negligible leaf wastes into nutrient-rich biofertilizer.

Key words: Vermicomposting, Leaf wastes, *Eudrilus eugeniae*, *Eisenia foetida* and Fourier transform infrared spectroscopy.

1. INTRODUCTION

Lately in India there is a rapid increase in industrialization, population and economic development which leads to significant increase in municipal solid waste generation. According to Centre Pollution Control Board, 2000 India produces about 500 g of municipal solid waste/day/person in urban areas. Among this some organic waste end up in the land which enriches the solid fertility. However, the high accumulation of leaf litter seen in sidewalks, lawns and play grounds leads to severe problem to the municipal solid waste disposal [1]. In countries like India, the leaf litter were often set on fire. By burning, there may be a possible loss of carbon, nitrogen and phosphorus content [2]. In spite of burning, leaf litter can be composted and hence that the NPK content would not get lost. On the other hand, vermicompost is believed to have high content of enzymes and plant growth hormones, which stimulates the plant growth [3]. Vermicomposting is a process of biological decomposition of organic material through the joint action of earthworm and microorganism. As vermicompost is pathogen free, it enhances the plant growth and crop yield. In this view we explored the

possibility of generating vermicompost from the leaf litter.

Tectona is a genus of tropical hardwood tree under the mint family, Lamiaceae. This is often called as teak and is native to South East Asia mainly India, Pakistan, Bangladesh. This tree is commonly found as a component of monsoon forest vegetation. The trees grows up to 30-40 m (10-120 feet). *Tectona grandis* is an economically important species, which is a source of most commercial teak wood products. *Casuarina* belongs to the family Casuarinaceae, native to Australasia and India. They are evergreen shrub growing up to 35 m tall. *Casuarina* species act as a food source for larvae of hepialid moth and *Endoclita malabarica*. This tree yields large quantities of leaf litter thereby adding burden to the municipal solid waste. On the other hand this waste are often set on fire, thereby causing air pollution.

In our study, the comparison of the vermicompost prepared from the *T. grandis* and *Casuarina* leaf litter with the help of two different earthworm *Eisenia fetida* and *Eudrillus eugenia* was investigated. This also

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focuses to minimize the air pollution due to burning of the leaf litter, thereby converting it into good organic manure through vermicomposting process.

2. MATERIALS AND METHODS

Leaf litters were collected from Bharathidasan University campus, Tiruchirappalli, Tamil Nadu, India. Leaf litter were mixed with cow dung in 1:1 ratio. Set A contains leaf litter (*T. grandis*) + cow dung (1:1 ratio) along with *E. fetida*, Set B contains the same composition with *E. eugenia*. Set C contains leaf litter (*Casuarina*) + cow dung along with *E. fetida* and Set D contain in same composition with *E. eugenia*.

The experiments were carried out in the vermin reactors (20 cm × 30 cm). Sampling were done once in every 15 days. Atmospheric moisture was maintained in the vermibin at 60% [4]. After the complete decomposition, the earthworms and cocoons were removed manually, and the vermicompost was further analyzed.

2.1. Chemical Analysis

Total carbon and total nitrogen in the vermicompost were determined by [5] method and by Kjeldahl method [6]. The pH level was determined by pH meter (model 600 Eutech). Electrical conductivity (EC) was measured by Electrical Conductivity meter (Model L180 Elico). In addition, total phosphorus level was also determined by vanadate molybdate reagent method as per Tandon [7].

2.2. Morphological Determination

Microbial plate count was done as per [8]. About 1 g of vermicompost was stirred with 100 ml sterile distilled water in a 250 ml conical flask. The supernatant was serially diluted to 10^3 , 10^4 and 10^5 times and the total heterotrophic population of bacteria was estimated using nutrient agar medium. Isolated organism were identified by morphological determination and also by biochemical tests like indole, meth red, vorges prosker, catalyse, citrate, oxidase, urease, H_2S production, motility and gram staining [9].

2.3. Statistical Analysis

The data were represented as mean and standard deviation of three replicates. One-way analysis of variance (ANOVA) was evaluated and the significance level ($p < 0.05$) was analyzed using SPSS statistics tool- version 16.0 - <http://www.spss.com>.

2.4. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of control and four different vermicompost samples were recorded using Perkin Elmer. FTIR spectrometer in the spectral range of $4000-400\text{ cm}^{-1}$. The samples were mixed with spectroscopic grade KBr and were made in the form of pellets at a pressure of about 1 MPa. The pellets were about 13 mm in diameter and 1 mm in thickness.

3. RESULTS AND DISCUSSION

3.1. Changes in Physicochemical Parameters

After 25 days of vermicomposting process, the leaf litter was decomposed into manure. The obtained vermicompost was dark in color. The physicochemical characteristics of Type A, B, C and D were represented in Figure 1a and b. Both earthworms *E. fetida* and *E. eugenia* seems to be able to accelerate the process of decomposition of leaf litter.

3.2. Comparison of Physic Chemical Characteristics of Different Types

The pH of the vermicompost is found to be increased when initial values were compared final values with the in all types. It ranged from 8.11 ± 0.06 to 9.14 ± 0.163 in Type A; 7.14 ± 0.962 to 9.67 ± 0.522 in Type B; 8.12 ± 0.256 to 9.02 ± 0.112 in Type C; 5.16 ± 0.937 to 9.46 ± 0.049 in Type D (Figure 1b). The increase in pH range may be due certain associates gut microbes present in earthworm which responsible for the mineralization of organic matter [10].

EC is a good indicator for the safety disposal of vermicompost for agricultural purpose. In our experiment, the EC values have got increased from initial to final. In Figure 1b Type A EC value has increased from $0.63\ \mu\text{s/cm} \pm 0.006$ to $1.36\ \mu\text{s/cm} \pm 0.005$; in Type B it is from $0.87\ \mu\text{s/cm} \pm 0.006$ to $1.67\ \mu\text{s/cm} \pm 0.006$; in Type C it is from $0.66\ \mu\text{s/cm} \pm 0.005$ to $1.27\ \mu\text{s/cm} \pm 0.006$ and in type D it is from $0.82\ \mu\text{s/cm} \pm 0.009$ to $1.72\ \mu\text{s/cm} \pm 0.009$ [11] reported that the increase in EC might be due to the release of different mineral salts in available form such as phosphate, ammonium, potassium during the degradation of organic matter.

Similarly, Figure 1a data revealed that the loss in total organic carbon was high in Type B (28.5%) and Type D (28.7%) when compared to Type A (26.1%) and Type C (22%). This is because the earthworm might have modified the substrate condition along with the joint action of microbes by releasing CO_2 during respiration. The loss in organic carbon during vermicomposting process has been reported by [12,13].

In contrast, the total kjeldahl nitrogen (TKN) content were found to be increased in all the type is represented in Figure 1b. In Type A TKN is increased from $0.62\% \pm 0.007$ to $1.76\% \pm 0.008$; in Type B it is from $0.62\% \pm 0.007$ to $1.12\% \pm 0.004$; in Type C it is from $0.62\% \pm 0.007$ to $1.12\% \pm 0.047$; in Type D it is from $0.60\% \pm 0.007$ to $1.12\% \pm 0.047$. This may be due to the release of nitrogenous excretory substances by the earthworms while feeding the substrate and also because of release of growth stimulating hormones and enzymes during the decomposition of organic matter [14].

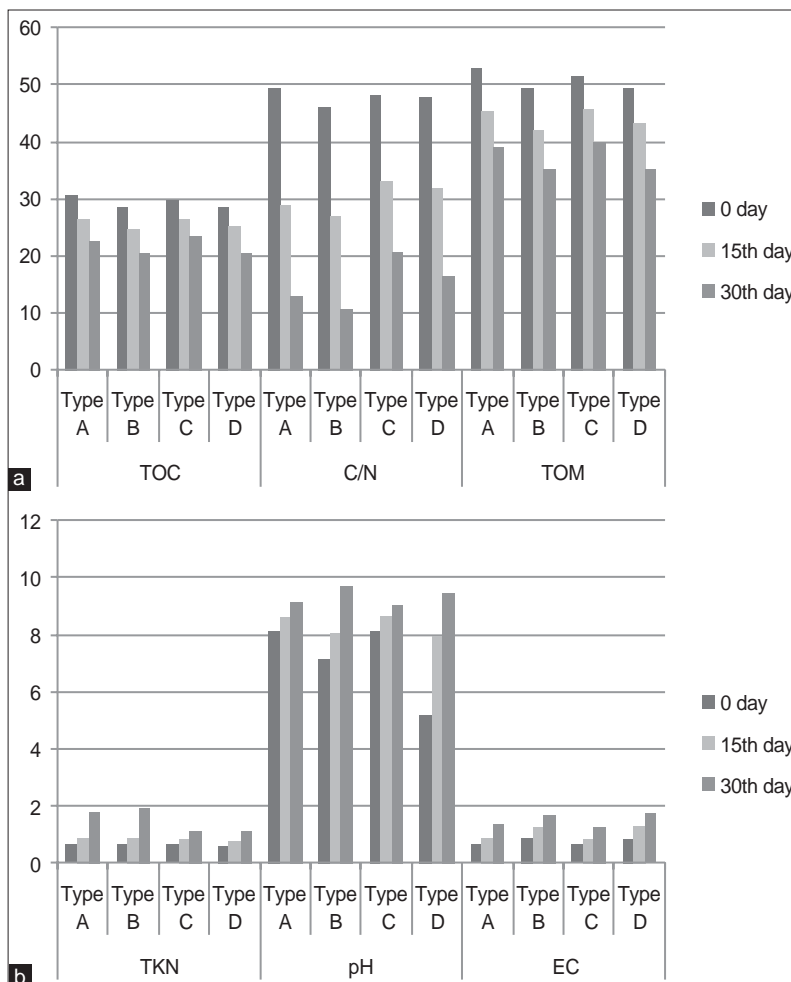


Figure 1: (a,b) Comparisons chart of physicochemical parameters of different types of leaf litter.

According to [15], the rate of mineralization and stabilization reflects the C/N ratio during vermicomposting process. In Figure 1a, the C/N ratio of all type range from 49.5 ± 0.004 to 12.86 ± 0.004 (Type A); 46.2 ± 0.034 to 10.67 ± 0.051 (Type B); 48.22 ± 0.005 to 20.8 ± 0.007 (Type C) and 47.8 ± 0.057 to 16.4 ± 0.07 (Type D) [16]. Reported that the C/N ratio < 20 indicates the satisfactory degree of maturity of organic waste. In our results C/N ratio < 20 is seen in all the four types of vermicompost prepared.

The loss of organic matter also implies the loss of C/N ratio. The total organic matter has got reduce from initial to final 52.9 ± 0.008 - 39.08 ± 0.008 and 49.7 ± 0.008 - 35.34 ± 0.008 in Type A and B. Similarly, 51.54 ± 0.008 - 40.16 ± 0.008 and 49.47 ± 0.008 - 35.16 ± 0.008 in Type C and D (Figure 1a). The loss of organic matter is propositional to the loss of carbon and gain of nitrogen in the vermicomposting process [17,14].

3.3. Isolation and Identification of Micro Organism

Naturally occurring microorganisms are the primary decomposers that accomplish composting. These microorganisms include bacteria, fungi, actinomycetes

and protozoa. They break up waste debris and facilitate the decomposition. Microbial activity is considered to be greatly stimulated by favorable condition (moisture content, pH, high concentration of mucus) which would have enhanced the microbial activity resulting in the digestion of soil organic matter [18].

The number of bacteria in all types wastes were isolated using nutrient agar plating method. During vermicomposting process, the bacterial diversity was higher than in the latter phase. Totally 5 different strains were isolated from vermicompost. Table 1 shows the morphological characteristics of the organism. The biochemical results for the isolated organisms were represented in Table 2 and in Figure 2. Finally, the bacterial strains like *Bacillus thuringiensis*, *Bacillus anthracis*, *Bacillus funiculas*, *Bacillus cerus*, *Lactobacillus patheries* were identified.

3.4. FTIR Analysis

The infrared spectra of Type A and Type B were interrupted in Figure 3 and Type C and D were interrupted in Figure 4. Significant broad bands were centered at 3831 - 3806 cm^{-1} were observed

Table 1: Morphological characterization of isolated organism.

Morphology	Shape	Colour	Size	Texture	Elevation	Opacity
<i>Bacillus thuringiensis</i>	Irrugular	White	Small	Mucus	Concave	Opaque
<i>Bacillus anthracis</i>	Circular	White	Medium	Mucus	Concave	Opaque
<i>Bacillus fumiculus</i>	Circular	Pale white	Medium	Mucus	Concave	Transparent
<i>Bacillus cerus</i>	Circular	Pale white	Large	Dry	Convex	Opaque
<i>Lactobacillus pantheries</i>	Circular	Yellow	Small	Dry	Convex	Transparent

B. thuringiensis: *Bacillus thuringiensis*, *B. anthracis*: *Bacillus anthracis*, *B. fumiculus*: *Bacillus fumiculus*, *B. cerus*: *Bacillus cerus*, *L. pantheries*: *Lactobacillus pantheries*

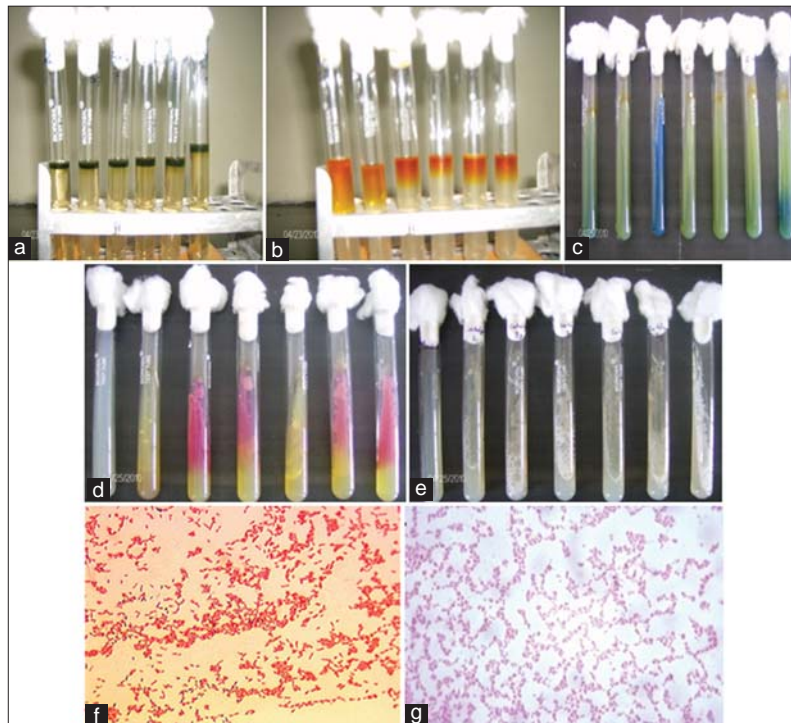


Figure 2: Biochemical tests for isolated organism, (a) Indole test, (b) Methyl red test, (c) Citrate utilization test, (d) Urease test, (e) Catalase test, (f) Gramnegative, (g) Grampositive.

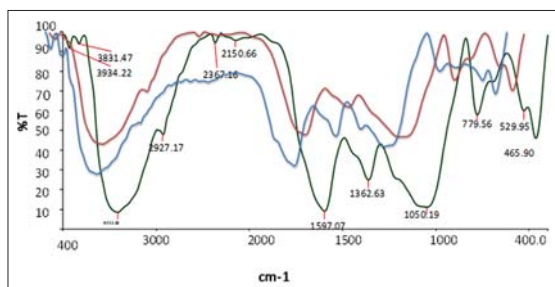


Figure 3: Comparison of Fourier transform infrared spectroscopy spectra of Type A and B.

in each spectrum, which corresponding to O-H hydroxyl group. The stretches of vibration 2815-2813 cm^{-1} is reported to be the presences of amide group. The peak value around 1649-1521 cm^{-1} is found due to be the presence of C=C aromatic structure. Hence, during composting the protein,

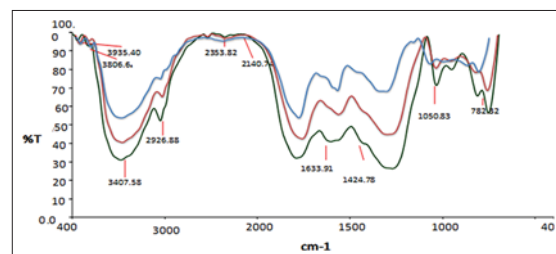


Figure 4: Comparison of Fourier transform infrared spectroscopy spectra of Type C and D.

cellulose and hemicelluloses has got mineralized. The increase in the aromatic C to aliphatic C ratio is considered as an indicator of an increasing degree of organic matter huminification in natural condition of biodegradation [19] which could be associated to the stability and maturity of compost and their transformation to highly humified substrate [20].

Table 2: Biochemical characterization of isolated organism.

Strain name	Indole	MR	VP	Catalyse	Citrate	Urease	Oxidase	H ₂ S	Motility	Gram staining
<i>B. thuringiensis</i>	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	Motile	+ve
<i>B. anthracis</i>	-ve	-ve	+ve	+ve	+ve	±	+ve	-ve	Motile	+ve
<i>B. fumiculus</i>	-ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve	Motile	+ve
<i>B. cerus</i>	-ve	-ve	+ve	+ve	+ve	±	+ve	-ve	Motile	+ve
<i>L. pantheries</i>	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	Non motile	+cocci

B. thuringiensis: *Bacillus thuringiensis*, *B. anthracis*: *Bacillus anthracis*, *B. fumiculus*: *Bacillus fumiculus*, *B. cerus*: *Bacillus cerus*, *L. pantheries*: *Lactobacillus pantheries*

While comparing all the types the intensity of peak in Type B and D is less in Type A and C. Therefore we conclude that the rate of decomposition of leaf litter is better in *E. eugenia* than in *E. fetida*.

4. CONCLUSION

The present study substantiated the feasibility of utilization of leaf litter such as *T. grandis* and *Casuarina* through vermicomposting process. Our results demonstrate that the decomposition of the waste materials using *E. eugenia* was better, by showing significant reduction in C:N ratio while compared with that by *E. fetida*. The FTIR spectroscopy analysis in the vermicompost of different types showed the degradation of lipids, carbohydrate and nitrogenous substances. This study also indicates the significance of the microorganisms present in the vermicompost. Finally, it was concluded that if leaf litter and cow dung are blended in appropriate quantities, it would yield a good quality vermicompost. By this way the waste dumped in the landfills as well the burning of leaf litter would be appreciably reduced resulting in safer environment.

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***Bibliographical Sketch**



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