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VERMICONVERSION OF MIXED LEAVES LITTER (PRE-DIGESTED WITH FUNGAL CONSORTIUM) BY USING *EUDRILUS EUGENIAE*, *PERIONYX EXCAVATUS* AND *LAMPITO MAURITII*

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ABSTRACT

The main objective of this research work is to utilize fungal consortium containing *Aspergillus oryzae, Aspergillus fumigates* and *Rhizopus oryzae* for the partial degradation of mixed leaves litter. The Vermicomposting ability of *Eudrilus eugeniae, Perionyx excavatus* and *Lampito mauritii* under monoculture (E I, E II and E III, respectively) and polyculture (E IV) conditions by using pre-digested mixed leaves litter in combination with cow dung (1:1 ratio) was studied. The conversion ratio of waste into vermicompost was in the order: E III > E IV> E I > E II. In the finished product the chemical nutrients and biological composition were measured. The vermicompost obtained from E III and E IV experimental trays were rich in macro and micro nutrients and microbial populations Therefore it may be concluded that the mixed leaves litter must be pre-digested with the chosen fungal consortium and be converted into vermicompost by using *L. mauritii* under Monoculture and *E. eugeniae, P. excavatus* and *L. mauritii* under Polyculture conditions.

KEY WORDS: ***Fungal consortium, Mixed Leaves Litter, Eudrilus eugeniae, Perionyx excavatus, Lampito mauritii and Vermicompost***

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INTRODUCTION

Waste is a valuable raw material which can be converted into useful products by making use of appropriate processing technology[1]. Recycling and refuse of solid wastes help to reduce the problem of waste disposal. By utilizing the solid waste, vermicompost can be produced which eventually can bring down pollution level and keep the environment clean. At present, the potential of vermicomposting as a viable alternative for waste management is gaining momentum in India[2]. Application of vermicompost can improve the physicochemical and biological properties of soils[3]. Leaves litter of various plants is seen in many places, which is a good source of waste for composting. Instead of converting them into compost they are burnt in public places, which ultimately result in air pollution in and around human habitations. In order to avoid such polluting activity, an attempt has been made to convert these wastes into valuable product *i.e.,* vermicompost. When the leaf litter is subjected to composting process, the ligno-cellulose usually constitutes an important component of the total organic matter and they decompose slowly. These organic constituents are difficult to break down in a normal composting process and can take considerable period of time. During vermicomposting technique, the earthworms require pre-decomposed wastes for their feeding. In order to derive maximum benefits out of this process, it would be desirable to decrease the pre-decomposition time of the leaf litter initially with certain efficient fungi in the form of fungal consortium. Therefore, combined approach of partial composting and vermicomposting is deemed as one of the most efficient technologies for adoption during present day situations and future. Therefore, the utilization of fungal consortium becomes absolutely essential for pre-digestion of large quantity of leaf litter available around us and to obtain the pre-digested food within a short span of time due to quick decomposition of complex chemical constituents into simple forms with the help of enzymes synthesized by fungi and the same product can be used as a food for earthworms. The microorganisms naturally present in the wastes usually hasten the decomposition process. In this study, we investigated a biological method of accelerating the decomposition of chief chemical constituents by using a novel fungal consortium. It is the best alternative of the present day's environmental degradation to make proper use of the available unutilized organic biodegradable wastes in order to convert them into compost within a short period. The three earthworm species *viz., Eudrilus eugeniae, Perionyx excavatus* and *Lampito mauritii* which are known to be voracious feeders and have other attributes suitable for vermicomposting, high fecundity, high rate of growth, preference for the temperature, etc., were employed. These include an *epigeic* (Phytophagous) earthworm species – *E. eugeniae* and *P. excavatus* and an *anecic* (Geophytophagous) earthworm species – *L. mauritii* [4].

Therefore, the study is aimed at

1. To explore the feasibility of fungal consortium for the partial decomposition of mixed leaves litter for the production of quality vermicompost.
2. To test the vermicomposting ability of *Eudrilus eugeniae, Perionyx excavatus* and *Lampito mauritii* under monoculture and polyculture conditions by using pre-digested mixed leaves litter with the chosen fungal consortium.

MATERIALS AND METHODS

Mixed leaves litter and cow dung was collected from Puthanampatti village of Tiruchirappalli district, Tamil Nadu, India. The earthworm species *i.e., Eudrilus eugeniae, Perionyx excavatus* and *Lampito mauritii* was collected from Vermiculture Yard of our college and these species were identified and confirmed by using morphological characters given by Talashilkarand Dosani[5] and Blakemore[6]. The fungal consortium which contains *Rhizopus oryzae, Aspergillus oryzae* and *Aspergillus fumigates* was utilized for the pre-digestion of mixed leaves litter and the fungal strains *Rhizopus oryzae* (MTCC 8784)*, Aspergillus oryzae* (MTCC1122) and *Aspergillus fumigates* (MTCC870) were procured from CSIR-Institute of Microbial Technology, Chandigarh. The fungal consortium was grown in 1% molasses solution for 7 days. Two discs of fungal mycelium of all the three fungal strains were subcultured in PDA for mass cultivation and incubated at 30±2◦C for 6 days. After 6 days of growth, 5% (v/v) of spore suspension of fungal consortium was mixed with 1 kg of moistened mixed leaves litter for bioconversion[7]. Similar layers with these combinations were laid until the heap reached one meter height. Further, the moisture content of the substrate was maintained as 65% by periodic sprinkling of water. This setup was maintained for 23 days. Plastic trays of 45 × 15 × 30 cm with a hole at the bottom were used as experimental trays. The feed materials were prepared by mixing the pre-digested mixed leaves litter with cow dung in 1:1 ratio and filled in plastic trays. The moisture content was maintained around 40% throughout the study period by periodic sprinkling of adequate quantity of water. After 2 to 3 days, adult earthworms were introduced in to each experimental tray. The experimental design was given in Table 1.

Table 1
Experimental design for vermicompost production

S. No.	Experiment Name	Name of the earthworm species used	Number of earthworms introduced in each tray
1	E I	*E. eugeniae*	108
2	E II	*P. excavatus*	108
3	E III	*L. mauritii*	108
4	E IV	*E. eugeniae + P. excavatus + L. mauritii*	36+36+36
5	Control	-	-

All these experiments were replicated thrice. When the compost was ready by its physical appearance as ascertained by the development of a dark brown to black colour, watering was stopped. A sample of vermicompost was collected from each experimental trays and air dried at room temperature (28º C). All the samples were stored in a zip lock polythene cover for further analyses.

Table 2
Methods used for analyzing various physico-chemical, biological and enzyme parameters of the vermicompost and compost

S. No.	Parameter Analyzed	Methodology	Reference
1	pH	Digital pH meter (Elico)	Tandon[8]
2	Electrical Conductivity	Digital Electrical Conductivity Meter (Elico)	Tandon[8]
3	Moisture	-	Tandon[8]
4	Organic Carbon	Potassium Dichromate Oxidation Method	Walkley and Black[9]
5	Total Nitrogen	Micro Kjeldahl Method	Tandon[8]
6	Total Phosphorus	Spectrophotometric Method	Tandon[8]
7	Total Potassium	Flame Photometric Method	Tandon[8]
8	C:N ratio	-	Anon[10]
Quantification of Microbial Populations			
9	Colony Forming Units of Bacteria, Fungi and Actinomycetes	Serial Dilution Plate Method	Tandon[8]

The total number of colony forming units of bacteria, fungi and actinomycetes present in the vermicompost samples were estimated by serial dilution method. Nutrient Agar for bacteria, Potato Dextrose Agar for fungi and Soil Extract Agar for actinomycetes were used[8].

STATISTICAL ANALYSES

Paired samples "t" test was used to determine difference between initial and final products in each treatment at 0.05%, 0.01% and 0.001% levels of significance. All these analyses were done by using SPSS (Statistical Package for Social Science) program version 16.0 for windows.

RESULTS AND DISCUSSION

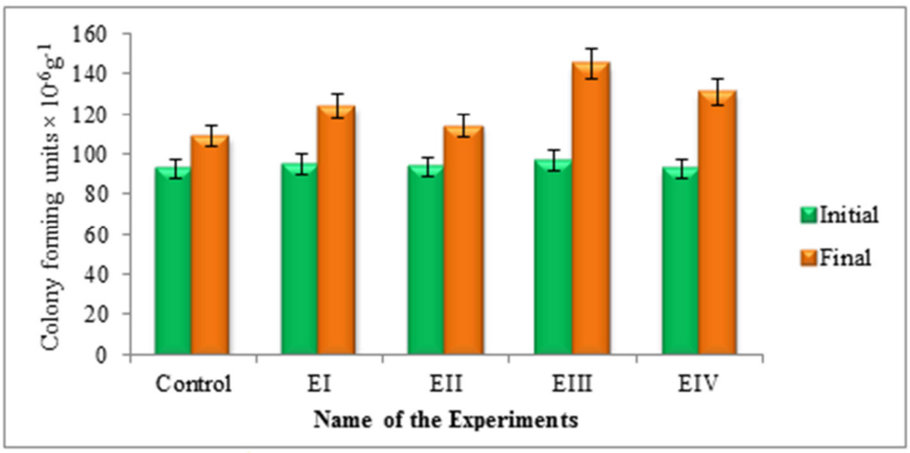
The average weight of vermicompost and compost obtained after vermicomposting of mixed leaves litter were in the order: 3200g (E III)>3000g (E IV)>2500g (E I)>2100g (E II)>1800 (Control).The mean percent conversion was in the order: 80% (E III) >75% (E IV) >62.5% (E I) >52.5% (E II) >45% (Control).It is understood from the results that the maximum quantity of vermicompost was produced by *L. mauritii* under monoculture condition followed by polyculture conditions with the combination of three earthworm species. Hence it may be concluded that *L. mauritii* under monoculture condition and polyculture conditions with a combination of three species of earthworms may be recommended to vermicompost producers (over the other monoculture conditions) in order to get maximum bioconversion ratio of the wastes (vide Table 3).The vermicompost production rate generally depends on the characteristics of feed, environmental conditions and type of earthworm species[4].

Table 3
The quantity of composition of mixed leaves litter (pre-digested with fungal consortium) and cow dung in 1:1 concentration and their bioconversion into vermicompost by utilizing E. eugeniae, P. excavatus and L. mauritii. Each value represents the mean of three observations.

Particulars	EI	EII	EIII	EIV	Control
Weight of pre-digested mixed leaves litter in each tray (g)	2000	2000	2000	2000	2000
Weight of cow dung in each tray (g)	2000	2000	2000	2000	2000
Total weight of pre-digested mixture in each tray (g)	4000	4000	4000	4000	4000
Number of adult earthworms introduced in each tray	108	108	108	108	-
Mean total weight of vermicompost or compost obtained from each tray (g)	2500	2100	3200	3000	1800
Mean percentage of bioconversion of vermicompost/compost in each tray (%)	62.5	52.5	80	75	45

[1]For experiment combinations see Table 1.

The pH of the end product was significantly (t test; p<0.001) lower in all the experiments than the initial material except control (p=0.216) (Table 4). pH is one of the most important properties that affect the availability of nutrients. Macronutrients tend to be less available to the plants with low pH, and micronutrients tend to be less available to plants with high pH[11]. Thus, it is necessary for the compost to have an ideal pH when added to the soils so as to promote absorption of nutrients[12]. The EC values in the end product and initial material were significantly (t test; p<0.001) different from each other in all the experiments (Table 4). The increase in EC has been related to the accumulation of compounds with negative charges (such as lignin derivatives of the like of humic substances) and the increase of carboxylic and phenolic groups during the humification of organic matter[13]. The EC values of the vermicompost produced in the study did not exceed the threshold value of 4 dSm^{-1} and it indicates that this can be safely applied to soil[13]. With a low EC (<4 dSm^{-1}), the organic fertilizers release the mineral salts slowly, which is required for plant growth[15,16]. The moisture content in the end products and initial materials in all the experiments were significantly (t test; $p<0.001$) different from each other (Table 4). Tandon[8] suggested that the moisture content of good quality vermicompost is between 20 and 30%. The moisture content observed in this range is desirable for nitrogen fixing and phosphate solubilising bacteria for their survival. Low moisture conditions may also delay sexual development; it was found that earthworms of the same age developed clitella at different times under different moisture conditions[16].Our results are in accordance with these earlier works.[18-20,14] There was a significant (t test; $p<0.001$) decrease in the contents of organic carbon in the end products harvested from all the experimental trays than initial materials (Table 4). The reduction of carbon in vermicompost is the result of respiration and mineralization of the organic matter mainly by microorganisms and earthworms. Since vermicomposting is a combined action of earthworms and microorganisms, earthworms through their fragmenting action modify the substrate condition which consequently increase the surface area for microbial action[21] thus promote carbon loss through respiration and in similar pattern the oxidation of organic matter within the vermicomposting unit. Suthar[11] States that excreta and body fluid of earthworms like mucus encourage microbial multiplication which in turn promotes rapid respiration that minimizes the carbon level of the waste. There was a highly significant (t test; $p<0.001$) increase in the Total Nitrogen content of the end product harvested from all the experiments than initial materials (Table 4).The enhancement of total N in the vermicompost was probably due to mineralization of the organic matter containing proteins[22,23] and conversion of ammonium nitrogen into nitrate[24,25]. Earthworms can boost the nitrogen levels of the substrate during digestion in their gut adding their nitrogenous excretory products, mucus, body fluids, enzymes and even through the decaying dead tissues of worms in vermicomposting subsystem[12]. Significant variations were observed in the total Phosphorus content of the end product than initial materials (t test; $p<0.001$) (Table 4). The rise of TP might be due to the action of earth worms phosphatases and phosphorus solubilizing microorganisms in the worm cast. In supporting this suggestion Lee[26]stated that as the organic residues passes along the earthworms gut, the unavailable form of phosphorus in the organic matter was converted to available forms for plants. The total potassium content had statistically significant (t test; $p<0.001$) variations between final product and initial materials (Table 4). Large number of symbiotic micro flora present in the gut and the cast of earthworms in collaboration with secreted mucus and water increased the degradation of ingested organic matter and the release of assailable metabolites. These metabolites enhanced the enrichment of the vermicompost with exchangeable potassium[27]. The results of t test on the C:N ratio varied significantly (t test; $p<0.001$) between the end product and the initial materials (Table 4). Decrease in C:N ratio reflecedchanges in the forma and properties of organic matter during bioconversion, reduction in C:N ratio during the vermicomposting process became one of the most widely used indicators of vermicompost maturation[16]. According to Morais and Queta[28] a C:N ratio below 20 is an acceptable maturity level, while a ratio of 15 or lower is highly referable for agronomic purpose, therefore, the present results obtained from mixed leaves litter (pre-digested with fungal consortium) treated by *E. eugeniae, P. excavatus* and *L. mauritii* under monoculture and polyculture conditions, showed the C:N ratio within the acceptable limit for agricultural usage. The results of t test on the population of bacteria, fungi and actinomycetes varied significantly (t test; $p<0.001$) between end product and the initial materials (Figs. 1,2 & 3). Availability of half digested nutrient rich organic wastes by earthworm activity contributed for the proliferation of aerobic decomposing heterotrophic microbes. These results are in conformity with the results of earlier works like Kale *et al.*[29] who had reported higher counts of actinomycetes and bacteria when the *E. eugeniae* and *P. excavatus* worked organic waste mixed with soil. Parthasarathi and Ranganathan[30] had reported an increase of bacterial population in *L. mauritii* and *E. eugeniae* worked vermicompost when compared to control.



[1]For experiment combinations see Table 1

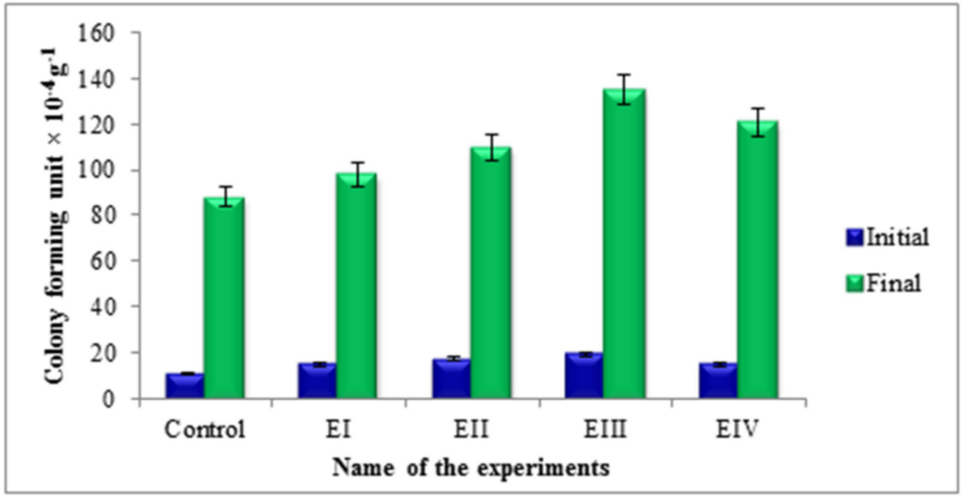
Figure 1

The quantity of bacterial population of initial and end product of the experiments

Table 4
Physico-chemical properties (Mean ± S.D., n = 3) of initial and end products of the experiments

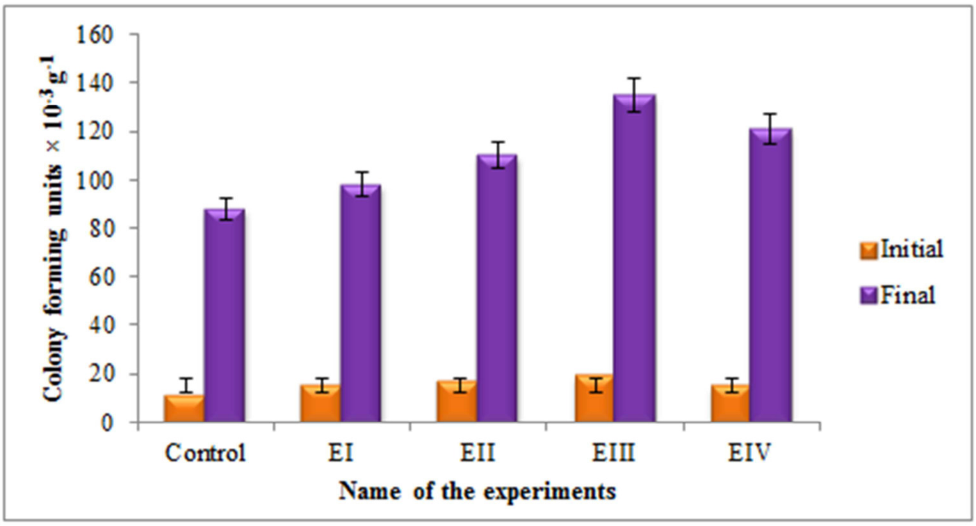
[1]Experiments	pH			EC			Moisture			Organic Carbon		
	Initial	Final	T test (Sig.)	Initial	Final	T test (Sig.)	Initial	Final	T test (Sig.)	Initial	Final	T test (Sig.)
Control	8.16±0.02	8.05±0.03	0.216^{NS}	1.55±0.03	1.95±0.02	0.05*	30.43±0.02	29.15±0.01	0.001***	28.35±0.00	18.95±0.03	0.001***
EI	8.26±0.02	7.95±0.03	0.01**	1.07±0.01	2.97±0.01	0.001***	33.41±0.02	21.17±0.02	0.001***	26.66±0.32	19.63±0.15	0.001***
EII	8.26±0.02	7.24±0.02	0.01**	1.04±0.03	2.85±0.04	0.001***	30.73±0.20	27.66±1.52	0.001***	25.38±0.03	19.94±0.04	0.001***
EIII	8.05±0.03	7.06±0.02	0.01**	1.95±0.02	2.83±0.02	0.01**	23.26±0.02	18.13±0.02	0.001***	18.86±0.03	15.68±0.01	0.001***
EIV	8.06±0.02	7.06±0.03	0.001***	1.66±0.03	2.04±0.02	0.001***	29.36±0.01	20.06±0.02	0.001***	26.17±0.02	19.21±0.02	0.001***
[2]Standard for Vermicompost 1	6.5 – 7.5			Not more than 4			14.0 – 25.0			Minimum 18%		
[1]Experiments	Total Nitrogen			Total Phosphorus			Total Potassium			C:N ratio		
	Initial	Final	T test (Sig.)	Initial	Final	T test (Sig.)	Initial	Final	T test (Sig.)	Initial	Final	T test (Sig.)
Control	1.04±0.03	1.28±0.01	0.01**	1.15±0.04	2.16±0.01	0.001***	2.77 ± 0.01	3.05 ± 0.01	0.001***	27:1	15:1	0.001***
EI	1.15±0.03	1.03±1.03	0.05*	1.20±0.01	2.06±0.02	0.05*	1.24 ± 0.02	2.87 ± 0.01	0.001***	26:1	17:1	0.001***
EII	0.94±0.01	1.19±0.01	0.001***	1.19±1.19	1.04±0.02	0.05*	1.64 ± 0.02	2.82 ± 0.02	0.001***	27:1	17:1	0.001***
EIII	1.13±0.04	2.25±0.04	0.001***	1.15±0.02	3.07±0.01	0.001***	1.17 ± 0.01	3.48 ± 0.00	0.001***	23:1	10:1	0.001***
EIV	1.03±0.02	1.33±0.02	0.001***	1.18±0.01	2.23±0.02	0.001***	2.48 ± 0.01	3.30 ± 0.01	0.001***	18:1	12:1	0.05*
[2]Standard for Vermicompost 1	>1			>1			>1			10:1 – 20:1		

*[1]For experiment combinations see Table 1; [2]Source: Tandon (2005), *, ** and *** indicates statistically significant at p<0.05, p<0.01, p<0.001.*



[1]For experiment combinations see Table 1.

Figure 2
The magnitude of fungal population of initial and end products of the experiments



[1]For experiment combinations see Table 1

Figure 3
The propensity of actinomycetes population of initial and end products of the experiments

CONCLUSION

The fungal consortium (includes *A. oryzae, A. fumigates* and *R. oryzae*) was required for the quick conversion of mixed leaves litter into vermicompost/compost. All the earthworm species under monoculture and polyculture were found suitable in the present study for bioconversion of mixed leaves litter into vermicompost. The highest bioconversion observed were in the order: E III> E VI > E I> EII. The vermicompost obtained from E III and E IV experimental trays were rich in macro and micro nutrients and microbial populations. Therefore it may be concluded that the mixed leaves litter must be pre-digested with fungal consortium and be converted into vermicompost by using *L. mauritii* under Monoculture and *E. eugeniae, P. excavatus* and *L. mauritii* under Polyculture conditions.

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AUTHORS CONTRIBUTION STATEMENT

Dr. P. Neelanarayanan and Dr. J. Viji contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

CONFLICT OF INTEREST

Conflict of interest declared none.

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