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PRODUCTION OF VERMICOMPOST BY UTILIZING PADDY (*ORYZA SATIVA*) STRAW (PRE-DIGESTED WITH *TRICHODERMA VIRIDE*) AND *EUDRILUS EUGENIAE, PERIONYX EXCAVATUS* AND *LAMPITO MAURITII*

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ABSTRACT

The paddy straw is one of the most abundant agricultural by-products in Tamil Nadu, India. It contains approximately 37% cellulose, 24% hemicelluloses and 15% lignin. In the present investigation the paddy straw was pre-digested with *Trichoderma viride* and subsequently the same was utilized for the production of vermicompost by using *Eudrilus eugeniae, Perionyx excavatus* and *Lampito mauritii* under monoculture conditions.The conversion ratio of waste into vermicompost was found to be high (71%) in the trays in which *E. eugeniae* was used. Further, it was observed in the end product the *E. eugeniae* produced more number of cocoons and young ones when compared to the other two cultures. The vermicompost harvested from *E. eugeniae* experimental trays showed remarkable levels of chemical nutrients and higher density of microbial population *viz.,* Bacteria, Actinomycetes and Fungi than the other experimental trays. The results of the present study apparently suggest that in though all the three earthworm species can be used for converting paddy straw (pre-digested with *T. viride*) into value added vermicompost, *E. eugeniae* in is very effective.

KEY WORDS: **Paddy straw, *Trichoderma viride, Eudrilus eugeniae, Perionyx excavatus, Lampito Mauritii,* Chemical nutrients, Bacteria, Actinomycetes and Fungi**

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INTRODUCTION

Paddy straw is one of the most abundant cellulosic wastes on the earth. In India, total annual production of rice is estimated to be 136.5 million tons [1]. About 1-1.5 kg of straw is produced from every kilogram of the grain harvested [2] and thus, 136.5-150 million tons of paddy straw is estimated to be produced annually. In India, approximately 85-95 million tons of paddy straw is disposed off by burning. One ton of paddy straw burning releases 3 kg particulate matter, 60 kg CO, 1460 kg CO_2, 199 kg ash and 2 kg SO_2 [3]. Burning of paddy straw causes lung and respiratory diseases in humans [4].Repeated burning of paddy straw also results in soil erosion. Paddy straw consists of cellulose (35-40 %), hemi-cellulose (20-24 %), lignin (8-12 %), ash (14-16 %) and extractives (10-12 %). Although, paddy straw has high cellulose content but the lignin complex and silica incrustation shields the microbial action for biogas production. Therefore, the paddy straw needs to be pre-treated in order to enable cellulose to be more accessible to the microbial/enzymatic attack. Many physical (mechanical and non-mechanical), chemical (alkaline hydrolysis, acid hydrolysis, oxidative delignification and solvent extraction), physico-chemical (ammonia fibre explosion, CO2 and steam explosion) and biological pre-treatments (ligno-cellulolytic micro-organisms and the enzymes) have been proposed in the recent years [5]. However, the physical and chemical pre-treatments require high energy, corrosion resistant and high pressure reactors which eventually increase the cost of pre-treatment. Furthermore, the chemical pre-treatment can be detrimental to the environment because of the formation and release of methanogens apart from generating acidic or alkaline water, which needs pre-disposal treatment to ensure environment safety [6].

Thus, an alternative approach is microbial pre-treatment which employs the use of microorganisms especially fungi to increase the digestibility of paddy straw. In biological pre-treatment processes, in general, microorganisms such as brown, white and soft rot fungi are used to degrade lignin and hemi-cellulose in waste materials. The advantages of biological pre-treatment of waste materials include less manpower, low energy requirement, less time and space and ultimately deemed as safe to environs and low cost technology. Most of the white-rot fungi degrade lignin and cellulose simultaneously [5].It is understood from the review of literature that the following fungi such as *Fusarium sp.*[7], *Armillaria* sp., *Polyporus* sp. and *P. Chrysosporium* [8], *Aspergillus awamorii, Paecilomyces fusisporus* and *Trichoderma viride* [9] have been used for the degradation of paddy straw. However, no work has been reported on the vermiconversion of paddy straw pre-digested with *T. viride* and hence the present investigation. The present study was undertaken to pre-digest the chosen agro-waste paddy straw with *T. viride* and subsequently to assess the vermicomposting potential of three earthworm species *i.e., Eudrilus eugeniae, Perionyx excavatus* and *Lampito mauritii* under monoculture conditions and the magnitude of chemical nutrients and micro-flora in the vermicompost produced by these earthworms.

MATERIALS AND METHODS

Collection of plant and animal waste

The selected agro-waste *i.e.,* Paddy straw was collected from Puthanampatti, Tiruchirappalli District, Tamil Nadu, India. Cow dung was collected from a nearby dairy yard of our college campus.

Collection of Earthworm Species

The vermicomposting earthworm species, *E. eugeniae*, *P. excavatus* and *L. mauritii* were obtained from our college vermished.

Preparation of T. viride inoculum

10 ml of molasses was taken in a conical flask and 90 ml of distilled water was added and mixed well. To this 1 ml of pure culture of *T.*

viride was added and mixed with 1 litre of jaggery solution (1 kg of jaggery + 1 litre of water). This preparation was mixed well and maintained for 7 days and used as an inoculum.

Pre-digestion of the chosen Organic wastes

The paddy straw was spread on a clean floor, which was open to sunlight for 5 days. Watering was done regularly twice in a day on the paddy straw. Similar method was adopted for curing cow dung. The sun dried paddy straw and cow dung were transferred to a shady place where it was cured for 5 days. Later 1kg of paddy straw was spread on a clean floor and to this 50 ml of *T. viride* was uniformly sprinkled. Above to this layer again 1kg of paddy straw was spread and this process was repeated until the heap reaches a height of about 1 meter. The moisture content in the heap was maintained at about 60-70% by sprinkling water. All these layers were covered by wet pieces of jute bags in order to maintain the moisture content. This set up was maintained for 30 days.

Preparation of Experimental Trays

Plastic trays of 45×15×30 cm were bought and holes were made to drain the excess water in the experimental medium. Vermibeds were prepared by mixing the pre-decomposed paddy straw along with cow dung in 50:50 concentration and they were filled in twelve trays, individually. Of these, three trays each were utilized for inoculating *E. eugeniae, P. excavatus* and *L. mauritii,* individually as monoculture. The remaining three trays were maintained as control in order to make comparisons with vermicompost. Healthy and clitellate individuals of all the three species of earthworms were collected from the vermished of our college. Hundred adult worms of *E. eugeniae, P. excavatus* and *L. mauritii* were introduced into the respective designated experimental trays. It was observed that the worms entered into the media immediately after the inoculation. These trays were kept in an undisturbed shady place. Watering was done regularly twice in a day in order to maintain the temperature and moisture content of the medium during the entire composting period. On 18th day the experiment was terminated as the pre-digested food was converted into vermicompost and the same was harvested and sieved with 3mm mesh size sieve.

Chemical Nutrient analysis

The vermicompost and compost materials were then analysed for different physico-chemical parameters. The pH was measured by using a digital pH meter (Elico make Model No. 120) and Electrical conductivity was measured by using digital conductivity meter (Systronics make Model No. 304). The Moisture content was determined by adopting the method suggested by Tandon [10]. The organic carbon was determined by partial oxidation method of Walkley and Black [11]. Total nitrogen, total phosphorous and total potassium were determined by micro kjeldhal, spectrophotometric and flame photometric methods suggested by Tandon [10], respectively. Total Calcium and Total magnesium were estimated by versenate method as suggested by Trivedy and Goel [12]. The Flame photometric and Spectrophotometric methods of Tandon [10] were used for the estimation of Total sodium and total sulphur, respectively. C:N ratio was calculated by dividing the percentage of organic carbon with percentage of total nitrogen [13].

Quantification of Microorganisms

The total number of colony forming units of bacteria, fungi and actinomycetes present in the control and vermicompost samples were estimated by serial dilution method [14]. Nutrient Agar for bacteria, Potato Dextrose Agar for fungi and Soil Extract Agar for actinomycetes were used. The obtained data were subjected to appropriate statistical analyses.

RESULTS AND DISCUSSION

Magnitude of composition of pre-digested food (Paddy (*Oryza sativa*) Straw,– pre-digested with *T. viride*) and its bioconversion into vermicompost by *E. eugeniae, P. excavatus* and *L. mauritii* is presented in Table 1. The mean number of days required for bio-conversion was 15 (*E. eugeniae*), 27 (*P. excavatus*), 35 (*L. mauritii*) and 58 (Control). The average total weight of vermicompost obtained after vermicomposting of paddy straw were 2870g (*E. eugeniae*), 2125g (*P. excavatus*), 2055g (*L. mauritii*) and 1985g (Control). The mean percent conversion of vermicompost was 72% for *E. eugeniae*, 53% for *P. excavatus*, 51% for *L. mauritii* and 50% for Control. The mean number of cocoons and young ones enumerated were to the tune of 89 and 82 (*E. eugeniae*), 75 and 66 (*P. excavatus*) and 51 and 41 (*L. mauritii*) (Table 1).

Table 1

Magnitude of composition of pre-digested food (Paddy (Oryza sativa)Straw,– pre-digested with T. viride) and its bioconversion into vermicompost by E. eugeniae, P. excavatus and L. mauritii. Each value represents the mean of three observations.

Sl. No.	Particulars	Composting organisms			
		E. eugeniae	*P. excavatus*	*L. mauritii*	Control
1.	Weight of cured predigested paddy straw(g)	2000	2000	2000	2000
2.	Weight of cured cow dung (g)	2000	2000	2000	2000
3.	Total weight of pre-digested mixture (g)	4000	4000	4000	4000
4.	Number of adult worms introduced	100	100	100	-
5.	Total weight of compost/vermicompost obtained (g)	2870	2125	2055	1985
6.	Percent conversion of vermicompost	71%	53%	51%	50%
7.	Number of days taken for conversion	15	27	35	58
8.	Mean number of cocoons observed in each tray	89	75	51	-
9.	Mean number of young ones observed in each tray	82	66	41	-

The physicochemical characteristics of vermicompost and compost are given in Table 2.

pH

The pH value of the vermicompost produced by all the three species of earthworms utilized in the present investigation was found to be within/close to the standard value for vermicompost (Table 2). However, the control had 7.92 ±0.22. Earlier, Ndegwa and Thompson [15]and Kaushik and Garg [16] reported similar results in vermicomposting experiments, and suggested that the mineralization of N and P compounds, the release of CO_2 and organic acids from microbial metabolism, and the production of humic and fulvic acids, as possible causes of the decrease in pH during vermicomposting process.

Electrical Conductivity

The electrical conductivity value was comparatively higher in vermicompost than control (Table 2). The maximum increase was observed in the vermicompost produced by *L. mauritii* (1.51 ± 0.05). The electrical conductivity (EC) reflects the salinity of an organic amendment. High salt concentration may cause phytotoxicity problems and therefore EC is a good indicator of the suitability and safety of a compost or vermicompost for agricultural purposes [17]. A sharp increase due to the release of soluble salts like ammonium and phosphate after the degradation of the most labile compounds in the thermophilic stage of composting has also been reported [18]. 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.

Moisture

The vermicompost showed lower moisture level than control (Table 2). The moisture content of vermicompost obtained from all experiments was around 21 – 26%. Tandon [10] suggested that the moisture content of good quality vermicompost should be between 20 and 30%. In this range the nitrogen fixing and phosphate solubilising bacteria can thrive well.

Table 2
The physicochemical characteristics of vermicompost produced by E. eugeniae, P. excavatus and L. mauritii utilizing Paddy straw (predigested with T. viride) in 50:50 concentration and control. Each values represents the mean (Mean ± S.D.) of three observations.

S.No.	Parameters	Composting organisms				
		E. eugeniae **Mean ± S. D.**	***P. excavatus*** **Mean ± S. D.**	***L. mauritii*** **Mean ± S. D.**	**Control Mean ± S. D.**	**Standard for vermicompost[1]**
1	pH	7.23 ± 0.05	7.21 ± 0.05	7.61 ± 0.01	7.92 ±0.22	6.5 – 7.5
2	Electrical Conductivity (dSm-1)	1.36 ± 0.01	1.42 ± 0.02	1.51 ±0.05	0.94 ± 0.14	Not more than 4
3	Moisture (%)	24.68 ± 0.04	21.1 ± 0.03	26.21 ± 0.04	38.7 ± 0.02	14.0 – 25.0
4	Organic Carbon (%)	28.63 ± 0.02	21.48 ± 0.05	29.21 ± 0.01	30.16 ± 0.01	Minimum 18%
5	Total Nitrogen (%)	2.11 ± 0.01	1.99 ± 0.01	1.95 ± 0.01	0.85 ± 0.15	>1
6	Total phosphorus (%)	1.88 ± 0.05	1.86 ± 0.03	1.15 ± 0.05	0.18 ± 0.03	>1
7	Total potassium (%)	2.15 ± 0.01	1.35 ± 0.03	1.12 ± 0.04	0.97 ± 0.01	>1
8	Total Calcium (%)	1.98 ± 0.01	1.95 ± 0.01	1.86 ± 0.04	1.70 ± 0.04	-
9	Total Magnesium (%)	1.78 ± 0.04	1.68 ± 0.04	1.52 ± 0.03	1.24 ± 0.01	-
10	Total Sodium (%)	1.48 ± 0.05	1.27 ± 0.01	0.81 ± 0.01	0.79 ± 0.01	-
11	Total Sulphur (%)	1.97 ± 0.05	1.12 ± 0.01	1.01 ± 0.05	0.87 ± 0.05	-
12	C: N ratio	14:1	11:1	15:1	30:1	10:1 – 20:1

S. D. – Standard Deviation
[1]Source: Tandon (2005 a, b).

Our results were in accordance with earlier works [19, 20]. 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 [21].

Organic Carbon
The organic carbon was found to be low in the final product *i.e.,* vermicompost when compared to control (Table 2). The maximum reduction (21.1 ± 0.03) was recorded in the vermicompost harvested from the experimental trays inoculated with *P. excavatus*. The observed results are supported by those of other authors [22, 20, 23] who have reported 20–45% loss of carbon as CO_2 during vermicomposting of municipal, industrial wastes and agro wastes. According to Dominguez [24] vermicomposting is a combined operation of earthworm and microorganisms in which earthworm fragments and homogenizes the ingested material through muscular action of their foregut and also adds mucus and enzymes to ingested material and thereby increases the surface area for microbial action while, microorganisms perform the biochemical degradation of waste material providing some extracellular enzymes required for organic waste decomposition within the worm's gut. Moreover, this biological mutuality caused C loss in the form of CO_2 from the substrates during the decomposition and mineralization of organic waste [25, 26]. The conversion of some part of organic fractions of waste into worm biomass can also reduce the C loss from the substrate.

Total Nitrogen
The total nitrogen content in the end product *i.e.,* vermicompost was higher than control (Table 2). The maximum increase was noticed in the vermicompost produced by *E. eugeniae* (2.11 ± 0.01) followed by *P. excavatus* (1.99 ± 0.01) and *L. mauritii* (1.95 ± 0.01). Suthar [27] suggested that decaying tissues of dead worms also add a significant amount of N to the vermicomposting sub-system. The earthworms also enhance the nitrogen levels of the substrate by adding their excretory products, mucus, body fluid, enzymes and even through decaying tissues of dead worms in vermicomposting sub-system [28].The observed pattern for nitrogen enhancement in cast appears to be related with quality of the substrate used for worm feed. Flegel and

Schreder [29] demonstrated a significant correlations of the enzymes activities (dehydrogenases, acid and alkaline phosphomonesterase; indicating microbial decomposition in worm's gut) in the earthworm casts with their organic C and total nitrogen content.

Total Phosphorus

The extent of total phosphorous content was comparatively higher in the vermicompost than the control (Table 2). The vermicompost produced by *E. eugeniae* showed the greater content of total phosphorus (1.88 ± 0.05) than other two earthworm species used. The observed increase in Total Phosphorous was attributed to direct action of worm gut enzymes on the waste and indirectly by stimulation of the micro flora. According to Lee [30], the passage of organic residue through the gut of earthworm, results in phosphorous converted to forms, which are more available to plants. Le Bayon and Binet [31], observed earthworm-mediated phosphatase enhancement in soils. They concluded that earthworms were responsible for additional alkaline phosphatases in substrate, produced in the worm gut and excreted through cast deposition and further release of P may be by P-solubilizing microorganisms in casts [32].

Total Potassium

Vermicomposted material showed higher total potassium level than control (Table 3). The maximum increase was observed in the vermicompost harvested from the experimental tray inoculated with *E. eugeniae* (2.15 ± 0.01). A similar increase in potassium was reported by Selvamuthukumaran and Neelanarayanan [20, 23]. Acid production by the microorganisms is the major mechanism for solubilizing the insoluble potassium. The enhanced number of micro flora present in the gut of earthworms in the case of vermicomposting might have played an important role in this process and increased potassium over the control [22]. Similarly Delgado *et al.*[33] reported higher TK content in the vermicomposts prepared while using sewage sludge as feed mixture. Suthar [27] suggested that earthworm processed waste material contains high concentration of exchangeable K, due to enhanced microbial activity during the vermicomposting process, which consequently enhances the rate of mineralization. In contrast Orozco *et al.*[34] opined a decrease in TK in coffee pulp waste after vermicomposting. These differences in the results can be attributed to the differences in the chemical nature of the initial feed mixtures [35].

Total Calcium and Total Magnesium

The concentration of Total Calcium and Total Magnesium had increased levels in vermicompost when compared to control (Table 2). It is suggested that gut processes associated with calcium metabolism are primarily responsible for enhanced content of inorganic calcium content in worm casts. However, the similar pattern of calcium enhancement is well documented in the available literature [35, 36]. Similar observations have been reported by Elvira *et al* .[37] during the vermicomposting of paper-pulp mill sludge by *E. andrei.* The higher concentration of Mg in vermicompost reported in present study was also in consistence with the findings of earlier workers [38, 39, 40] and Ca [38, 39]. The increased level of Ca and Mg in the vermicompost may be due to the increased microbial and enzyme activity in the gut of earthworms [41].

Total Sodium

The total sodium concentration was increased in all the three vermicompost produced by the chosen earthworms when compared to control (Table 2). Similar observations have been reported by Murali and Neelanarayanan [42] during the vermicomposting of coir waste by *E. eugeniae.*

Total Sulphur

The total sulphur content was observed to be higher in vermicompost than control (Table 2). Ramalingam and Ranganathan [43] opined that sulphur is an essential element for the synthesis of amino acids and vitamins. The present findings corroborate to those of

Selvamuthukumaran and Neelanarayanan [20], who demonstrated that higher S concentration in the vermicompost prepared from leaves litter.

C:N ratio

The C:N ratio had decreased levels in vermicompost when compared to control (Table 2). The C:N ratio traditionally considered as a parameter to determine the degree of maturity of compost. C:N ratio below 20:1 is indicative of acceptable maturity, while a ratio of 15:1 or lower being preferable [44].

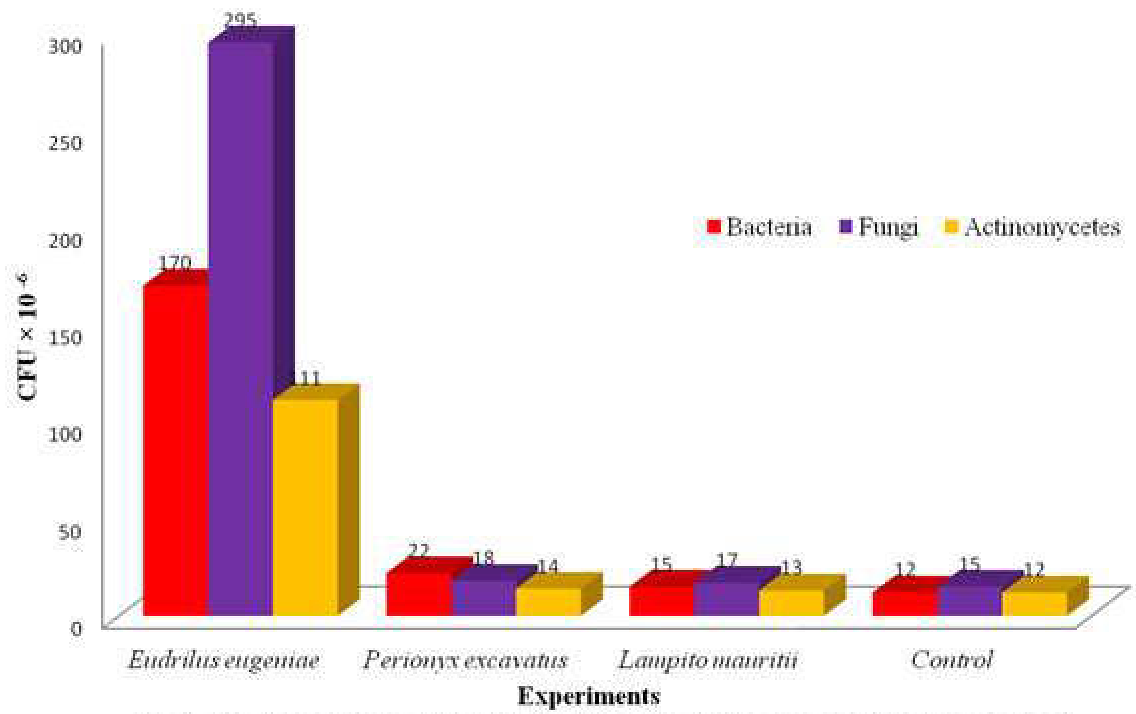

Fig. 1. Density of microbes (CFU × 10^{-6}) in compost processed by different species of earthworms

Microbial Population

In the present study, in general, an increase in the number of bacteria, fungi and actinomycetes colonies were observed (Fig. 1). The maximum increase was noticed in the vermicompost produced by *E. eugeniae* followed by *P. excavatus* and *L. mauritii.* 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* .[45] who had reported higher counts of actinomycetes and bacteria when the *E. eugeniae* and *P. excavatus* worked organic waste mixed with soil. Jambhekar [46] noticed a considerable increase in total viable counts of actinomycetes and bacteria in the vermicompost than the control. Parthasarathi and Ranganathan [47] had reported an increase of bacterial population in *L. mauritii* and *E. eugeniae* worked vermicompost when compared to control. The observed increase in microbial population in the vermicompost may be due to the nutrient rich organic wastes which were utilized for the vermicomposting process during the present study. Earlier, reported similar such observations. Similar observations have been reported by Tiwari *et al.*[40] and Selvamuthukumaran and Neelanarayanan [20] during the vermicomposting of pine apple leaves litter and leaf litter by *P. excavatus* respectively.

CONCLUSION

The results of the present study apparently suggest that *T. viride* may be used for the partial degradation of paddy straw and it is evident from the results that the chemical nutrients and microflora values were observed with desired level in the vermicompost produced by using all the three chosen earthworm species. Hence it may be concluded, in general, all these three earthworm species may be used to produce vermicompost and in particular, *E. eugeniae* was found to be better for vermicompost production for the following reasons:

- Highest rate of bioconversion,
- Lowest number of days required for the bioconversion,
- Number of cocoons and young ones produced was found to be high,
- The quantity of macro and micronutrients in the vermicompost was found to be within the good quality vermicompost range values and
- Increased number of micro flora.

In the present study, in general the total time taken for the bioconversion of raw paddy straw into vermicompost was 53 days. Further, research work is required in order to find out a method in which the bioconversion of the raw paddy straw into vermicompost is possible in about 25-30 days or even lesser than this time limit.

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