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MICROBIAL DIVERSITY AND ACTIVITY DURING RECYCLING OF COFFEE HUSK BLENDED WITH DIFFERENT ORGANIC WASTE USING INDIGENOUS EARTHWORM *PERIONXY CEYLANENSIS*

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ABSTRACT

Coffee husk (CH) amended with cow dung (CD) and pressmud (PM) and effect of indigenous earthworm *Perionyx ceylanensis* were performed to evaluate biological potential during vermicomposting. A total of six different vermicomposters were maintained for this study and the experiments were monitored for 60 days. The results suggested that the total microbial population of vermicompost produced were significantly higher than initial substrate. Similarly, the microbial activities of vermicompost obtained from all the vermicomposter were significantly increased after vermicomposting. Results also revealed that *Perionyx ceylanensis* considerable effects on microbial population and activity during vermicomposting of CH amended with organic waste than CH alone. Periodical analysis of above mentioned microbial population and enzyme activity of final vermicompost indicated that equal proportion (1:1:1 ratio) of CD, PM and CH are probably the optimum composition to obtain best quality vermicompost for agronomic use.

Keywords: coffee husk, vermicomposting, *Perionxy ceylanensis*, microbial population and activity

1.INTRODUCTION

Vermitechnology has been used for the management of agro based industrial waste. It is well established that organic wastes can be ingested by earthworms and egested as peat like material termed as vermicompost [2, 12]. It is much more fragmented, porous and microbially active than parent material [6] due to humification and increased decomposition. During vermicomposting, organic matter is transformed into a rich humic product by the action of microorganisms and earthworms. Nevertheless, and in spite of this major role, most studies in vermicomposting have focused on physico-chemical parameters to evaluate both process evolution and compost quality. Properties like cat-ion exchange capacity, C:N ratio or humic fraction ratio have traditionally been used for the monitoring of composting/vermicomposting processes, while biological and biochemical parameters have recently arisen as good indicators both during and at the end of the aerobic biotransformation of organic wastes [4,13]. Starting material is one of these factors, since earthworms adapted to the nature and concentration of the available carbon substrates will grow and reproduce to a higher extent [13]. Therefore, characterizing microbial communities and enzyme activities during vermicomposting process may provide valuable information regarding the evolution of the process, the rate of biodegradation and finally, the maturity of the product [1].

Coffee husk contains some amount of caffeine and tannins, which can make it toxic and slow degradation in nature, resulting the disposal problem. However, coffee husk is rich in lignocelluloses materials, which makes it an ideal substrate for microbial processes. Several solutions and alternative uses of coffee husk/pulp have been attempted. Coffee husk and coffee pulp have been used as a raw material for bioprocess to produce biogas, enzymes, mushroom and compost [1]. However, CH along with CP useful nutrients, and are therefore used as organic fertilizer [14, 22]. However uncontrolled decomposition and excess applications of CH to soil can cause environmental problems due to their extremely high levels of protein (10.1%), reduc ed sugar (12.4%), ash (8. 3%), and caffe in (1. 3%), low pH, and heat generation. Therefore, there is an urgent need to recycle the Coffee husk without environmental impact.

Several epigeic earthworm, e.g., *Eisenia fetida, Eudrilus eugeniae, Perionyx excavates* and *Perionyx sansibaricus* have been identified as detritus feeders and can be used potentially to minimize the anthropogenic wastes from different sources [12]. Growth and reproduction of *E. eugeniae* were studied by Neuhauser *et al.* [16] using sludge and horse manure, using a mixture of animal and vegetable waste materials by Loehr *et al.* [12] and using cow dung by Kale and Bano [9]. Further, Kale *et al.* [10] reported the better growth of *E. eugeniae* in press mud. Ramalingam

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[19]studied the growth, reproduction and life cycle of *E. eugeniae* and *L. mauritii* using pressmud. Karmegam and Daniel [11] studied the growth and reproduction of *E. eugeniae* in leaf litter substrates. The indigenous earthworms (*Perionyx ceylanensis*) which were commonly found in Indian soils, has appeared as an efficient tool for organic waste reduction [27]. The aim of this work is to study the evolution of some important enzymatic activities, as well as of the total microbial communities, during the vermicomposting of Coffee husk amended with pressmud and cow dung using indigenous earthworm species (*P.ceylanensis*) and to determine the influence of earthworms and nature of the CH on these parameters in order to produce large scale vermicompost.

2.MATERIALS AND METHODS

Collection of organic waste and earthworm species

Coffee husks were collected from coffee processing areas located in Yercaud, Tamilnadu, India.

Press mud (PM) was obtained from effluent treatment plant of E.I.D. Parry Sugar Mill located at Nellikkuppam, Tamil Nadu, and India. Fresh Cow dung (CD) was collected from the agricultural farm, Faculty of Agriculture, Annamalai University, Tamil Nadu, India. Native earthworm species *Perionxy ceylanensis* of different age groups were cultured and developed outside the laboratory on partially degraded cow dung as feed, respectively. *Perionxy ceylanensis* (25-29 days) were randomly picked from the stock culture and used for the purpose of this experiment.

Experimental design

Six vermicomposters (cement tank) were established having 2 kg of feed mixture each containing CD, PM and CH alone (control) and CD, PM mixed with CH in different rations (Table1). Each vermicomposter was established in triplicate. The feed mixtures were turned manually every day for two weeks in order to stabilize the feed so that it becomes palatable to worms. After two weeks fifty species of worms were introduced in each vermicomposter, separately. The moisture content was maintained at 65-75% during the experiment. The vermicomposter were covered with moist jute to prevent moisture loss. The 0 day (Initial) refers to the day of inoculation of earthworms after stabilization of two weeks. Samples (initial substrate and vermicompost) for periodical analysis were taken before inoculating earthworms and at the end of experimentation.

Analysis of total microbial populations and activity

The different microbial colonies developing on the plates were estimated by counting. Microbial biomass was analyzed by the chloroform fumigation–extraction method [28]. The number of colony forming unit (CFU) on the surface of the media was counted and expressed as CFU $\times 10^6 g^{-1}$, according to the method described by Baron et al. [3]. To determine the microbial activity (in terms of dehydrogenase activity), samples were collected from initial substrate and vermicompost of all the vermicomposters and worm gut. Dehydrogenase activity was determined according to the method described by Stevenson [25]. Cellulse, protease, urease and phosphatase activities were calculated according to the method described by Garcia et al. [8].

Statistical analysis

The objective of statistical analysis was to determine any significant differences among the parameters analyzed in different vermicomposters during the vermicomposting process. One-way ANOVA was used to analyze the significant differences among different vermicomposters. Tukey's *t*-test was used as a post hoc analysis to compare the means (SPSS Package). The probability levels used for statistical significance were $P < 0.05$ for the tests.

3.RESULTS AND DISCUSSION

The total microbial population (bacteria, fungi and actinomycetes) in different combination of CH, CD and PM mixture (initial), worm gut during vermicomposting using *Perionxy ceylanensis* and vermicompost were observed (Table 2-4**).** In the present observation total microbial population in vermicomposts made by both worms was significantly higher in CD+PM+ CH (1:1:1ratio) and it was followed by CD, PM, CD+ CH (1:1ratio), PM+ CH (1:1ratio) and CH, respectively. Among the different vermicomposters, CD+PM+ CH in1:1:1ratio and CD (control) were found to have significantly ($p<0.05$) higher microbial population than other vermicomposters (Table 2). Similarly, in the present analysis total microbial population in gut of *Perionxy ceylanensis* were higher in CD+PM+ CH (1:1:1 ratio) vermicomposter and it was followed by CD, PM, CD+ CH, PM+ CH and CH vermicomposters, respectively.

Microorganisms are the key factor in nutrient transformation and addition of bulking material in initial organic waste resulted in enrichment in the nutrient status of vermicomposts [19]. Fracchia *et al*. [7] have proposed a symbiotic relationship between earthworms and their gut microflora enhance the nutrient content of vermicomposts. In the present study, The significantly increased level of microbial population and their activity in the final product of *Perionxy ceylanensis* could be due to the higher nutrient concentration in the initial substrate material and vermicompost, multiplication of microbes while passing through the gut of worms, optimal moisture and large surface area of casts ideally suited for better feeding, multiplication and activity of microbes. It may be concluded that CH mixed with organic amendments (CD and PM in 1:1:1 ratio) is ideally suited for vermicomposting.

Dehydrogenase activity is considered as a parameter for microbial activity, which is related a group of enzymes that catalyze metabolic reactions producing ATP through the oxidation of organic matter. It has been often used to monitor the biological activity of composting and vermicomposting process [24, 29]. Results suggested that the dehydrogenase activity of vermicompost obtained from all the vermicomposters with *Perionxy ceylanensis* were increased significantly and especially in CD and CD+PM+ CH (1:1:1ratio) vermicomposters (Table 4). Similarly there was an increase in cellulase, protease and phosphatase in all the vermicomposters after vermicomposting. The highest increase in cellulase, protease and phosphatase were observed in 100% CD (control), 1:1:1 ratio of CD, PM and

CH (Tables 3 and 4). The availability of adequate oxygen, moisture, temperature, pH, the quantity and quality of organic matter and the amount of elemental nutrients are essential for the microbial growth and activity during vermicomposting [18]. Hence it was concluded that specific environment in vermicomposting, organic matter composition and the earthworm gut condition as well as selective effects of the earthworm gut fluid and surface excreta are probably the major dynamic forces for the observed pattern of microbial community and enzyme activity in vermicompost [5, 17]. The results of this study confirm that coupled microbial population and enzyme activities are helpful approaches for evaluating the impact of vermicomposting on CH, as well as for characterizing the derived finished products (vermicompost).

Table -1: Details of vermicomposters used for experimentations

Vermicomposter	Ratio	Description
CD (control)	-	100% cow dung
PM (control)	-	100% press mud
CH	-	100% Coffee husk
CD+ CH	1:1	1 part cow dung + 1 part Coffee husk
PM+ CH	1:1	1 part press mud + 1 part Coffee husk
CD+PM+ CH	1:1:1	1 part cow dung +1 part press mud+ 1 part Coffee husk

All values are reported as mean ± standard deviation between six replicates; values in the same column with different letters are significantly different (ANOVA; Tukey's test, $p < 0.01$).

Table -2: Total microbial population count (CFU $\times 10^6$ g^{-1}) in initial substrate, gut of worms and vermicompost of different vermicomposter of CH amended with different organic waste using *Perionxy ceylanensis*

Vermi composter	Initial Substrate	*Perionxy ceylanensis*	
		Gut of worm	Vermicompost
CD	3.50 ± 0.62^{c}	5.74 ± 0.44^{cd}	5.30 ± 0.32^{cd}
PM	3.45 ± 0.37^{c}	5.59 ± 0.53^{c}	5.21 ± 0.23^{c}
CH	2.36 ± 0.42^{a}	4.42 ± 0.41^{a}	4.07 ± 0.22^{a}
CD+ CH (1:1 ratio)	3.40 ± 0.29^{c}	5.66 ± 0.53^{c}	5.14 ± 0.41^{c}
PM+ CH (1:1 ratio)	3.19 ± 0.48^{b}	5.24 ± 0.71^{b}	5.07 ± 0.34^{b}
CD+PM+ CH (1:1:1ratio)	3.54 ± 0.51^{c}	5.85 ± 0.49^{cd}	5.75 ± 0.39^{d}

All values are reported as mean ± standard deviation between six replicates; values in the same column with different letters are significantly different (ANOVA; Tukey's test, $p < 0.01$).

Table - 3: Cellulase and protease during vermicomposting of CH amended with different organic waste using *Perionxy ceylanensis*

Vermicomposter	Cellulase (mg glucose g^{-1} oven dry substrates for 24 hrs incubation)		Protease (mg glutamic acid g^{-1} oven dry substrates for 24 hrs incubation)	
	Initial substrate	Vermicompost	Initial substrate	Vermicompost
CD	4.70 ± 0.19^{d}	6.50 ± 0.43^{e}	4.64 ± 0.45^{d}	6.26 ± 0.53^{e}
PM	4.29 ± 0.43^{c}	5.64 ± 0.55^{c}	3.30 ± 0.51^{bc}	5.19 ± 0.59^{c}
CH	2.95 ± 0.46^{a}	3.90 ± 0.47^{a}	2.54 ± 0.23^{a}	3.73 ± 0.25^{a}
CD+ CH (1:1 ratio)	3.96 ± 0.33^{bc}	5.94 ± 0.69^{d}	3.95 ± 0.58^{c}	5.38 ± 0.46^{d}
PM+ CH (1:1 ratio)	3.64 ± 0.66^{b}	4.46 ± 0.47^{b}	3.09 ± 0.31^{b}	4.35 ± 0.35^{b}
CD+PM+ CH (1:1:1 ratio)	4.78 ± 0.59^{d}	6.40 ± 0.62^{e}	4.64 ± 0.49^{d}	6.24 ± 0.41^{e}

All values are reported as mean ± standard deviation between six replicates; values in the same column with different letters are significantly different (ANOVA; Tukey's test, $p < 0.01$).

Table -4: Phosphatase and dehydrogenase activity during vermicomposting of CH amended with different organic waste using *Perionxy ceylanensis*

Vermicomposter	Phosphatase (mg phenol g^{-1} oven dry substrate sample for 24 hrs incubation)		Dehydrogenase activity (µl / 5 g substrate unit)	
	Initial substrate	Vermicompost	Initial substrate	Vermicompost
CD	3.24 ± 0.52^{e}	5.10 ± 0.31^{c}	9.25 ± 0.75^{d}	15.65 ± 0.32^{c}
PM	2.56 ± 0.43^{c}	4.39 ± 0.51^{bc}	8.54 ± 0.54^{c}	14.44 ± 0.41^{b}
CH	1.73 ± 0.37^{a}	2.29 ± 0.32^{a}	6.59 ± 0.49^{a}	13.80 ± 0.32^{a}
CD+ CH (1:1 ratio)	2.78 ± 0.61^{d}	3.75 ± 0.29^{c}	8.71 ± 0.62^{c}	14.89 ± 0.45^{bc}
PM+ CH (1:1 ratio)	2.25 ± 0.49^{b}	3.29 ± 0.37^{b}	8.25 ± 0.46^{b}	15.46 ± 0.55^{b}
CD+PM+ CH (1:1:1ratio)	3.16 ± 0.36^{e}	4.88 ± 0.49^{c}	9.19 ± 0.81^{d}	15.34 ± 0.41^{c}

All values are reported as mean ± standard deviation between six replicates; values in the same column with different letters are significantly different (ANOVA; Tukey's test, $p < 0.01$).

4.CONCLUSION

The present work on the feasibility analysis of vermicomposting CH waste by *Perionxy ceylanensis* has clearly indicated that CH could be converted to valuable manure with desirable microbial population and enzyme activity status in a short period of time. Among the various amendment combinations, 1:1:1 ratio of CD, PM and CH gave the best result in terms microbial population and enzyme activity. Therefore, it could be concluded that *Perionxy ceylanensis* are potential species for rearing and bio-stabilization of CH for large scale vermicompost production for sustainable agriculture.

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