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Effect of indigenous earthworm *Lampito mauritii* (Kinberg) and *Perionyx excavates* (Perrier) on microbial diversity and activity during bioconversion of poultry waste

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ABSTRACT

Influence of poultry waste (PW) amended with cow dung (CD) and pressmud (PM) and comparative studies of indigenous earthworm L. mauritii *and* P. excavates *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 by both worms were significantly higher than initial substrate. Similarly, the microbial activities of vermicompost obtained from all the vermicomposter for both species of worms were significantly increased after vermicomposting. Results also revealed that both the worms had considerable effects on microbial population and activity during vermicomposting of PW amended with organic waste than PW 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 PW are probably the optimum composition to obtain best quality vermicompost.*

Key words: poultry waste, vermicomposting, earthworms, microbial population and activity

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INTRODUCTION

Vermicomposting has been used for the management of agro 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].

The dramatic development of the poultry industry over the last 20 years created a serious waste disposal problem. India is one of the largest producers of poultry in the world and the poultry manure availability is estimated to be

12.1 million tons [26]. In the poultry farm large amount of droppings that accumulated in the litter turns it into importance sources of contamination *i.e.* odorous gases including amines, amides, mercaptans, sulphides and disulphides. These noxious gases can cause respiratory disease in animals and humans [23]. However, poultry droppings along with litter have useful nutrients, and are therefore used as organic fertilizer [14, 22]. However uncontrolled decomposition and excess applications of PW to soil can cause environmental problems due to their extremely high levels of nitrogen as ammonia, low pH, and heat generation. Therefore, there is an urgent need to recycle the poultry waste 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 [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 (*L. mauritii* and *P. excavates*) which were commonly found in Indian soils, has appeared as an efficient tool for organic waste reduction [27]. *L. mauritii and P. excavates* was, and still remains, the favored earthworm species for laboratory trail experiments on vermicomposting due to its wide tolerance of environmental variables [12]. 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 poultry waste amended with pressmud and cow dung using indigenous earthworm species (*L. mauritii* and *P. excavatus*) and to determine the influence of earthworms and nature of the poultry waste on these parameters in order to produce large scale vermicompost.

MATERIALS AND METHODS

Organic waste and earthworm species

Poultry waste (PW; droppings) was collected from Indian feeds farm, Perumalkovilmedu, Namakkal district, Tamil Nadu, India. Press mud (PM) was obtained from effluent treatment plant of E.I.D. Parry Sugar Mill located at Nellikkuppam, Tamil Nadu, India. Fresh Cow dung (CD) was collected from the agricultural farm, Faculty of Agriculture, Annamalai University, Tamil Nadu, India. Native earthworm species *Lampito mauritii* (Kinberg) and *Perionyx excavates* (Perrier) of different age groups were cultured and developed outside the laboratory on partially degraded cow dung as feed, respectively. Earthworms *L.mauritii* (30-35 days old) and *P. excavates* (25-30 days) were randomly picked from the culture and used for the purpose of this experiment.

Experimental design

Six vermicomposters (cement tank) were established having 3kg of feed mixture each containing CD, PM and PW alone (control) and CD, PM mixed with PW in different rations (Table1). Each vermicomposter was established in triplicate. The feed mixtures were turned manually every day for 14 days in order to stabilize the feed so that it becomes palatable to worms. After 14 days 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 14 days. Samples (initial substrate and vermicompost) for periodical analysis were taken before inoculating earthworms and at the end of experimentation.

Determination 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.

RESULTS AND DISCUSSION

The total microbial population (bacteria, fungi and actinomycetes) in different combination of PW, CD and PM mixture (initial), worm gut during vermicomposting using *L. mauritii* and *P. excavates* and vermicompost were observed (Table 1-6)**.** In the present observation total microbial population in vermicomposts made by both worms was significantly higher in CD+PM+PW (1:1:1ratio) and it was followed by CD, PM, CD+PW (1:1ratio), PM+PW (1:1ratio) and PW, respectively. Among the different vermicomposters, CD+PM+PW in1:1:1ratio and CD (control) were found to have significantly ($p<0.05$) higher microbial population than other vermicomposters for both species of worms (Table 2). Similarly, in the present analysis total microbial population in gut of *L. mauritii* and *P.excavatus* were higher in CD+PM+PW (1:1:1ratio) vermicomposter and it was followed by CD, PM, CD+PW, PM+PW and PW vermicomposters, respectively.

Table -1: Description of vermicomposters used for experimentations (*Lampito mauritii* and *Perionyx excavates*)

Vermicomposter	Ratio	Description
CD (control)	-	100% cow dung
PM (control)	-	100% press mud
PW	-	100% poultry waste
CD+PW	1:1	1 part cow dung + 1 part poultry waste
PM+PW	1:1	1 part press mud + 1 part poultry waste
CD+PM+PW	1:1:1	1 part cow dung +1 part press mud+ 1 part poultry waste

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 PW amended with different organic waste using *L. mauritii* and *P.excavatus*

Vermi composter	Initial Substrate	*L. mauritii*		*P.excavatus*	
		Gut of worm	Vermicompost	Gut of worm	Vermicompost
CD	3.58 ± 0.62^{c}	5.71 ± 0.44^{cd}	5.32 ± 0.32^{cd}	5.89 ± 0.29^{cd}	5.57 ± 0.51^{d}
PM	3.49 ± 0.37^{c}	5.59 ± 0.53^{c}	5.21 ± 0.23^{c}	5.76 ± 0.43^{c}	5.47 ± 0.32^{c}
PW	2.39 ± 0.42^{a}	4.42 ± 0.41^{a}	4.07 ± 0.22^{a}	4.43 ± 0.31^{a}	4.32 ± 0.37^{a}
CD+PW (1:1 ratio)	3.44 ± 0.29^{c}	5.65 ± 0.53^{c}	5.17 ± 0.41^{c}	5.71 ± 0.49^{c}	5.63 ± 0.49^{c}
PM+PW (1:1 ratio)	3.15 ± 0.48^{b}	5.23 ± 0.71^{b}	5.07 ± 0.34^{b}	5.32 ± 0.25^{b}	5.23 ± 0.33^{b}
CD+PM+PW (1:1:1ratio)	3.50 ± 0.51^{c}	5.89 ± 0.49^{cd}	5.71 ± 0.39^{d}	5.97 ± 0.33^{d}	5.83 ± 0.67^{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 PW amended with different organic waste using *L. mauritii*

Vermicomposter	Cellulase (mg glucose g^{-1} oven dry substrates for 24hrs incubation)		Protease (mg glutamic acid g^{-1} oven dry substrates for 24hrs incubation)	
	Initial substrate	Vermicompost	Initial substrate	Vermicompost
CD	4.74 ± 0.19^{d}	6.52 ± 0.43^{e}	4.63 ± 0.45^{d}	6.25 ± 0.53^{e}
PM	4.29 ± 0.43^{c}	5.65 ± 0.55^{c}	3.39 ± 0.51^{bc}	5.17 ± 0.59^{c}
PW	2.93 ± 0.46^{a}	3.94 ± 0.47^{a}	2.54 ± 0.23^{a}	3.73 ± 0.25^{a}
CD+PW (1:1 ratio)	3.91 ± 0.33^{bc}	5.98 ± 0.69^{d}	3.93 ± 0.58^{c}	5.38 ± 0.46^{d}
PM+PW (1:1 ratio)	3.69 ± 0.66^{b}	4.47 ± 0.47^{b}	3.07 ± 0.31^{b}	4.31 ± 0.35^{b}
CD+PM+PW (1:1:1ratio)	4.74 ± 0.59^{d}	6.45 ± 0.62^{e}	4.62 ± 0.49^{d}	6.21 ± 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).

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 *L. mauritii* and *P. excavatus* 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 PW mixed with organic amendments (CD and PM in 1:1:1 ratio) is ideally suited for vermicomposting.

Table - 4: Phosphatase and dehydrogenase activity during vermicomposting of PW amended with different organic waste using *L. mauritii*

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	3.21 ± 0.52^{e}	4.96 ± 0.31^{c}	9.22 ± 0.75^{d}	15.55 ± 0.32^{c}
PM	2.55 ± 0.43^{c}	3.39 ± 0.51^{bc}	8.57 ± 0.54^{c}	14.43 ± 0.41^{b}
PW	1.73 ± 0.37^{a}	2.27 ± 0.32^{a}	6.59 ± 0.49^{a}	12.82 ± 0.32^{a}
CD+PW (1:1 ratio)	2.71 ± 0.61^{d}	3.72 ± 0.29^{c}	8.68 ± 0.62^{c}	14.87 ± 0.45^{bc}
PM+PW (1:1 ratio)	2.29 ± 0.49^{b}	3.22 ± 0.37^{b}	7.91 ± 0.46^{b}	14.42 ± 0.55^{b}
CD+PM+PW (1:1:1ratio)	3.14 ± 0.36^{e}	4.87 ± 0.49^{c}	9.17 ± 0.81^{d}	15.39 ± 0.41c

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 - 5: Cellulase and protease during vermicomposting of PW amended with different organic waste using *P. excavates*

Vermicomposter	Cellulase (mg glucose g^{-1} oven dry substrates for 24hrs incubation)		Protease (mg glutamic acid g^{-1} oven dry substrates for 24hrs incubation)	
	Initial substrate	Vermicompost	Initial substrate	Vermicompost
CD	4.74 ± 0.19^{d}	6.73 ± 0.29^{d}	4.63 ± 0.45^{d}	6.33 ± 0.47^{d}
PM	4.29 ± 0.43^{c}	5.81 ± 0.47^{c}	3.39 ± 0.51^{bc}	5.19 ± 0.62^{c}
PW	2.93 ± 0.46^{a}	3.22 ± 0.51^{a}	2.54 ± 0.23^{a}	3.85 ± 0.29^{a}
CD+PW (1:1 ratio)	3.91 ± 0.33^{bc}	5.97 ± 0.48^{cd}	3.93 ± 0.58^{c}	5.41 ± 0.43^{cd}
PM+PW (1:1 ratio)	3.69 ± 0.66^{b}	4.60 ± 0.36^{b}	3.07 ± 0.31^{b}	4.39 ± 0.38^{b}
CD+PM+PW (1:1:1ratio)	4.74 ± 0.59^{d}	6.61 ± 0.41^{d}	4.62 ± 0.49^{d}	6.33 ± 0.47^{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 - 6: Phosphatase and dehydrogenase activity during vermicomposting of PW amended with different organic waste using *P. excavates*

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	3.21 ± 0.52^{e}	6.77 ± 0.45^{d}	9.22 ± 0.75^{d}	15.72 ± 0.42^{d}
PM	2.55 ± 0.43^{c}	5.79 ± 0.23^{c}	8.57 ± 0.54^{c}	14.47 ± 0.31^{b}
PW	1.73 ± 0.37^{a}	3.35 ± 0.41^{a}	6.59 ± 0.49^{a}	12.89 ± 0.56^{e}
CD+PW (1:1 ratio)	2.71 ± 0.61^{d}	5.99 ± 0.17^{cd}	8.68 ± 0.62^{c}	14.95 ± 0.59^{c}
PM+PW (1:1 ratio)	2.29 ± 0.49^{b}	4.72 ± 0.51^{b}	7.91 ± 0.46^{b}	14.38 ± 0.29^{b}
CD+PM+PW (1:1:1ratio)	3.14 ± 0.36^{e}	6.77 ± 0.29^{d}	9.17 ± 0.81^{d}	15.51 ± 0.36^{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).

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 *L. mauritii* and *P.excavatus* were increased significantly and especially in CD and CD+PM+PW (1:1:1ratio) vermicomposters (Table 4 and 6). 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 PW for both species of worm (Tables 3, 4, 5, and 6). 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 PW, as well as for characterizing the derived finished products.

CONCLUSION

The present study on the feasibility analysis of vermicomposting PW waste by *L. mauritii* and *P. excavatus* has clearly indicated that PW 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 PW gave the best result in terms microbial population and enzyme activity. Therefore, it could be concluded that *L. mauritii* and *P. excavatus* are potential species for rearing and bio-stabilization of PW for large scale vermicompost production.

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