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Growth and reproduction dynamics of exotic and indigenous earthworm species during biotransformation of coffee pulp amended with sugar mill wastes

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Abstract
Biotransformation of agro-industrial waste into stabilized product is of double interest: on the one hand, a waste is transformed into value added product and on the other it minimizes a pollutant that is a result of increasing industrialization. Coffee pulp (CP) is an abundant agro-industry solid waste as a result of coffee processing. The present study investigated the growth and reproduction of earthworms *E. fetida* and *P. excavatus* during biotransformation (vermicomposting) of CP amended with sugar industry by products (press mud and bagasse). CP was mixed with press mud (SPM) and bagasse (SBG) in different ratio to produce six different treatments for *E. fetida* and *P. excavatus*, respectively. Results revealed that CP which was amended with SPM and SBG, especially SPM, encouraged the growth and reproduction of both worm as compared to the treatment with CP alone (TE6 and TP12). Significantly, both worms showed the better growth and reproduction performances in first three treatments (TE1 – TE3 for *E. fetida* and TP7 – TP9 for *P. excavatus*) possibly due to higher content of nutrients in the amendment material. The earthworm mortality during experimentation was higher in the treatments those contained more CP ratios. Overall, TE3 and TP9 treatments (1:1:1: ratio of CP, SPM and SBG) appeared to be an ideal combination for enhancing maximum bio-potential of *E. fetida* and *P. excavatus* to managing coffee pulp with sugar industrial waste.

Keywords: sugar industrial waste, coffee pulp, vermicomposting, earthworms, reproduction

Introduction

Agro-industrial wastes that are rich in organic matter content and free from lethal substances or ions could be suitable substrates for biotransformation using earthworms (Adi and Noor, 2009) [1]. Sugar industry is one of the main agro processing industries in India with installation of 1062 factories of large to small capacities and Indian directorate of Economics and statistics (DES) has reported the production of approximately 280 million tons of sugarcane per year. However, it is one of the most polluting industries. Murty *et al*. (2006) [2] has reported pollution concentrations for some factories in India as high as 1154 mg/l for biological oxygen demand (BOD), 5915 mg/l for chemical oxygen demand (COD), and 5759 mg/l for suspended solids (SS) against the minimum national standards (MINAS) in India. Further, sugarcane mills mainly use activated sludge process for wastewater treatment, which generates huge quantity of sludge commonly known as filter cake or press mud. For about 134 million tons of sugarcane crushed, 4.0 million tones of pres mud are generated (Yadav, 1995) [3]. Due to the unaffordable cost of sludge disposal, it is either dumped in open or along roadsides or railway tracks where it causes obnoxious impacts on the ambient surroundings (Sen and Chandra, 2007) [4]. Bagasse is also a by-product of sugar mills and these are lignocellulosic in nature and are formed by three main polymeric constituents-cellulose, hemicellulose and lignin. Huge quantity of these bagasse remained unutilized and either left to natural degradation or burnt in the field leading to severe environmental aggression and wastage of resource (Pandey *et al.*, 2000) [5].

Coffee is one of the worldwide agricultural products and is the second chief product traded in the world subsequently to oil. Annually, huge amount of coffee by-products are generated throughout coffee processing. Further, coffee processing units those are situated in coffee growing areas pretense threat to the environment because of unsafe discarding of coffee pulp and effluents leading to pollution of water and land around the processing units (Pushpa and Manonmani, 2008) [6]. Coffee pulp could be useful because of its high content of carbohydrates and proteins. Large potentialities exist for recycling of pulp of coffee that can be composted and used as manure for several crops. However, the presence of caffeine, tannins and polyphenols limits its utilization (Jayam and Manivannan, 2017) [7]. Therefore an eco-friendly and economically sustainable management of coffee pulp and sugar mill waste is necessary to protect and conserve the environment. Several researches have been conducted on the potential use of earthworms in nutrient recovery from agro-industrial wastes. It has been well established that epigeic earthworms can accelerate the waste stabilization process to a significant level with production of an enhanced quality of vermicompost as compared with those prepared through conventional composting methods (Khwairakpam and Bhargava, 2009; Kaviraj and Sharma, 2003; Suthar and Singh, 2008) [8, 9, 10].

Biotransformation i.e. vermicomposting is a stabilization of organic waste materials involving the joint action of earthworms and microorganisms. Benitez *et al*., (2000) [11] concluded that during composting using earthworms, inoculated worms maintain aerobic condition in the organic wastes, alter a portion of the organic material into worm biomass and respiration products, and expel the remaining partially stabilized product (vermicompost). It is much more fragmented, porous and microbially strong than parent material due to humification and increased decomposition organic waste (Prabhakaran and Manivannan, 2014) [12]. Further, initial vermicomposting process of any organic biomass is generally characterized by growth and reproduction of earthworms, determining different physico-chemical parameters and increases in humic substances. The suitability of any earthworm species for waste management practices mainly depends upon their efficiency to convert a considerable amount of plant metabolites into more available forms. The growth and reproduction of earthworms during vermicomposting trial also provides some important information related to the biology of this species. Therefore, the main objective of this study was to assess the growth and reproduction performance of exotic and indigenous earthworm species **(***E. fetida* and *P. excavatus*) on different ratios of coffee pulp amended with sugar mill wastes for large scale vermicomposting practices.

Materials and methods

Earthworm cultures

Two composting species of earthworms exotic *E. fetida* and indigenous *P. excavatus* were chosen for the study. Stock earthworms *E. fetida* and *P. excavatus* were cultured in the laboratory using cow dung under ambient temperature and were randomly picked for experimentation.

Collection of organic waste

The coffee pulp (CP) was collected from the T.T.L P. Ltd, coffee plantation processing industry at Yercaud in Salem district, Tamilnadu, India. Sugar industry by product press mud (SPM) and bagasse (SBG) were called from E.I.D. Parry's Sugar Mill located at Nellikuppam, Cuddalore District, Tamil Nadu, India. Fresh SPM was kept under shade for 2-3 weeks to remove the foul smell before using for the experimental process. One month old SBG was sundried separately for 15 days to remove the odor and noxious gases. The partially degraded SPM and SBG were then blended with CP in different ratios to increase the C/N ratio.

Experimental design

In the present study, different proportions of coffee pulp (CP) with amendment material sugar industry waste press mud (SPM) and bagasse (SBG) mixtures were prepared (Table 1). A total of twelve experimental treatments containing different proportions of CP, SPM and SBG were established for both worms. The waste mixtures, CP, SPM and SBG of different proportions were transferred to respective plastic troughs with 40cm diameter x 60cm depth. After transferred in to the plastic troughs all the mixture compositions in different treatments were allowed for seven days of initial natural decomposition. All the experimental treatments were kept in six replicate for each treatment in a completely randomized block design. The troughs were kept under shade and moisture content was maintained around 65-75% for all treatments by periodic sprinkling of water. The temperature in the laboratory was maintained at around 25 ± 2°C, which is the optimum temperature for earthworms during vermicomposting (Khwairakpam and Bhargava, 2009) [13]. After the completion of pre-inoculation period of seven days, the clitellated *E. fetida* and *P. excavatus* were weighed (15gm kg^{-1} of substrate) and inoculated in to respective treatment containing 3 kg of substrate.

Growth and reproduction study

Growth rate, cocoon and hatchlings production and mortality of *E. fetida* and *P. excavatus* in the treatments were recorded periodically for 90 days. The feed in the container was turned out, then earthworms, hatchlings and cocoons were separated from the feed by hand sorting method, after which they were counted and weighed after washing with water and drying them by tissue papers. Then all the worms, hatchlings and cocoons were returned to their respective treatment. The worms were weighed with full gut content. At the end of vermicomposting period the earthworms, hatchlings and cocoons were separated and the final vermicompost from each container was air-dried at room temperature and packed in airtight plastic vials for further analysis. One-way ANOVA was used to analyze the significant differences among different treatments. Tukey's *t*-test was used as a post hoc analysis to compare the means (SPSS Package) and the probability levels used for statistical significance were $P < 0.05$.

Results and discussion

The significant changes in growth, reproduction and mortality of all the treatments for *E. fetida* and *P. excavatus* over the studied period are illustrated in Tables 2 - 6. Generally *E. fetida* and *P. excavatus* showed significant ($P<0.05$) difference in biomass production and reproduction potential i.e., maximum biomass achieved (mg $worm^{-1}$), biomass gain (mg $worm^{-1}$), growth rate (mg $worm^{-1}$ day^{-1}), total number of cocoons, total hatchlings number and reproduction rate (cocoon $worm^{-1}$ day^{-1}) among different treatments. However, *E. fetida* showed considerable higher individual weight achieved in TE3 treatment followed by TE2, TE1, TE5 and TE4 treatments and *P. excavatus* showed significant higher individual weight in TE9 treatment followed by TP8, TP7, TP11 and TP10 treatments (Table 2) at the end of experiment. However, biomass gain (mg $worm^{-1}$) in 1:1:1 ratio of CP, SPM and SBG treatment for *E. fetida* and *P. excavatus* was significantly higher than other treatments studied. The maximum growth rate (mg $worm^{-1}$ day^{-1}) for *E. fetida* was in TE3 (9.2±0.5) treatment followed by TE2 (8.8±0.4), TE1 (8.3±0.6), TE4 (8.0±0.5) and TE5 (7.4±0.3) treatments and for *P. excavatus* was in TE9 (7.0±03) treatment followed by TP8 (6.7±0.4), TP7 (6.4±0.7), TP11 (5.9±0.5) and TP10 (5.6±0.2) treatments (Table 3). However, difference among TE3, TE2 and TE1 treatments for *E. fetida* and TP9, TP8 and TP7 treatments for *P. excavatus* in respect to biomass gain and growth rate was not statistically significant. The growth rate has considered excellent comparative index to compare the growth and biomass of earthworms in different feed combinations during vermicomposting. The maximum biomass gain and growth rate in the treatments may be due to the extra palatability and acceptability of the substrates by *E. fetida* and *P. excavatus* and the minimum biomass in the treatments with higher proportion of CP was probably due to the less organic carbon content and presence of various growth-retarding substances in it (Ndegwa and Thompson, 2000) [14]. The result from the present work, in the context of change in individual weight of earthworms with the stocking density corroborates with the results of other researchers (Tamizhazhagan *et al*., 2016; Tripathi and Bhardwaj, 2004) [15, 16]. Therefore, the difference in growth rate among different treatments in the present study seems to be closely related to organic waste quality.

The total cocoon production and hatchlings numbers varied among treatments and maximum and minimum cocoons obtained at the end were in TE3 (329±9.6) for *E. fetida* and TP9 (282±11.5) for *P. excavatus*. Cocoon production (worm^{-1}) and reproduction rate (cocoon worm^{-1} day^{-1}) varied significantly among different treatments for both species of worms ($p<0.05$). Similarly, total number of hatchlings was recorded highest in TE3 treatment for *E. fetida* and TP9 treatment for *P. excavatus* and minimum was observed in TE4, TE5 for *E. fetida* and TP10, TP11 for *P. excavatus*, respectively. Moreover statistically, the difference between TE1 and TE4 in *E. fetida* and TP7, TP8, TP10 and TP11in *P. excavatus* for reproduction rate was not significant (Table 5). Consequently, the observed results of this study also suggested that higher proportions of CP with SPM and SBG and CP alone in the treatments were not suitable for growth and reproduction for both worms. Hence, it may be concluded that production of cocoons in the feed mixtures during vermicomposting could be related to the quality of the substrate, which was one of the important factors and the microbial biomass and enzyme activities during vermicomposting are also important in determining the production of cocoons (Suthar, 2008) [17]. In the present study, biochemical characters of feeding stock may be a primary importance for rearing of earthworms on organic waste resources. So, the difference in cocoon and hatchlings production among the treatments for both worms could be due to variation in quality of the substrate combinations (Suthar, 2007) [18].

E. fetida and *P. excavatus* statistically showed a different pattern of worm mortality among different treatments (Table 6). However, difference among TE3, TE2 and TE1 for *E. fetida* and TP9, TP8 and TP7 treatments in respect to total worm mortality was not statistically significant ($p<0.05$), respectively. Yadav and Garg (2009) [19] have reported that survival and growth of earthworms in feed mixtures is drastically influenced by the chemical environment and ambient climatic variability and the food consumption rate in earthworms during initial period also determines the survival rate of earthworms in the treatments. According to Dominguez *et al*., (2001) [20] pre-composting is also essential to avoid the earthworm mortality during vermicomposting. In the present study, the earthworm mortality was higher in the treatments, which contained more proportion of CP or 100% of CP and hence it has been found that higher concentration CP affects the survival rate of earthworms in waste decomposing system. The data suggests that mixing of some other materials (e.g. sugar industrial wastes) in CP before vermicomposting not only accelerated the rates of decomposition but at the same time also reduced the rate of worm mortality during decomposition process.

Conclusion

Vermicomposting process of any organic biomass is generally characterized by measurement of the growth and reproduction of earthworm, determining different physicochemical parameters and production of humic substances. In this study, vermicomposting of CP amended with SPM and SBG in different ratios using *E. fetida* and *P. excavatus* has been examined for its suitability for growth and reproduction. Results revealed that both worms did not feed on raw CP and accepted it as a diet only when sugar industry waste (SPM and/or SBG) was amended with it. Mixing of sugar industry waste at equal proportions with CP accelerated the better growth medium for vermiculture. *E. fetida* and *P. excavatus* appeared to adapt the degrading activity of the substrate to a much greater level than the sole use of CP in vermicomposting. Among the two species of worms, *E. fetida* exhibits better biomass production, growth rate, cocoons and hatchlings production than *P. excavatus*. Although, much work is still required on physico-chemical parameters and humic substances by this species using coffee pulp amended with sugar industry waste, before recommendation for large scale vermicompost production.

Table 1: Description of different treatments using coffee pulp amended with sugar industrial waste

Treatment No.	Treatment description	proportion
	Eisenia fetida	
TE1	Coffee pulp + Bagasse	1:1
TE2	Coffee pulp + Press mud	1:1
TE3	Coffee pulp + Bagasse + Press mud	1:1:1
TE4	Coffee pulp + Bagasse	2:1
TE5	Coffee pulp + Press mud	2:1
TE6	Coffee pulp	100%
	Perionyx excavatus	
TP7	Coffee pulp + Bagasse	1:1
TP8	Coffee pulp + Press mud	1:1
TP9	Coffee pulp + Bagasse + Press mud	1:1:1
TP10	Coffee pulp + Bagasse	2:1
TP11	Coffee pulp + Press mud	2:1
TP12	Coffee pulp	100%

Table 2: Biomass production by *E. fetida* and *P. excavatus* in different treatments during vermicomposting

Treatments	Mean initial biomass (start), worm^{-1} (mg)	Maximum weight achieved (end), worm^{-1} (mg)
Eisenia fetida		
TE1	127±3.5	872±11.5^{b}
TE2	125±2.5	918±12.4^{c}
TE3	126±3.6	952±7.5^{cd}
TE4	125±3.0	793±12.4^{a}
TE5	124±4.0	848±10.5^{b}
TE6	124±3.8	ND
Perionyx excavatus		
TP7	112±2.5	687±7.5^{b}
TP8	111±1.8	709±8.6^{bc}
TP9	112±2.0	747±4.3^{c}
TP10	112±1.9	618±7.6^{a}
TP11	110±2.5	637±12.2^{a}
TP12	113±1.5	ND

Results are reported as mean ± standard deviation among six replicates; results in the similar column with different letters are significantly different (Tukey's test, $P < 0.05$); ND-Not Detected.

Table 3: Biomass enhances and growth rate of *E. fetida* and *P. excavatus* in different treatments using coffee pulp amended with sugar industrial waste

Treatments	Net biomass gained, worm^{-1} (mg)	Mean growth rate, worm^{-1} day^{-1} (mg)
Eisenia fetida		
TE1	745±7.4^{b}	8.3±0.6^{b}
TE2	793±7.6^{c}	8.8±0.4^{bc}
TE3	826±5.6^{d}	9.2±0.5^{c}
TE4	668±5.5^{a}	7.4±0.3^{a}
TE5	724±6.2^{b}	8.0±0.5^{ab}
TE6	ND	ND
Perionyx excavates		
TP7	575±4.6^{b}	6.4±0.7^{ab}
TP8	598±4.9^{bc}	6.7±0.4^{ab}
TP9	635±4.2^{c}	7.0±03^{b}
TP10	506±5.3^{a}	5.6±0.2^{a}
TP11	527±7.5^{ab}	5.9±0.5^{a}
TP12	ND	ND

Results are reported as mean ± standard deviation among six replicates; results in the similar column with different letters are significantly different (Tukey's test, $P < 0.05$); ND-Not Detected.

Table 4: Number of cocoons and hatchlings production by *E. fetida* and *P. excavatus* in different treatments using coffee pulp amended with sugar industrial waste

Treatments	Total no. of cocoons	Total no. of hatchlings
Eisenia fetida		
TE1	269±8.5^{b}	175±3.7^{b}
TE2	282±5.9^{bc}	191±10.4^{bc}
TE3	329±9.6^{c}	239±15.6^{c}
TE4	220±8.2^{a}	123±9.5^{a}
TE5	260±11.2^{b}	173±7.6^{b}
TE6	ND	ND
Perionyx excavates		
TP7	222±7.6^{b}	155±6.9^{b}
TP8	251±10.5^{c}	179±5.8^{bc}
TP9	282±11.5^{d}	215±10.4^{c}
TP10	162±4.7^{a}	92±5.2^{a}
TP11	150±5.9^{a}	81±4.7^{a}
TP12	ND	ND

Results are reported as mean ± standard deviation among six replicates; results in the similar column with different letters are significantly different (Tukey's test, $P < 0.05$); ND-Not Detected.

Table 5: Reproduction rate of *E. fetida* and *P. excavatus* in different treatments using coffee pulp amended with sugar industrial waste

Treatments	Cocoon production worm^{-1}	Reproduction rate worm^{-1} day^{-1}
Eisenia fetida		
TE1	6.1±0.08^{b}	0.06^{b}
TE2	6.3±0.04^{bc}	0.07^{c}
TE3	7.3±0.03^{c}	0.08^{d}
TE4	4.9±0.05^{a}	0.05^{a}
TE5	5.8±0.04^{b}	0.06^{b}
TE6	ND	ND
Perionyx excavates		
TP7	5.0±0.02^{b}	0.04^{a}
TP8	5.6±0.04^{bc}	0.04^{a}
TP9	6.3±0.05^{c}	0.07^{b}
TP10	3.6±0.03^{a}	0.04^{a}
TP11	3.4±0.04^{a}	0.04^{a}
TP12	ND	ND

Results are reported as mean ± standard deviation among six replicates; results in the similar column with different letters are significantly different (Tukey's test, $P < 0.05$); ND-Not Detected.

Table 6: Mortality rate of earthworms in different treatments during experimentation

Treatments	Total mortality after 84 days (%)
Eisenia fetida	
TE1	12.4±1.5^{b}
TE2	4.5±0.7^{a}
TE3	2.1±0.02^{a}
TE4	28.5±3.4^{d}
TE5	23.7±2.1^{c}
TE6	100±0.0^{e}
Perionyx excavates	
TP7	12.7±1.8^{b}
TP8	12.6±1.5^{b}
TP9	3.2±0.3^{a}
TP10	31.5±3.2^{cd}
TP11	25.9±2.5^{c}
TP12	100±0.0^{d}

Results are reported as mean ± standard deviation among six replicates; results in the similar column with different letters are significantly different (Tukey's test, $P < 0.05$); ND-Not Detected.

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References

1. Adi A, Noor ZM. Waste recycling: utilization of coffee grounds and kitchen waste in vermicomposting. Bioresour Technol, 2009; 100:1027-1030.
2. Murty MN, Kumar S, Paul M. Environmental Regulation, Productive Efficiency and Cost of Pollution Abatement: A Case Study of the Sugar Industry in India. 2006; 79:1-9.
3. Yadav RL, Verma RP. Crop residue management to conserve soil organic matter content in sugarcane-based crop rotations. Bioresour Technol, 1995; 51:241-245.
4. Sen B, Chandra TS. Chemolytic and solid -state spectroscopic evaluation of organic matter transformation during vermicomposting of sugar industry wastes. Bioresource Technology, 2007; 98:1680-1683.

5. Pandey A, Soccol CR, Nigam P, Soccol VT. Biotechnological potential of agro-industrial residues. I: Sugarcane bagasse. Bioresource Technology, 2000; 74:69-80.
6. Pushpa SM, Manonmani HK. Bioconversion of Coffee Industry Wastes with White Rot Fungus Pleurotus florida. Research Journal of Environmental Sciences, 2008; 2:145-150.
7. Jayam S, Manivannan S. Effect of earthworms activity on humus composition during biological stabilization of coffee pulp amended with pressmud. International Journal of Biology Research, 2017; 4:137-141.
8. Khwairakpam M, Bhargava R. Bioconversion of filter mud using vermicomposting employing two exotic and one local earthworm species. Bioresour Technol, 2009; 100:5846-5852.
9. Kaviraj Sharma S. Municipal solid waste management through vermicomposting employing exotic and local species of earthworms. Bioresour Technol, 2003; 90:169-173.
10. Suthar S, Singh S. Vermicomposting of domestic waste by using two epigeic earthworms Perionyx excavates and Perionyx sansibaricus. Int J Envion Sci. Tecnol, 2009; 5(1):99-106.
11. Benítez E, Nogales R, Masciandaro G, Ceccanti B. Isolation by isoelectric focusing of humic urease complexes from earthworm Eisenia foetida-processed sewage sludges. Biol Fertil Soils, 2000; 31:489-493.
12. Prabhakaran D, Manivannan S. Effect of inoculating lignocellulolytic fungus on nutrient changes during different phases of composting of poultry droppings amended with bagasse. Int J Curr Microbiol App Sci., 2014; 3(9):582-595.
13. Khwairakpam M, Bhargava R. Vermitechnology for sewage sludge recycling. J Hazard Mater, 2009; 161:948-954.
14. Ndegwa PM, Thompson SA. Integrating composting and vermicomposting, the treatment and bioconversion of biosolids. Bioresour Technolo, 2000; 76:107-112.
15. Tamizhazhagan V, Pugazhendy K, Sakthidasan V, Revathi K, Baranitharan M. Investigation of microbial count in the soil and earthworm gut Eudrilus Eugeniae. Innovare Journal of Agri Sci. 2016; 4(3):7-9.
16. Tripathi G, Bhardwaj P. Comparative studies on biomass production, life cycles and composting efficiency of Eisenia foetida Savigny and Lampito mauritii Kinberg. Bioresour Technol, 2004, 275-278.
17. Suthar S. Bioremediation of aerobically treated distillery sludge mixed with cow dung by using an epigeic earthworm Eisenia fetida. Environmentalist, 2008; 28:76-84.
18. Suthar S. Vermicomposting potential of Perionyx sansibaricus Perrier in different waste materials. Bioresour Technol, 2007; 98:1231-1237.
19. Yadav A, Garg VK. Feasibility of nutrient recovery from industrial sludge by vermicomposting technology. Journal of Hazardous Materials, 2009, 262-268.
20. Dominguez J, Edwards CA, Ashby J. The biology and population dynamics of Eudrilus eugeniae Kinberg Oligochaeta in cattle waste solids. Pedobiologia, 2001; 45:341-353.