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Research Article

EFFECT OF BACTERIAL SUSPENSION ON GROWTH AND REPRODUCTION POTENTIAL OF EARTHWORMS USING SAGO WASTE AMENDED WITH FILTER MUD DURING BIOCONVERSION

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ABSTRACT

The research work was designed to assess the growth and reproduction potential of indigenous species of earthworm *Perionyx ceylanensis* and *Lampito mauritii* using locally available agro-industry wastes, i.e., sago bagasse (SB) and sugarcane filter mud (SFM) with bacterial inoculation. The growth and reproduction of *P. ceylanensis* and *L. mauritii* was measured by studying parameters, such as, maximum biomass gain, net biomass gain worm^{-1}, growth rate of worms and number of cocoons produced and number of hatchlings emerged. Totally six experimental piles were prepared using 1:1 ratio of SB and SFM for inoculation of both earthworms separately and four experimental piles were inoculated with bacterial strain before inoculation of earthworms (EBP3, EBP4, EBL5 and EBL6). Results of this study revealed that the biomass gain and growth rate of both worms were significantly higher ($P<0.05$) in *Cellulomonas* sp. inoculated piles compared to *Pseudomonas* sp. and without inoculation of bacterial strain piles. The cocoon and hatchlings production were also significantly greater ($P<0.05$) in *Cellulomonas* sp. inoculated piles. Further, higher rate of worm mortality was recorded in without inoculation of bacterial strain piles. Hence, it has been concluded that inoculation of *Cellulomonas* sp. in vermicomposting before inoculation of earthworms for initial stabilization is a better medium for growth and reproduction of earthworms as well as large scale vermicompost production.

Keywords: Earthworms, Vermicomposting, Bacterial inoculation, Reproduction, Agro waste.

INTRODUCTION

Conversion of agro industrial organic waste into valuable products using earthworms has been widely documented as the most efficient, sustainable and environmentally friendly methods for soil application. Conversion through composting and vermicomposting methods were used widely since its economical and simple process, more than ever developing countries like India and composting and vermicomposting technologies are raising rapidly and precious tools in pollution prevention and management (Mahesh Kumar, 2015). Likewise, with regard to the concerns on global warming, conversion of organic waste into voluble one is playing a most important role. However, the optimization of the several biological methods for decentralized systems still needs to be investigated more (Krishnan & Manivannan,2017). Accordingly, several studies have been made on the use of earthworms in conversion processes using a mixture of organic waste materials (Gajalakshmi *et al*., 2002; Meena & Renu, 2009). Further, the ability of epigeic earthworms for agricultural, animal, and agro industrial wastes management has reported by several researchers (Garg & Kaushik, 2005; Suthar, 2010; Yadav & Garg, 2011). But utilization of sago solid waste blended with sugar industry waste filter mud combination and microbial inoculants for rapid stabilization during vermicomposting process is yet to be proven.

Sago is a familiar for human consumption starch in the form of globules is obtained by processing the tubers of tapioca and India acquires consequence in the widespread tapioca scenario due to its highest productivity. In the same way within India, Tamil Nadu state stands primary in respect of processing of tapioca into sago throughout India

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and it meet about 80 % of country's demand now. In India, sago industry is one of the major small scale sectors with more than 800 sago starch units located in Salem and Namakkal Districts of Tamil Nadu and the processing of sago generates huge quantities of biodegradable solid and liquid wastes which are very much macrobiotic, foul smelling and acidic in nature (Mahesh Kumar, 2015). On the other hand, sago waste is a rich fibrous residue and it is usually disposed of subsequent to the extraction of starch from the sago trunk (Mahesh Kumar, 2015) also reported that every 100 kg of sago starch in pith, about 10 kg of sago bagasse is generated and this sago waste are likely to be discarded into rivers or open dumps without any facilities for waste management and this practice may cause soil and water pollution. However, unavailability of land and public awareness has made such open dumps expensive and unfeasible. Apart from this, disposal of solid wastes in open dumps leads to wastage of organic and inorganic nutrients present in the waste which might be recovered and used as manure in agricultural fields (Meena & Renu, 2009). Moreover, filter mud produced huge quantity from sugar industry waste serve a excellent amendment for vermicomposting of different organic waste because of it is having rich nutrients and microbial populations (Manivannan, 2004; Suthar, 2010). Keeping this in view studies were conducted on the vermicomposting of sago industry solid waste amended with filter mud in different combinations employing with microbial inoculants and epigeic earthworms, *Perionyx ceylanensis* and *Lampito mauritii*.

MATERIALS AND METHODS

Collection of Sago bagasse (SB) and Sugar industry Filter mud (SFM)

The sago bagasse (SB) was collected from a sago factory in Athur, Salem district, Tamil Nadu (India). The sago bagasse was cured in direct sunlight for one week with periodic spinning with optimum moisture and to fetch its moisture content from 30 to 40%. Filter mud of Sugar cane industry (one month old and partially dehydrated) was collected in small gunny bags from Sugar Mill, Nellikkuppam, Tamilnadu. Collected SB and SFM immediately were brought into the laboratory for experimentation.

Culture of earthworm species and bacterial inoculants

Native species *Perionyx ceylanensis* was selected to compare with another native species anecic *Lampito mauritii* for their degradation efficiency and vermicompost production. The degradation efficiency of worms was assessed in terms of superiority of the final product based on humification process, nutrient status and microbial population and growth and reproduction of worms. Both earthworms were obtained from the stock culture maintained in the laboratory, Department of Zoology, Annamalai University, Tamilnadu, India. Earthworms were cultured in the cement tanks containing cattle dung. Moisture content (70-80%) was constantly maintained throughout the study period. Two bacterial strains i.e. *Pseudomonas* sp. and *Cellulomonas* sp. were tested through *in-vivo* for vermicomposting of SFM amended with SB. These bacterial stains were identified in Plant Biotechnology and Biochemistry Laboratory, Annamalai University, Annamalainagar.

Preparation of bacterial strain

Pseudomonas sp. and *Cellulomonas* sp. strains were alone cultured in Luria Broth media. A loop full of 4-day old colony of each strain was separately transferred to sterilize 200 ml Luria Broth in a 500 ml conical flask kept on orbital shaker at 28ºC for 5 days. The culture flask was placed on shaker at 50 for 15 minutes at the end of incubation. The content of each flask was used as bacterial suspension and was mixed properly with composting pile of SB with SFM.

Experimental setup

Each experimental pile contained 1:1 ratio of sago bagasse (SB) and sugar industry filter mud (SFM) and the moisture content in each pile was adjusted to 70% before and during the composting process. Each pile was manually mixed using spade for about 10 minutes to turn the pile and provide aeration before inoculation of earthworms. The experiments were performed in six piles with six replicates using cement container (60 cm x 180 cm x 60 cm) and each composting piles contains 5kg of SB and SFM in aforementioned ratio (w/w dry weight basis) (Table 1). Pure cultures of *Pseudomonas* sp. and *Cellulomonas* sp. were inoculated (50 ml/kg substrate having 10^{-6} cells per ml) in to the respective experimental piles. At the initial (0 day), the bacterial suspension was sprayed on the raw material as per the procedure of (Sarker *et al.*, 2013). All the amended materials were turned after inoculation of bacterial strains to spread the inoculated microbes in the piles. Control pile was used having no inoculation.

After 15 days, *P. ceylanensis* and *L. mauritii* were collected from the stock cultures and weighed without voiding their gut content and inoculated in to respective piles (EP1, EL2, EBP3, EBP4, EBL5 and EBL6) at the rate of 20 No's of respective worms kg^{-1} of waste in the pile (Manivannan, 2004). All the piles were maintained at the laboratory room temperature of 27±3°C and the moisture content in each pile was adjusted to about 75% during the composting process. Mean individual biomass gain of both worms and number of cocoons produced during experimentation was calculated at the end (75 days). The worms were monitored frequently for population mortality in different experimental piles and total mortality was calculated at the end of the experiment.

RESULTS AND DISCUSSION

P. ceylanensis and *L. mauritii* showed drastic among different experimental piles for growth rate, mean reproduction rate and mortality rate of all the experimental piles (Tables 2-7). In general, *P. ceylanensis* and *L.*

mauritii showed significant ($P < 0.05$) difference in biomass production and reproduction rate i.e., maximum biomass achieved per worm, biomass gain, growth rate, total number of cocoons and hatchlings among different experimental piles. On the other hand, *P. ceylanensis* showed maximum and minimum individual weight gain on EBP4 (781±13.6 mg) and EP1 (614±6.4 mg) and *L. mauritii* showed maximum and minimum individual weight gain on EBL6 (859±16.8 mg) and EL2 (728±14.5 mg), respectively (Tables 2 and 3). Moreover, at the end live individual weight was observed in the following order: EBL6 > EBL5 > EBP4 > EBP3 > EL2 > EP1 for both species of worms. However, biomass gain (mg worm^{-1}) in *Cellulomonas* sp. inoculated experimental piles for *P. ceylanensis* and *L. mauritii* was significantly higher than other experimental piles studied.

Table 1. Different experimental piles prepared from 1:1 ratio of sago bagasse amended with sugarcane filter mud.

Experimental Piles	Organic amendment 1:1 ratio	Inoculation	
		Bacteria	Earthworms
EP1	SB + SFM	-	*P. ceylanensis*
EL2	SB + SFM	-	*L. mauritii*
EBP3	SB + SFM	*Pseudomonas* sp.	*P. ceylanensis*
EBP4	SB + SFM	*Cellulomonas* sp.	*P. ceylanensis*
EBL5	SB + SFM	*Pseudomonas* sp.	*L. mauritii*
EBL6	SB + SFM	*Cellulomonas* sp.	*L. mauritii*

Table 2. Changes in biomass of *P. ceylanensis* in different experimental piles (mean±SEM, n=3).

Experimental Piles	Initial biomass of *P. ceylanensis* (Worm^{-1} mg)	Max. biomass gained by *P. ceylanensis* (Worm^{-1} mg)
EP1	105±3.5	614±6.4
EBP3	111±6.3	772±17.2
EBP4	108±7.4	781±13.6

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, $P < 0.05$).

Table 3. Changes in biomass of *L. mauritii* in different experimental piles (mean ± SEM, n=3).

Experimental Piles	Initial biomass of *L. mauritii* (Worm^{-1} mg)	Max. biomass gained by *L. mauritii* (Worm^{-1} mg)
EL2	149 ± 11.0	728±14.5
EBL5	148 ± 10.4	851±14.3
EBL6	145 ±11.5	859±16.8

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, $P < 0.05$).

Table 4. Biomass increase and growth rate of *P. ceylanensis* in different experimental piles (mean±SEM, n=3).

Experimental Piles	Net biomass increase / worm (mg)	Growth rate of *P. ceylanensis* Worm^{-1}day^{-1}
EP1	509±2.9	7.5±0.03
EBP3	661±5.9	8.5±0.12
EBP4	673±6.2	8.9±0.07

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, $P < 0.05$).

Table 5. Biomass increase and growth rate of *L. mauritii in* different experimental piles (mean±SEM, n=3).

Experimental Piles	Net biomass increase / worm (mg)	Growth rate of *L. mauritii* $Worm^{-1}day^{-1}$
EL2	583±3.5	7.2±0.04
EBL5	703±3.9	8.2±0.05
EBL6	714±8.3	8.6±0.03

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, $P < 0.05$).

Table 6. Reproduction and mortality of *P. ceylanensis* in different experimental piles after 90 days (mean± SEM, 3)

Experimental Piles	Cocoon production	Total no. of hatchlings emerged	Total mortality rate (%)
EP1	361±21	82±06	12.5± 5.0
EBP3	494±25	139±19	5.2 ± 0.5
EBP4	518±18	142±26	5.0 ± 0.9

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, $P < 0.05$).

Table 7. Reproduction and mortality of *L. mauritii* in different experimental piles after 90 days (mean±SEM, n=3)

Experimental Piles	Cocoon production	Total no. of hatchlings emerged	Total mortality rate (%)
EL2	127±18	62±05	18.3 ± 3.1
EBL5	135±11	84±11	6.3 ± 0.7
EBL6	141±13	83±08	6.5 ± 0.6

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, $P < 0.05$).

The maximum growth rate (net biomass increase) for *P. ceylanensis* was in EBP4 (673±6.2 mg) treatment followed by EBP3 (661±5.9 mg) and EP1 (509±2.9 mg) experimental piles and for *L. mauritii* was in EBL6 (714±8.3 mg) experimental piles followed by EBL5 (703±3.9 mg) and EL2 (583±3.5 mg) experimental piles (Tables 4 and 5). However, growth rate did not show significant difference among different experimental piles (EBP4 and EBP3 for *P. ceylanensis* and EBL6 and EBL5 for *L. mauritii*). The growth rate of earthworms during vermicomposting has considered an excellent comparative indicator to assess the growth and biomass gain of worms in different feed mixtures. The maximum biomass increase and growth rate in the experimental piles during experiment may be due to the extra deliciousness and suitability of the feed mixtures by the worms and the minimum biomass in the experimental piles may be due to higher proportion of un stabilized feed materials and presence of higher growth-retarding substances in it (Ndegwa & Thompson, 2000).

Moreover, piles in which earthworm showed better growth patterns, were probably with supplying of easily stabilized organic matter, non-assimilated carbohydrates, and very less concentration of growth-retarding substances, which favors earthworm growth (Tamizhazhagan *et al*., 2016; Tripathi & Bhardwaj, 2004). Therefore, the difference in growth rate among different experimental piles in the present study seems to be closely related to organic waste quality and stability influenced by selective bacterial inoculation. In the present study, total cocoon production and hatchlings numbers varied among different experimental piles and maximum and minimum cocoons obtained at the end were in EBP4 (518±18) and EP1 (361±21) for *P. ceylanensis* and EBL6 (141±13) and EL2 (127±18) for *L. mauritii*, respectively. Similarly, reproduction rate varied significantly among different experimental piles for both species of worms ($p<0.05$). Hence, it may be concluded that production of cocoons numbers during vermicomposting could be related to the stability and quality of the substrate material, which was one of the significant factor and the enhancement of microbial biomass and enzyme activities are also important for cocoons production.

Likewise, total number of hatchlings was recorded highest in EBP3 (142±26) for *P. ceylanensis* and EBL5 (84±11) for *L. mauritii* and minimum was observed in without inoculation of bacterial strains for *P. ceylanensis* (EP1) and *L. mauritii* (EL2), respectively (Table 6 and 7) at the end of experiment. The difference in cocoon production

and hatchlings emergences among the experimental piles for both worms could be due to disparity in quality of the substrate combinations and stabilization of waste material in initial degradation (Surindra Suthar, 2010), Jayan and Manivannan, 2017). Therefore, the observed results of this work also suggested that inoculation of *Cellulomonas sp.* and *Pseudomonas sp.* in the experimental piles were suitable for growth and reproduction for both worms. Inoculation of microbial strain before vermicomposting in to the substrate material, it stabilizes and partially degrade the material.

In this study, both the worms statistically showed a different performance on mortality rate among different experimental piles. However, difference among EBP4 and EBL6 for *P. ceylanensis* and EBL6 and BEL5 for *L. mauritii* in respect to total worm mortality was insignificant ($p<0.05$), respectively (Table 6 and 7) Initial degradation by pre-composting method is also essential steps to avoid the earthworm mortality during vermicomposting in different organic waste. In the present study, the earthworm mortality was higher in the experimental piles may be due to non stabilized particles in the feed material and hence it has been found that inoculation of selective bacterial strain in this study influences the survival and growth rate of earthworms during vermicomposting.

CONCLUSION

This study concludes that if sago bagasse is mixed with filter mud in appropriate quantities and inoculated with selected bacterial strains for initial degradation then it can be vermicomposted by *P. ceylanensis* and *L. mauritii.* Results revealed that earthworms feed slowly on without inoculation of bacterial strain and immediately accepted it as a diet only with inoculated of bacterial strain and a considerable amount of earthworm biomass and cocoon production was also produced in the piles. Addition of sugarcane filter mud at equal proportions with sago bagasse accelerated the better growth medium for vermiculture after stabilized by *Cellulomonas sp.* and *Pseudomonas sp.*, appeared to adapt the degrading activity of the substrate during vermicomposting.

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