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Vermiremediation of Sugarcane (Saccharum Officinarum) Trash – Pre-Digested with Pseudomonas fluorescens) By Utilizing Eudrilus eugeniae, Perionyx excavatus and Lampito mauritii under Monoculture Condition

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Abstract

Sugarcane is one of the major cash crops cultivated in India. After harvest of sugarcane, large amount of trash are generated and left in the field for natural degradation which takes several months. These trash are also disposed off simply by burning in the field which results in loss of nutrients as well as causes atmospheric pollution due to the emission of toxic gases. In order to mitigate this problem, sugarcane trash was pre-digested with *Pseudomonas fluorescens* and subsequently the same was utilized for the production of vermicompost by using conventional composting earthworms *viz.*, *Eudrilus eugeniae*, *Perionyx excavatus* and *Lampito mauritii* under monoculture conditions. The sugarcane trash were collected and cut into small pieces and mixed with *P. fluorescens* for pre-digestion. After 45 days, the partially decomposed sugarcane trash was mixed with cow dung in 50:50 concentration and they were filled in plastic trays, individually. Simultaneously, a control was also prepared with the same concentration and maintained. All these experiments were carried out in triplicates. Hundred

healthy *E. eugeniae*, *P. excavatus* and *L. mauritii* adult worms were introduced into each of these trays individually excepting the control trays. After 17 days of the experimental period vermicompost and compost (from control) was harvested. The conversion ratio of waste into vermicompost was found to be high (73%) 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 other two cultures. Harvested vemicompost and compost obtained from control were subjected to quantification of chemical nutrients analyses. The vermicompost harvested from *E. eugeniae* experimental trays consisted of desirable levels of chemical nutrients than the other experimental trays. It is obvious from the results that in general all the three earthworm species can be used for converting sugarcane trash (predigested with *P. fluoresecens*) into value added vermicompost and *E. eugeniae* in particular.

Keywords: Sugarcane trash, *Pseudomonas fluorescens*, *Eudrilus eugeniae*, *Perionyx excavatus*, *Lampito mauritii*, Monoculture and Chemical nutrients.

Introduction

Sugarcane is one of the important cash crops in India and plays essential role in both agricultural and industrial economy of the country. India is one of the largest producers of sugar and is in close competition with Brazil for the top position. In India, sugarcane is cultivated over an area of 4 million hectares and the production is estimated to be about 325 million tonnes with productivity of 70 tonnes per hectares. In Tamil Nadu, sugarcane is cultivated in an area of 3.22 lakh hectares with an average productivity of 101.8 tonnes. India need to produce more than 320 million tonnes of sugarcane to cater the crushing requirement of sugar factories operated in the country. Greater attention is given only in improving the sugar cane yield and not much in managing the cane trash. This trash contains 28.6% organic carbon, 0.35% to 0.42% nitrogen, 0.04 to 0.15% phosphorus, 0.50 to 0.42% potassium. In

India approximately 6.5 million tonnes of sugar cane trash are being produced every year and most of the residues are usually burnt in the field due to lack of proper composing techniques. Besides the loss of organic matter and plant nutrients, burning of crop residues also causes atmospheric pollution due to the emission of toxic gases methane, carbon dioxide that poses threat to human and ecosystem. Insitu composting of cane trash can be a good alternate to mitigate these problem. Earlier study confirmed that *Aspergillus flavipes, Penicillium chrysogenum, Cochliolous speifer, Rhizopus oryzae* and *Trichoderma viride* were found effective in sugarcane trash decomposition. Though the composting is better option for sugarcane decomposition but the time taken is little high. In recent years integrated system of composting, with bioinoculants and subsequent vermicomposting, to overcome the problem of lignocellulosic waste degradation of different crop residues and waste industrial by products is receiving worldwide attention of scientists (Wang and Christopher, 2003).

Earthworms have been used as an alternative tool to convert a great proportion of organic waste resource into a product with relatively higher concentration of plant nutrients, microbial population, soil enzymes and humic acids contents. Vermicomposting is a stabilization of organic waste materials involving the joint action of earthworms and microorganisms. Although, microbes are responsible for biochemical degradation of organic matter, earthworms are the important drivers of the process, conditioning the substrate and altering the biological activity (Aira *et al.*, 2005 and Suthar, 2007). Benitez *et al.* (1999) concluded that in vermicomposting process, inoculated earthworms maintain aerobic condition in the organic wastes, convert a portion of the organic material into worm biomass and respiration products, and expel the remaining partially stabilized product (vermicompost). Several epigeics (*Eisenia fetida*, *Eisenia andrei*, *Eudrilus eugeniae*, *Perionyx excavatus* and *Perionyx sansibaricus*) and some anecics (*Lampito mauritii*) have been identified as potential candidates to decompose organic waste materials (Suthar, 2009).

However, no work has been reported on the vermiconversion of sugarcane trash pre-digested with *P. fluorescens* and hence the present investigation. The present study was undertaken to pre-digest the chosen agro-waste sugarcane trash with *P. fluorescens* 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 in the vermicompost produced by these earthworms.

Materials and Methods

Collection of organic wastes and Earthworms

The selected agro-waste *i.e.*, Sugarcane trash was collected from Edumalai, Tiruchirappalli District, Tamil Nadu, India. Cow dung was collected from a nearby dairy yard of our college campus. The vermicomposting earthworm species, *Eudrilus eugeniae*, *Perionyx excavatus* and *Lampito mauritii* were obtained from our college vermished.

Preparation of Inoculum

10 ml of molasses was taken in a conical flask. Into that 90 ml of distilled water was added and mixed well. To this 1 ml of pure culture of *P. fluorescens* was added. Then this mixture was 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 Sugarcane trash

According to Mahimairaja *et al.* (2008), the sugarcane trash was cut into small pieces manually. The sugarcane trash was spread on a clean floor, which was open to sunlight for 5 days. Watering was done regularly twice in a day on the sugarcane trash. Similar method was adopted for curing cow dung. The sun dried sugarcane trash and cow dung were transferred to a shady place where it was cured for 5 days. Later 1kg of sugarcane trash was spread on a

clean floor. To this 50 ml of *P. fluorescens* inoculums was uniformly sprinkled. Above to this layer 1kg of sugarcane trash was spread. 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 64 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 sugarcane trash 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. 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 (Murali and Neelanarayanan, 2011).

Analyses of vermicompost and compost

The vermicompost and compost materials were then analysed for different physico-chemical parameters. The pH was measured by using 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 (2005). The organic carbon was determined by partial oxidation method of Walkley and Black (1934). Total nitrogen, total phosphorous and total potassium were determined by micro kjeldhal, spectrophotometric and flame photometric methods suggested by Tandon (2005), respectively. Total Calcium and Total magnesium were estimated by versenate method as suggested by Trivedy and Goel (1986). The flame photometric and spectrophotometric methods of Tandon (2005) 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 (Tandon, 2005).

Statistical Analysis

A one way ANOVA procedure was applied to the data to determine the significance of the different experiments.

Results and Discussion

Propensity of composition of pre-digested food (Sugarcane (Saccharum officinarum) trash — pre-digested with *P. fluorescens*) 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 bioconversion was 11 (*E. eugeniae*), 14 (*P. excavatus*), 21 (*L. mauritii*) and 34 (Control). The average weight of vermicompost obtained after vermicomposting of sugarcane trash was 2903g (*E. eugeniae*), 2700g (*P. excavatus*), 2505g (*L. mauritii*) and 1805g (Control). The mean percent conversion of vermicompost was 73% for *E. eugeniae*, 68% for *P. excavatus*, 63% for *L. mauritii* and 45% for Control. The mean number of cocoons and young ones enumerated were to the tune of 84 and 66 (*E. eugeniae*), 72 and 53 (*P. excavatus*) and 53 and 51 (*L. mauritii*) (Table 1). The extent of chemical composition of vermicompost and compost are given in Table 2.

pН

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). The near-neutral pH of vermicompost may be attributed by the secretion of NH₄⁺ ions that reduce the pool of H⁺ ions (Haimi and Huhta, 1987). Ndegwa *et al.* (2000) pointed out that a shift in pH is related to the mineralization of the nitrogen and phosphorous into nitrites/nitrates and orthophosphates and bioconversion of the organic material into intermediate compounds of the organic acids. Similar results on vermicomposting of leaf litters, poultry waste and paddy straw have been reported by Selvamuthukumaran and Neelanarayanan (2012a, b) and Viji and Neelanarayanan (2013).

Table 1. Propensity of composition of pre-digested food (Sugarcane trash, Saccharum officinarum – pre-digested with P. fluorescens) and its bioconversion into vermicompost by E. eugeniae, P. excavatus and L. mauritii

S. No.	Particulars	E. eugeniae	P. excavatus	L. mauritii	Control
1	Weight of pre-digested Sugarcane trash (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	100
5	Total weight of compost/vermicompost obtained (g)	2903	2700	2505	1805
6	Percent conversion of vermicompost	73	68	63	45
7	Number of days taken for conversion	11	14	21	34
8	Mean total number of cocoons observed in each tray	84	72	53	-
9	Mean total number of young ones observed in each tray	66	53	51	-

The results were obtained from five replicates.

Electrical Conductivity (EC)

A significant decrease was observed in electrical conductivity of vermicompost than control (p<0.05). The maximum reduction was observed in the vermicompost produced by E. eugeniae (1.21 \pm 0.06) followed by P. excavatus (1.39 \pm 0.12) and L. mauritii (1.63 \pm 0.06) (Table 2). 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 (Lazcano et al.,2008). During vermicomposting the minor production of soluble metabolites such as ammonium (NH₄), as well as precipitation of the dissolved salts may lead to lower EC values (Mitchell, 1997). In contrast to the present findings, Pattnaik and Reddy (2010) reported increased in Electrical Conductivity value in the vermicompost produced by E. eugeniae, P. excavatus and E. fetida compared to that of control. 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

Vermicompost samples showed significant decrease in moisture content when compared to control as per one way ANOVA analysis (p<0.05). The moisture content of vermicompost obtained from all experiments was around 21 – 26%. Tandon (2005) 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. Our results were in accordance with earlier works (Murali *et al.*, 2011; Selvamuthukumaran and Neelanarayanan, 2012a and Viji and Neelanarayanan, 2013). 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 (Dominguez *et al.*, 2002).

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Table 2. The propensity of chemical constituents of vermicompost produced by *E. eugeniae*, *P. excavatus* and *L. mauritii* utilizing sugarcane trash (predigested with *P. fluorescens*) in 50:50 concentration and control

S. No.	Parameters	E. eugeniae Mean ± S.D.	P. excavatus Mean ± S.D.	L. mauritii Mean ± S.D.	Control Mean ± S.D.	p value*	Standard values #
1	pН	7.34 ± 0.13	7.41 ± 0.03	7.51 ± 0.01	7.94 ± 0.03	0.00	6.5 – 7.5
2	Electrical Conductivity (dSm-1)	1.21 ± 0.06	1.39 ± 0.12	1.63 ± 0.06	1.89 ± 0.01	0.01	Not more than 4
3	Moisture (%)	18.80 ± 0.02	25.11 ± 0.02	27.61 ± 0.06	32.81 ± 0.07	0.00	14 – 25%
4	Organic Carbon (%)	21.23 ± 0.01	23.51 ± 0.02	24.08 ± 0.03	45.01 ± 0.02	0.00	Minimum 18%
5	Total Nitrogen (%)	1.88 ± 0.03	1.25 ± 0.04	1.18 ± 0.01	0.41 ± 0.04	0.03	>1%
6	Total Phosphorus (%)	1.17 ± 0.01	1.05 ± 0.01	0.98 ± 0.02	0.13 ± 0.01	0.02	>1%
7	Total Potassium (%)	3.69 ± 0.07	3.44 ± 0.25	3.29 ± 0.06	2.09 ± 0.01	0.00	>1%
8	Total Calcium (%)	2.45 ± 0.09	2.29 ± 0.09	2.01 ± 0.01	1.53 ± 0.01	0.01	-
9	Total Magnesium (%)	1.62 ± 0.12	1.53 ± 0.16	1.23 ± 0.13	0.34 ± 0.01	0.04	-
10	Total Sodium (%)	0.66 ± 0.01	0.54 ± 0.08	0.36 ± 0.01	0.11 ± 0.02	0.01	-
11	Total Sulphur (%)	1.69 ± 0.12	1.41 ± 0.01	1.52 ± 0.08	0.31 ± 0.05	0.00	-
12	C:N ratio	11:1	18:1	20:1	37:1	0.00	10:1 – 20:1

S. D. – Standard Deviation

The mean and standard deviation values were obtained from five individual observations; *Source: Tandon (2005 a, b).

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^{*}The difference between control and vermicompost was statistically significant (p<0.05 level by one way ANOVA).

Organic Carbon

A significant reduction in organic carbon was observed in vermicompost compared to compost (p<0.05). The maximum reduction (21.23 ± 0.01) was recorded in the vermicompost harvested from the experimental trays inoculated with *E. eugeniae*. Our results were in accordance with the findings of Muthukumarasamy *et al.* (1997), Parthasarathi and Ranganathan (2000), Khwairakpam and Bhargava (2009), Kale *et al.* (1982), Elvira *et al.* (1998) and Suthar, (2007).

Total Nitrogen

A significant increase was noticed in Total Nitrogen content of the vermicompost than control (p<0.05). The percent increase in the Total Nitrogen content was in the order: *E. eugeniae* $(1.88 \pm 0.03) > P$. excavatus $(1.25 \pm 0.04) > L$. mauritii (1.18 ± 0.01) (Table 2). The enhancement of N in vermicompost was probably due to mineralization of the organic matter containing proteins (Bansal and Kapoor, 2000; Kaushik and Garg, 2003) and conversion of ammonium nitrogen into nitrate (Suthar and Singh, 2008; Atiyeh et al., 2000). Earthworms can boost the nitrogen levels of the substrate during digestion in their gut adding their nitrogenous excretory products, mucus, body fluid, enzymes, and even through the decaying dead tissues of worms in vermicomposting subsystem (Suthar, 2007). According to Suthar (2008), the vermicompost produced by all the three earthworm species showed difference in total N content which could be attributed directly to the species specific feeding preference of individual earthworm species and indirectly to mutualistic relationship between ingested microorganisms and intestinal mucus.

Total Phosphorus

The Total Phosphorus content was significantly higher in vermicompost than compost (ANOVA: p<0.05 for all experiments). The vermicompost produced by *E. eugeniae* showed the greater content of total phosphorus (1.17 ± 0.01) (Table 2). Acid production during organic matter decomposition by the microorganisms is a major mechanism for solubility of insoluble phosphorous also; the percentage of large number of micro flora in the gut of earthworms might play an important role in increasing P in the process of vermicomposting (Kaviraj *et al.*, 2003). The present finding was agree with the reports of Kaushik and Garg, (2003), Suthar (2007) and Manna *et al.* (2003) who demonstrated similar increase in total phosphorus of vermicomposted materials. The worms during vermicomposting process converted the insoluble Phosphorus into soluble forms with the help of Phosphate solubilizing microorganisms through phosphatases present in the gut, making it more available to plants (Suthar and Singh, 2008; Padmayathiamma *et al.*, 2008; Ghosh *et al.*,1999).

Total Potassium

Statistically vermicomposted materials showed significant difference for Total Potassium than composted material (p<0.05) as per ANOVA analysis. The maximum increased was noticed in the vermicompost produced by E. eugeniae (3.69 \pm 0.07) followed by P. excavatus (3.44 \pm 0.25) and L. mauritii (3.29 \pm 0.06) (Table 2). The present findings corroborated to those of Selvamuthukumaran and Neelanarayanan (2012 a, b), Murali $et\ al.$, (2011) and Delgado $et\ al.$ (1995). The increase in potassium of the vermicompost in relation to that of the simple compost and substrate was probably because of physical decomposition of organic matter of waste due to biological grinding during passage through the gut, coupled with enzymatic activity in worm's gut, which may have caused its increase (Rao $et\ al.$, 1996). The

microorganisms present in the worm's gut probably converted insoluble potassium into the soluble form by producing microbial enzymes (Kaviraj and Sharma, 2003).

Total Calcium and Magnesium

The concentration of Total Calcium and Total Magnesium was significantly higher in vermicompost when compared to control (p<0.05) as per one way ANOVA analysis (Table 2). Similar observations have been reported by Elvira *et al.* (1998) 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 (Joshi and Kelkar, 1952; Kale and Bano, 1988; Tiwari *et al.*, 1989 and Viji and Neelanarayanan, 2013) and Ca (Joshi and Kelkar, 1952; Kale and Bano, 1988). 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 (Parthasarathi and Ranganathan, 2000).

Total Sodium

The total sodium concentration was increased in all the three vermicompost produced by the chosen earthworms when compared to control (Table 2). The difference between vermicomposted and composted material for Total Sodium was statistically significant (p<0.005). Similar observations have been reported by Murali and Neelanarayanan (2011), during the vermicomposting of coir waste by *E. eugeniae*, where as the present findings are in contradiction to the findings of Albanell *et al.* (1988), who reported decreased total sodium concentration during vermicomposting of tapioca solid waste.

Total Sulphur

The total sulphur was significantly increased when compared to control (p<0.05) (Table 2). The maximum increase was observed in the vermicompost harvested from the experimental

trays inoculated with E. eugeniae (0.66 \pm 0.01). Ramalingam and Ranganathan (2001) 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 (2012a), who demonstrated that higher S concentration in the vermicompost prepared from leaves litter.

Carbon Nitrogen Ratio (C:N ratio)

The C:N ratio had decreased levels in vermicompost when compared to control (Table 2). The difference between vermicomposted and composted materials for C:N ratio was significant (ANOVA: p<0.05 for all treatments). The maximum reduction (11:1) was noticed in the vermicompost produced by *E. eugeniae* where as the control had 37:1. 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. Plants cannot assimilate mineral N unless the C:N ratio is about 20:1, and this ratio is also an indicative of acceptable maturity of compost (Morais and Queda, 2003). The C:N ratio of the substrate material reflects the organic waste mineralization and stabilization during the process of composting or vermicomposting. Higher C:N ratio indicates slow degradation of substrate (Haug, 1993), and the lower the C:N ratio, the higher is the efficiency level of mineralization by the species. The loss of carbon through microbial respiration and mineralization and simultaneous addition of nitrogen by worms in the form of mucus and nitrogenous excretory material lowered the C:N ratio of the substrate (Suthar, 2007; Dash and Senapati, 1986; Talashilkar *et al.*, 1999; Christy and Ramalingam, 2005).

CONCLUSION

The results of the present study obviously suggest that *P. fluorescens* may be used for the partial degradation of sugarcane trash and it is evident from the results that the chemical nutrients 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 and
- The quantity of macro and micronutrients in the vermicompost was found to be within the good quality vermicompost range values.

In the present study, in general the total time taken for the bioconversion of raw sugarcane trash into vermicompost was 85 days. Further, research work is required in order to find out a method in which the bioconversion of the raw sugarcane trash into vermicompost is possible in about 25-30 days or even lesser than this time limit.

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