International Journal of Current Science Research
www.drbgrpublications.in

Volume: 2; Issue: 5; May-2016; pp 650-667. ISSN: 2454-5422

Vermicomposting of sunflower (Helianthus annuus l.) Cob (pre-digested with Aspergillus niger) without cow dung by utilizing conventional composting earthworms

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Abstract

In the present investigation the sunflower cob was pre-digested with *Aspergillus niger* and the same was utilized for the production of vermicompost without cow dung by using *Eudrilus eugeniae*, *Perionyx excavatus* and *Lampito mauritii* under monoculture condition. The conversion ratio of waste into vermicompost was found to be high (88%) in the trays in which *L. mauritii* was used. Further, it was observed in the end product the *L. mauritii* produced more number of cocoons and young ones when compared to the other two cultures. The maximum wet biomass was found in *E. eugeniae* (40±0.05mg). The vermicompost harvested from *L. mauritii* experimental trays showed remarkable levels of chemical nutrients and higher density of microbial population *viz.*, Bacteria, Actinomycetes and Fungi than the other experimental trays. The results of the present study apparently suggest that the sunflower cob (pre-digested with *A. niger*) into value added vermicompost without cow dung by utilizing the three earthworm species and in particular *L. mauritii* is very effective.

Key Words: Vermicompost, Sunflower Cob, *Aspergillus niger*, *Eudrilus eugeniae*, *Perionyx excavatus*, *Lampito mauritii* and Monoculture

Introduction

Agricultural wastes are the by products of various agricultural activities such as crop production, crop harvest, saw milling, agro-industrial processing and others. The major quantity of wastes generated from agricultural resources are sugarcane baggase, paddy and wheat straw and husk, wastes of vegetables, food products, jute fibre, groundnut shell, coconut husk and cotton stalk etc. It has been estimated that 136.5 to 150 million tonnes of paddy straw, 46 million tonnes of sunflower cob, 35 million tonnes of sugarcane trash, 6 million tonnes of groundnut shell, 5.64 million tonnes of maize cob, 1.39 million tonnes of coir wastes, etc., are annually available as organic wastes in India (Wati *et al.,* 2007). Majority of them are remain unutilized or more than 95% of farmers dispose these agricultural wastes simply by burning in the field which results in loss of nutrients as well as loss of invaluable organic matter. Besides the loss of organic matter and plant nutrients, the burning of crop residues also causes atmospheric pollution due to the emission of toxic gases such as methane, carbon dioxide that poses threat to human ecosystem and eventually human health (Wang and Christopher, 2003).

The chemical compositions of these agricultural wastes are cellulose, hemi-cellulose, lignin, pectin, starch and proteins. Out of which, cellulose, hemi-celluloses and lignin are the most important chemical constituents. When agricultural wastes are subjected to composting processes, the ligno-cellulose usually constitutes an important component of the total organic matter, and they are slowly decomposed (Tuomela *et al.,* 2000; Shi *et al.,* 2006; Yu *et al.,* 2007 and Tang *et al.,* 2008). The inoculation of lingo-cellulolytic enzymes producing microorganisms is a strategy that could potentially enhance the lingo-cellulose degradation in the selected agro-wastes. These enzymes released by the microorganisms during composting also play a key role in the biological and biochemical transformations of wastes. Microbial enzymes are responsible for the breakdown of several organic compounds characterized by a complex structure, finally produces a simple water-soluble compounds (Benitez *et al.,* 1999).

Disposal and environmental friendly management of these agricultural wastes has become a serious global problem. Therefore, much attention has been paid in the recent years to develop inexpensive, low-input and efficient technologies to convert these nutrient-rich organic wastes into value added products for sustainable agro-practices. Decomposition of complex organic waste resources into odour free humus like substances through the action of earthworm is termed as vermicomposting. However, it is obvious that vermicomposting is nothing but the stabilization of organic material 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 (Dominguez, 2004).

However, no work has been reported on the vermicomposting of sunflower cob (pre-digested with *A. niger)* without cow dung and hence the present investigation. The present study was undertaken to pre-digest the chosen agro-waste sunflower cob with *A. niger* and subsequently to assess the vermicomposting potential of three earthworm species *i.e., Eudrilus eugeniae, Perionyx excavatus* and *Lampito mauritii* under monoculture conditions without cow dung and the magnitude of chemical nutrients and micro-flora in the vermicompost produced by these earthworms.

Materials and Methods

Collection of organic wastes

The selected waste *i.e.,* Sunflower Cob was collected from Thuraiyur, Tiruchirappalli District, Tamil Nadu. Cow dung was also collected from nearby dairy yard.

Collection of earthworms

The earthworm *viz., Eudrilus eugeniae*, *Perionyx excavatus* and *Lampito mauritii* was obtained from our college vermished.

Isolation and Preparation of *A. niger* inoculum

Aspergillus niger was isolated from soil and was identified using Manual of the *Aspergilli* (Thom and Raper, 1945). 10 ml of molasses was taken in a conical flask and 90 ml of distilled water was added and mixed well. To this 1 ml of pure culture of *A. niger* was added and 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.

Fig. 1. *Aspergillus niger*

Fig. 2 *A. niger* in Potato Dextrose Broth medium

Fig. 3. *A. niger* in molasses medium

Pre-digestion of organic wastes

The sunflower cob was spread on a clean floor, which was open to sunlight for 5 days. Watering was done regularly twice in a day on the sunflower cob. The sun dried sunflower cob was transferred to a shady place where it was cured for 5 days. Later 1kg of sunflower cob was spread on a clean floor. To this 20 ml of *Aspergillus niger* was uniformly sprinkled. Above to this, a layer of 1kg of sunflower cob was spread. This process can be 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 17 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 with pre-decomposed sunflower cob 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. After 2 days of the preparation of the experimental media in trays, hundred healthy and clitellate individuals of all the three species of earthworm were collected from the vermished of our college and 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. On 35th day 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.

Chemical Nutrient analysis

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 (Anon, 2006).

Quantification of Microorganisms

The total number of colony forming units of bacteria, fungi and actinomycetes present in the vermicompost samples were estimated by serial dilution method (Allen, 1953). Nutrient Agar for bacteria, Potato Dextrose Agar for fungi and Soil Extract Agar for actinomycetes were used.

Statistical Analysis

One way ANOVA was used to analyze the significant difference between treatments.

Results and Discussion

The mean total weight of the vermicompost obtained after vermicomposting of sunflower cob were 3000g (*E. eugeniae*), 2600g (*P. excavatus*), 3500g (*L. mauritii*) and 1520g (Control) (Table 1). The percent conversion of vermicompost was 75% for *E. eugeniae*, 65% for *P. excavatus*, 88% for *L. mauritii* and 38% for control (Table 1). The mean number of cocoons and youngones enumerated were to the tune of 11 and 8 (*E. eugeniae*), 18 and 12 (*P. excavatus*), and 23 and 27 (*L. Mauritii*) (Table 1).

No mortality was observed in *L. mauritii* inoculated experimental trays. Neuhauser *et al.* (1980) reported that when earthworms received food below maintenance, it lost weight at a rate, which depended upon the quantity and nature of its ingestible substrates. The earthworms exhibited different patterns of cocoon production. The maximum number of cocoon production was observed in experimental trays inoculated with *L. mauritii* and minimum was observed in *E. eugeniae* inoculated experimental trays. Edwards *et al.* (1998), have reported that the difference rates of cocoon production in different organic wastes are related to the quality of the waste material used as feed. The difference between cocoon productions in different treatments could be related to the biochemical quality of the feed mixtures, which is one of the important factors in determining onset of cocoon production (Flack and Hartenstein, 1984).

The physico-chemical characteristics of vermicompost and compost are provided in Table 2. The pH value of the vermicompost produced by all the three species of earthworms utilized in the present study was found to be within the standard value for vermicompost. pH is an important parameter in the vermicompost for promoting plant growth. The decreasing trend in pH during vermicomposting corroborates with the findings of other researchers (Mitchell, 1997; Ndegwa *et al.,* 2000; Khwairakpam and Bhargava, 2009; Gupta and Garg, 2008). The decrease in pH during vermicomposting may be due to CO_2 and organic acids produced by microbial metabolism (Elvira *et al.,* 1998). Contrary to the present finding, an increasing trend was reported in vermicomposting residues from olive oil (Nogales *et al.,* 2000) and paper, yard and food waste mixtures (Komalis and Ham 2006).

The electrical conductivity value was higher in vermicompost than control. Electrical conductivity (EC) is an indicator of the change in the salt concentration in compost, and high concentrations mean better nutrients (especially potassium, calcium and magnesium) content, but if they are too high they decrease water accessibility (Lasaridi and Stentiford, 1998). The observed results are supported by those of other authors Viji and Neelanarayanan, 2013 and Selvamuthukumaran and Neelanarayanan, 2012). The vermicompost showed lower moisture level than control. 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. Similar observations have been reported by Viji and Neelanarayanan (2013) during the vermicomposting of paddy straw by *E. Eugeniae, P. excavatus* and *L. mauritii.*

The organic carbon had decreased levels in vermicompost when compared to control. Saradha (1997) reported a significant reduction in total organic carbon content in a vermicompost produced from olive oil industrial waste. Lower level of organic carbon was observed in the vermicomposted Parthenium plant than in non vermicomposted Parthenium (Yadav and Garg, 2011). Elvira *et al*. (1998) was identified that vermicomposting of pulp-mill sludge for 40 days decreased carbon content by 1.7-fold. Suthar (2009) also described that mineralization of organic matter in sewage sludge by earthworms leads to a considerable decrease in total organic carbon (TOC) content. The reduction of carbon in vermicompost is the result of respiration and mineralization of the organic matter mainly by microorganisms and earthworms. Since vermicomposting is a combined action of earthworm and microorganisms, earthworms through their fragmenting action modify the substrate condition which consequently increase the surface area for microbial action4 thus promote carbon loss through respiration and in similar pattern the oxidation of organic matter within the vermicomposting unit is enhanced by earthworm population in the vermicomposting unit. Suthar (2007) stated that excreta and body fluid of earthworms like mucus encourage microbial multiplication which in turn promotes rapid respiration that minimizes the organic carbon level of the waste.

Vermicomposted material showed higher total nitrogen level than control. Earthworm activity enriches the nitrogen profile of the vermicompost through microbial mediated nitrogen transformation, through addition of mucus and nitrogenous wastes secreted by earthworms. Decrease in pH may be an important factor in nitrogen retention as N_2 is lost as volatile ammonia at high pH values. Increase in nitrogen content in vermicompost of sugarcane trash and cow dung substrate as compared to control was reported by Ramalingam and Thilagar (2000). Atiyeh et al. (2000) reported that by enhancing nitrogen mineralization, earthworms have a great impact on nitrogen transformation, so that nitrogen retained in the nitrate form. In contrast, Hobson *et al.* (2005) reported that the reductions in N concentrations in vermicomposting due to *in vivo* denitrification within the worm's digestive tract. Part of the N-content in the initial substrate is also transformed into earthworm protein. Hartenstein and Hartenstein (1989) also reported 1.8 fold reduction of nitrogen in vermicomposting sludge.

Table 1. Magnitude of composition of pre-digested food (Sunflower (*Helianthus annuus L.*) cob – pre-digested with *A. niger*) and its bioconversion into vermicompost by *E. Eugeniae, P. excavatus* and *L. Mauritii*. Each value represents the mean of three observations.

S. No.	Particulars	Composting organisms			
		E. eugeniae	*P. excavatus*	*L. mauritii*	Control
1	Weight of predigested sunflower cob (g)	4000	4000	4000	4000
2	Number of adult earthworms introduced	100	100	100	-
3	Total weight of compost/vermicompost obtained (g)	3000	2600	3500	1500
4	Percent conversion of vermicompost	75	65	88	38
5	Mean number of adult earthworms observed in each tray	90	80	100	
5	Mean number of cocoons observed in each tray	11	18	25	-
6	Mean number of young ones observed in each tray	7	12	27	-

Table 2. The physico-chemical characteristics of vermicompost produced by *E. Eugeniae, P. excavatus* and *L. Mauritii* utilizing Sunflower cob (pre-digested with *A. niger*) and control. Each value represents the mean (Mean ± S.D.) of three observations.

S.No.	Parameters	Composting organisms				
		E. eugeniae	***P. excavatus***	***L. mauritii***	**Control**	**Standard for vermicompost[1]**
1	pH	7.6 ± 0.17	7.26 ± 0.11	7.2 ± 0.10	8.6 ± 0.10	6.5 – 7.5
2	Electrical Conductivity (dSm-1)	3.46 ± 0.15	3.40 ± 0.10	3.16 ± 0.11	1.26 ± 0.20	Not more than 4
3	Moisture (%)	20.12 ± 0.02 ^{NS}	13.23 ± 0.01	15.02 ± 0.01	27.43 ± 0.01	14.0 – 25.0
4	Organic Carbon (%)	25.14 ± 0.01	21.23 ± 0.02	24.34 ± 0.01	37.24 ± 0.01	Minimum 18%
5	Total Nitrogen (%)	1.21 ± 0.01	1.24 ± 0.01 ^{NS}	1.36 ± 0.31 ^{NS}	0.96 ± 0.03	>1
6	Total phosphorus (%)	1.02 ± 0.01	1.63 ± 0.04	1.82 ± 0.01	0.32 ± 0.01	>1
7	Total potassium (%)	1.43 ± 0.01	1.54 ± 0.05	1.62 ± 0.01	0.37 ± 0.03	>1
8	Total Calcium (%)	0.54 ± 0.01	0.62 ± 0.01	0.71 ± 0.02	0.45 ± 0.01	-
9	Total Magnesium (%)	0.34 ± 0.02	0.46 ± 0.01	0.53 ± 0.02	0.16 ± 0.04	-
10	Total Sodium (%)	0.43 ± 0.01	0.63 ± 0.02	0.83 ± 0.02 ^{NS}	0.26 ± 0.04	-
11	Total Sulphur (%)	0.24 ± 0.03	0.47 ± 0.02	0.56 ± 0.01	0.13 ± 0.02	-
12	C: N ratio	21:1	17:1	19:1	39:1	10:1 – 20:1

NS – Non Significant

The Total Phosphorus concentration was increased in all the three vermicompost produced by the chosen earthworms when compared to control. Total Phosphorus (P) being an essential nutrient is required by plants for photosynthesis, energy transferring mechanisms, better flowering, fruit growth, plant maturation and is taken up by plants in the form of inorganic ions: H_2PO_4 (Vasanthi et al., 2011). Our results are in agreement with the reports given by various workers who have proved vermicomposting to be an efficient technology for the conversion of unavailable forms of phosphorus to easily available forms for plants (Ghosh *et al.*, 1999).

Total Potassium had increased levels in vermicompost when compared to control. Delgado *et al*. (1995) in vermicomposting sewage sludge, Orozco *et al*. (1996) in vermicomposting coffee pulp and textile mill sludge, Dominguez and Edwards (1997) in vermicomposting pig slurry and in vermicomposting municipal solid wastes have reported the general rise of total potassium in the final product. Large number of symbiotic micro flora present in the gut and the cast of earthworms in collaboration with secreted mucus and water might increase the degradation of ingested organic matter and the release of assailable metabolites. These metabolites enhance the enrichment of the vermicompost with exchangeable potassium (Kaviraj and Sharma, 2003). Contrast to this finding, some researchers has reported lower content of TK in vermicompost. Elvira *et al.* (1998) and Ananthakrishnasamy *et al*. (2009) have reported lower level of potassium in vermicompost than the initial substrate. This probably reflects leaching of this soluble element by the excess water that drained through the mass. Benitez *et al*. (1999) pointed out that the leacheates collected during vermicomposting process had higher potassium concentrations.

The extent of total calcium content was comparatively higher in vermicompost than the control. The higher Ca content in vermicompost compared to that of compost and substrate is attributable to the catalytic activity of carbonic anhydrase present in calciferous glands of earthworms generating $CaCO_3$ on the fixation of CO_2 (Padmavathiamma *et al.*, 2008). This finding was supported by several previous vermicomposting research works on various organic wastes. Jadia and Fulekar (2008) in vermicomposting vegetable waste, Ansari (2009) in vermicomposting various mixtures of urban solid wastes, Pattnaik and Reddy (2010) in vermicomposting urban green wastes observed a considerable increment, but on the other hand, Elvira *et al* (1998) in vermicomposting sludges from paper mill and diary industries, Chaudhuri *et al* (2000) in vermicomposting kitchen wastes, observed lower amount of calcium than the initial substrate value. The decline might be the result of leaching of this soluble element by the excess water that is drained through (Elvira *et al*., 1998).

The extent of total magnesium content was comparatively higher in the vermicompost than the control. The higher concentration of Mg in vermicompost reported in present study was also in consistence with the findings of earlier workers (Pathmavathiamma *et al.,* 2008 and Tiwari *et al.,* 1989)**.**

The total sodium was found to be high in the final product i.e., vermicompost when compared to control. Similar observations have been reported by Murali and Neelanarayanan (2011) and Viji and Neelanarayanan (2013) during the vermicomposting of coir waste and paddy straw by *E. Eugeniae* and *E. eugeniae, P. excavatus* and *L. mauritii* respectively.

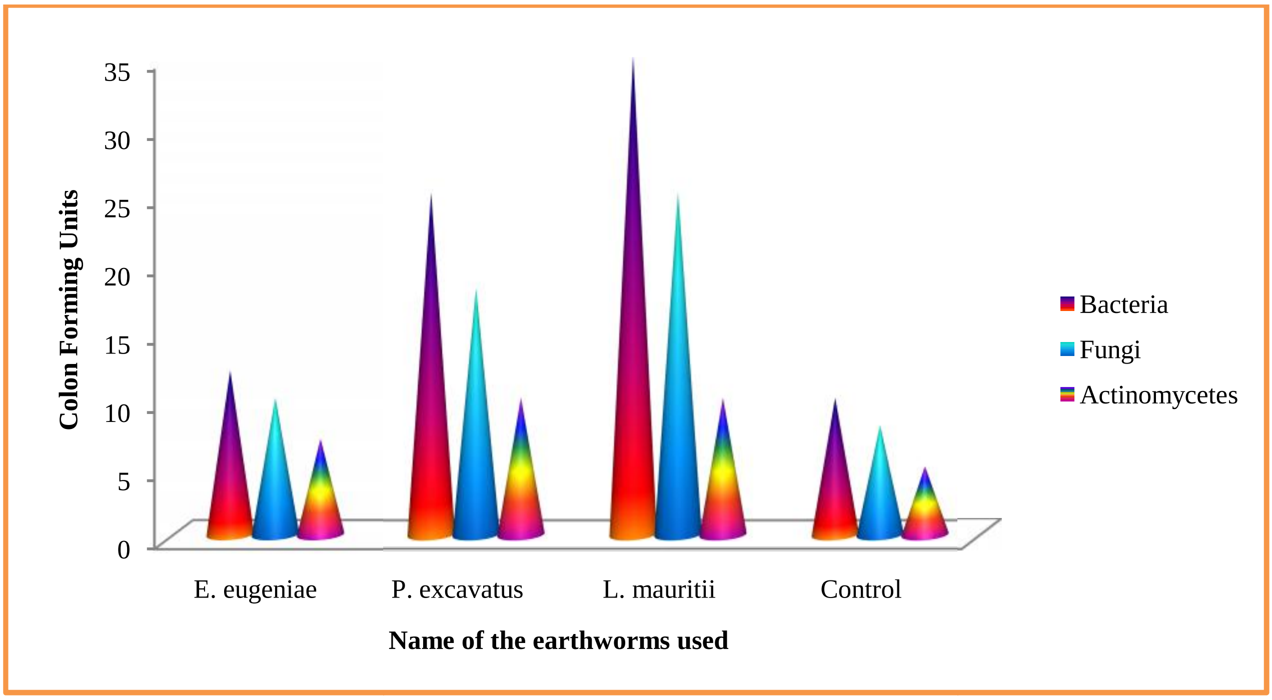
The total sulphur of the vermicompost produced by all the three species of earthworms utilized in the present investigation was found to be high. Ramalingam and Ranganathan (2001) declared that sulphur is an essential element for the synthesis of amino acids and vitamins. The present findings corroborated to those of Selvamuthukumaran and Neelanarayanan (2012), who demonstrated that higher S concentration in the vermicompost prepared from leaves litter.

The C:N ratio is an important criteria of good manure. It should lie between 10 and 20. The plant cannot assimilate mineral nitrogen unless the C:N ratio is 20:1 or less (Edwards and Bohlen, 1996). The loss of carbon as carbon dioxide in the process of respiration and production of nitrogenous excreta enhance the level of nitrogen, which lower the C:N ratio. A decline in C/N ratio to less than 20 indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity of organic wastes (Senesi, 1989). The decrease in C/N ratio over time might also be attributed to increase in the earthworm population (Ndegwa and Thompson, 2001), which led to rapid decrease in organic carbon due to enhanced oxidation of the organic matter.

Microbial Population

In the present investigation, in general an increase in the number of bacteria, fungi and actinomycetes colonies were observed. The earthworms carry the capacity to degrade the organic wastes with the help of enzymes present in the alimentary canal and also with the help of microorganisms present in the gut. Edwards and Lofty (1977) suggested that the size of the microbial population in casts depend on the quality and type of the food. Microorganisms constitute an important component of the earthworm diet (Lee, 1985).

Fig.1 Densityof microbes (CFU) in vermicompost processed by different species of earthworms



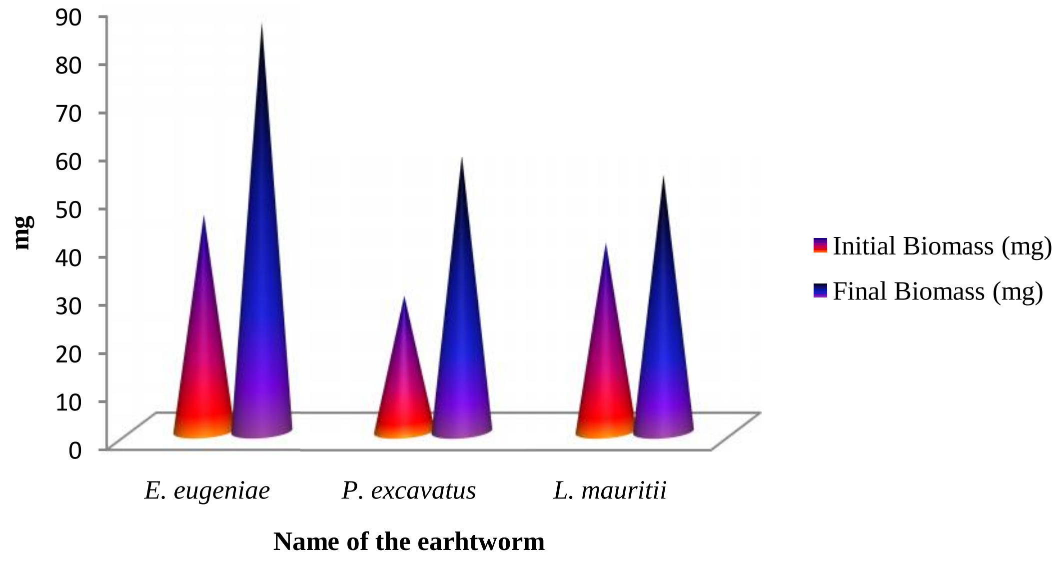
Bacteria – CFU × 10^6 g^{-1}; Fungi - CFU × 10^4 g^{-1} ; Actinomycetes - CFU × 10^3 g^{-1}

Earthworm Biomass

In the present study, an increase in wet biomass of composting organisms *i.e.*, *E. eugeniae*, *P. excavatus* and *L. mauritii* were recorded. *E. eugeniae* was gained the maximum net bio mass (40±0.05mg). According to Suthar (2007), the quality of substrate/bedding material or even fluctuating temperature or both might be responsible for attaining maximum biomass. Another factor like microbial density also contributes the increase in earthworm biomass. The organic waste palatability for earthworms is directly related to the chemical nature of the organic wastes that consequently affects the earthworm growth parameters (Suthar, 2006). Our results were in accordance with earlier works (Reinecke *et al.*, 1992 and Edwards, 1998).

The indigenous species, L. mauritii and P. excavatus exhibited better growth and reproduction performance compared to the other exotic species. The higher numbers of cocoons, young ones and adults collected from the vermicompost processed by L. mauritii was probably because its indigenous nature being acclimatized to the abiotic environmental conditions extremely well compared to other species.

Fig. 2 Total(wet) biomass of the earthworms



Conclusion

The results of the present study apparently suggest that *A. niger* may be used for the partial degradation of sunflower cob and it is evident from the results that pre-digested sunflower cob is potential resource material for the chosen three earthworm species biomass and nutrient rich vermicompost production. It is obvious from the results, the chemical nutrients and microflora values were observed with desired level in the vermicompost produced from sunflower cob without cow dung by using all the three chosen earthworm species. Hence it may be concluded, the partially decomposed sunflower cob alone may be used for the production of vermicompost and in general, all these three earthworm species may be used to produce vermicompost and in particular, *L. mauritii* 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,
- ✓ The quantity of macro and micronutrients in the vermicompost was found to be within the good quality vermicompost range values and
- ✓ Increased number of micro flora.

Acknowledgement

We are thankful to UGC-RGNF (No. F. 14 -2 (SC)/2009 [SA-III] dated 16.12.2010) for extending financial assistance to do this work. Authors are thankful to the Management and Principal of our college for the facilities extended for doing this research work. We thank the Staff members of Zoology Department, students and Research Scholars of Centre for Eco-friendly Agro-Technologies (Vermibiotechnology), Nehru Memorial College (Autonomous) for their help. We wish to thank Mr. N. Subash, Mr. S. Ramachandran, Mrs. Kamatchi and Mrs. A. Uma for their assistance during this composting study.

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