

Recycling of Mango Leaf Litter and Cowdung Using Epigeic and Anecic Earthworms in Mono- and Mixed-Culture Vermireactors

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Abstract: Waste recycling is a challenging problem in rapidly changing agro - environment. Vermitechnology can be effectively utilized to convert waste into valuable asset. Therefore, composting potential, biomass, growth and cocoon production of an epigeic, (*Eisenia fetida*) and an anecic (*Lampito mauritii*) species were studied in mono and mixed – culture vermireactors. Mango (*Mangifera indica*) (MLL) leaf litter with cow-dung in 1:1 (mass/mass) was subjected to vermicomposting for 90 days. Changes in nutrients of bedding material, earthworm growth and cocoon production were observed in mono and mixed culture systems. *L. mauritii* showed higher mass gain and growth rate as compared to *E. fetida* in both vermibeds. *E. fetida*, in monoculture bedding, produced more cocoons than those in mixed-culture bed, which may be due to interspecific competition between the two species. The same results were found for reproductive rate. Degree of waste decomposition and increase in earthworm population were strongly influenced by the quality of culture. Nutrient (organic carbon, nitrogen, phosphorus, potassium) availability varied with respect to earthworm species and type of culture. The worm- worked bedding from mixed-culture, monoculture bedding with *E. fetida* and monoculture with *L. mauritii* showed 4.3, 3.1 and 2.6 times decrease in C/N ratio, respectively as compared to their control values. Microbial counts exhibited remarkable changes as a function of decomposition period and culture type. The present study clearly suggests that mixed-culture of *E. fetida* and *L. mauritii* is more efficient than monoculture (single species) for recycling of organic wastes and production of vermimass and vermicompost.

Key words: Mixed culture • Monoculture • Leaf litter • Growth • Reproductive rate • Earthworm

INTRODUCTION

Rapid urbanization has increased the problem of solid waste management. Recycling of solid waste has become technically and ecologically challenging in the present scenario. The problem has further increased because most of the wastes are dumped randomly in unsustainable manner by dumping. This may lead to loss of nutrients from the waste rendering economic losses [1]. Leaf litter, food industries, livestock farming and poultry generate huge quantities of solid wastes which are rich in nutrients. Fresh organic wastes cannot be applied to soil as they effect plant growth due to nitrogen starvation and production of toxic metabolites until they have been sufficiently biostabilised [2]. Attentions are being paid to evolve economically viable technologies for organic

waste management [3-5]. Vermicomposting is an ecotechnological process that involves decomposition of complex organic waste into stabilized humus-like product (vermicompost) through action of earthworms. It involves the joint action of earthworms and microorganisms. Microbes are responsible for biochemical degradation of the waste. However, earthworms are the important drivers of the process conditioning the physical structure of substrate and altering biological activity of microbes in it. Potential of earthworm in waste management is mainly dependent on survival, growth and reproduction of species in bedding material. Earthworm production is influenced by the nature and availability of food [6]. The kind and amount of available food materials is directly affect the size of earthworm population, species richness, growth productiveness.

Earthworms prefer food which is rich in nitrogen, cellulose and micro-organism [7, 8]. Various experiments have been conducted on growth and reproduction of different species of earthworms using various waste materials [9-11]. Epigeic and anecic worms are considered as potential decomposers of organic matters and they are used efficiently in reduction of various organic waste resources [10-12]. It is interesting that earthworms belonging to different ecological categories have the ability to digest a variety of organic waste resources. In comparison to epigeic, anecic earthworms show different patterns of biological activity in substrates, mainly due to their burrowing activity. They exhibit remarkable differences in their feeding behaviors and niche selection patterns which ultimately lead to differences in the diversity of microbial communities in composting system.

Several epigeic (*Eisenia fetida*, *Eisenia andrei*, *Eudrilus eugeniae*, *Perionyx excavatus* and *Perionyx sansibaricus*) and few anecic earthworms (*Lampito mauritii* and *Lumbricus terrestris*) have been identified as decomposers of organic wastes [12-17]. These species have been tested in monoculture only. But information on mixed culture of earthworm is lacking. Therefore, it was intended to compare waste decomposition efficiency of *Eisenia fetida* and *Lampito mauritii* in mono and mixed culture. At the same time the influence of waste diets and local conditions on growth, reproduction and biology of earthworms were also studied. The specific objectives were: 1. to evaluate the decomposition and microbial load efficiencies of epigeic (*Eisenia fetida*) and anecic (*Lampito mauritii*) in mono- and mixed-culture reactors. 2. to evaluate the biomass and growth of both the species in different vermibeds.

MATERIALS AND METHODS

Earthworms and Organic Waste: Our studies aimed at one exotic (*Eisenia fetida*) and (*Lampito mauritii*) one native worm and their combinations to compare the suitability of these species for composting. During random survey in Haryana, India, *Lampito mauritii* was the only pure anecic species. Hence we decided to choose two composting species of earthworms viz., one exotic (*Eisenia fetida*) and one indigenous (*Lampito mauritii*) species for vermiculture experiment. *E. fetida* (epigeic) was obtained from local vermicomposting unit in Karnal, Haryana. *L. mauritii* (anecic) was collected from sewage sludge in Karnal.

The leaf-litter of *Mangifera indica* (L.) (MLL) and cow dung in 1:1 ratio on mass basis was used as an organic waste. MLL was collected from orchards present in a Kurukshetra University Campus (Haryana, India) and fresh cow dung (CD) was collected from nearby cattle sheds. Both were subjected to partial decomposition for 15 days in rectangular cemented tank (75cm x 60cm x 45cm) in order to make them palatable by the worms. Both species of earthworms were introduced in this bedding material separately as well as in combination depending upon the type of culture.

Experimental Design: Earthworm cultures were set-up in triplicate in plastic containers of (50cm diameter and 35cm depth) for monoculture and mixed-culture. About 2.5 kg of feed was added to the containers and garden soil was used as base material along with some concretes for aeration. Two monoculture reactors were set parallel and this had 20 individuals of *E. fetida* and *L. mauritii*, separately. Ten clitellated individuals (nearly of same size) of each species (*E. fetida* and *L. mauritii*) (total twenty) were released on experimental containers for mixed-culture. Control (without earthworms) was also set with the experimental set. Vermicomposting was allowed for a period of 90 days. All containers were covered with wet jute bags to retain moisture. The pH and moisture content of the bedding was measured throughout the experiment. The pH of bedding material was 6.5- 7.5. About 40-60% moisture content was maintained by sprinkling water from time to time. Culture pots were placed in shady and moist place and they were kept undisturbed during experimentation.

Nutrients Analysis: A nutrient analysis was done after every 15 days for 3 months. Samples from each vermibed (LLM + CD) were dried, ground and sieved. The pH was measured using digital pH meter (Systronic made) in 1/10 (w/v) aqueous solution. Moisture was determined by heating a sample at 105°C in hot air oven till constant mass was achieved. Organic carbon (OC) was estimated according to Walkley and Black [18]. Total nitrogen (TN) was measured by Micro-Kjeldhal method [19]. Available phosphorous (P) was determined by Olson's sodium bicarbonate extraction method [20]. Exchangeable potassium (K) was measured by ammonium acetate extractable method [21]. C/N ratio was also calculated. Percent nutrient changes were calculated employing following formula:

$$[(A - B/A) \times 100];$$

where A = value in the worm-worked substrate, B = value in the control substrate.

Enumeration of Microorganisms: Serial Dilution method was used for the enumeration of microorganisms from different bedding materials. Media used for total bacterial count and mould count were standard nutrient agar and standard PDA, respectively. After incubation, CFU / ml (colony forming units) were calculated as follows:

$$\text{CFU/ml} = \text{No of colonies} \times \text{Dilution factor} / \text{Dry mass of sample}$$

Biological Observations: Growth and cocoon production of earthworms were observed to evaluate the productivity of worms. Earthworms produced during experiment were separated from the substrate. They were washed in tap water to remove adhering material from their bodies and weighed. The mean of three triplicates was used to express the results. Then all worms were returned to the concerned container. Separated cocoons was counted and kept in separate container having bedding material. No fresh feed was added at any stage during the study period.

Statistical Analysis: Data were subjected to paired student t-test. The level of significance was set at 0.05.

RESULTS

Nutrient Enrichment: Monoculture and mixed -culture of *E. fetida* and *L. mauritii* in mango leaf litter and cowdung bedding for 90 days showed significant changes in nutrient profile. Mineralization and decomposition were higher in mixed culture as compared to monocultures (Table 1; Fig. 1). At the end of vermicomposting the organic waste mixture was odor free, nutrient and microbial rich and brownish black homogenous material. Vermicompost obtained from recycling of substrates exhibited almost neutral pH ranging from 7.0 to 7.3 in all cultures than their initial values. Organic carbon was 39.99 % before worms inoculation and it declined by 24.35% in mixed- culture over a period of 90 days. Whereas in monoculture of *E. fetida* and monoculture of *L. mauritii* organic carbon reduced by 26.71% and 28.60 %, respectively. The percentages of nitrogen, phosphorus

and potassium increased gradually at every fortnight. Initially the nitrogen was 1.05% which increased upto 2.70 % in mixed culture, 2.12 % in monoculture of *E. fetida* and 1.91% in monoculture of *L. mauritii* during the process of decomposition. Similarly, the percentage of phosphorus increased from 0.93 % to 1.29 % in mixed culture. While its values increased by 1.19 % and 1.16 % in monoculture of *E. fetida* and in monoculture of *L. mauritii*, respectively. Likewise on the last sampling date (day 90), the percentage of potassium raised upto 0.49%, 0.44 % and 0.42 % in mixed- culture, monoculture of *E. fetida* and monoculture of *L. mauritii*, respectively as compared to 0.36 % on 0 day (before worms inoculation). After 90 days of worm working, C/N ratios declined in all the three vermireactors which were 8.97 (mixed - culture), 12.60 (monoculture of *E. fetida*) and 14.71 (monoculture of *L. mauritii*) in different beddings.

Microbial Load: Total microbial load was highest in mixed-culture vermibed (*E. fetida* and *L. mauritii*) as compared to other vermibed (Table 2). Bacterial population of vermicompost varied from 2.88×10^6 to 6.68×10^7 cfu /g. The data pertaining to the observation on fungal colonies revealed variation from 1.8×10^6 to 5.5×10^6 . Most of the isolates produced oral, round and irregular shape and raised and flat colonies had smooth shiny surface with smooth margin. They differed in colour from off-white to creamish, yellow and orange but all were odorless. Brownish pigmentation was observed in some of the isolates. Maximum bacteria on staining appeared dark purple or blue in colour, thus they were gram- positive bacterium.

Reproductive Potential: *E. fetida* and *L. mauritii* showed drastic differences in growth and cocoon production pattern between monoculture and mixed-culture vermicomposting systems. *L. mauritii* demonstrated higher weight gain (376.7 ± 1.79 mg) and growth rate (4.18 ± 0.145 mg) that was significantly higher than those obtained by *E. fetida* in both vermireactors. Continous increase in growth rate was observed upto 9 weeks in monoculture and mixed-culture vermibeds. Afterwards the growth became more or less constant. The number of cocoon production in different composting unit also varied significantly (Table 3). *E. fetida* in monoculture reactor produced maximum (75.33 ± 7.684) number of cocoons than those produced in mixed-culture reactor. The same results were also observed for reproduction rate.

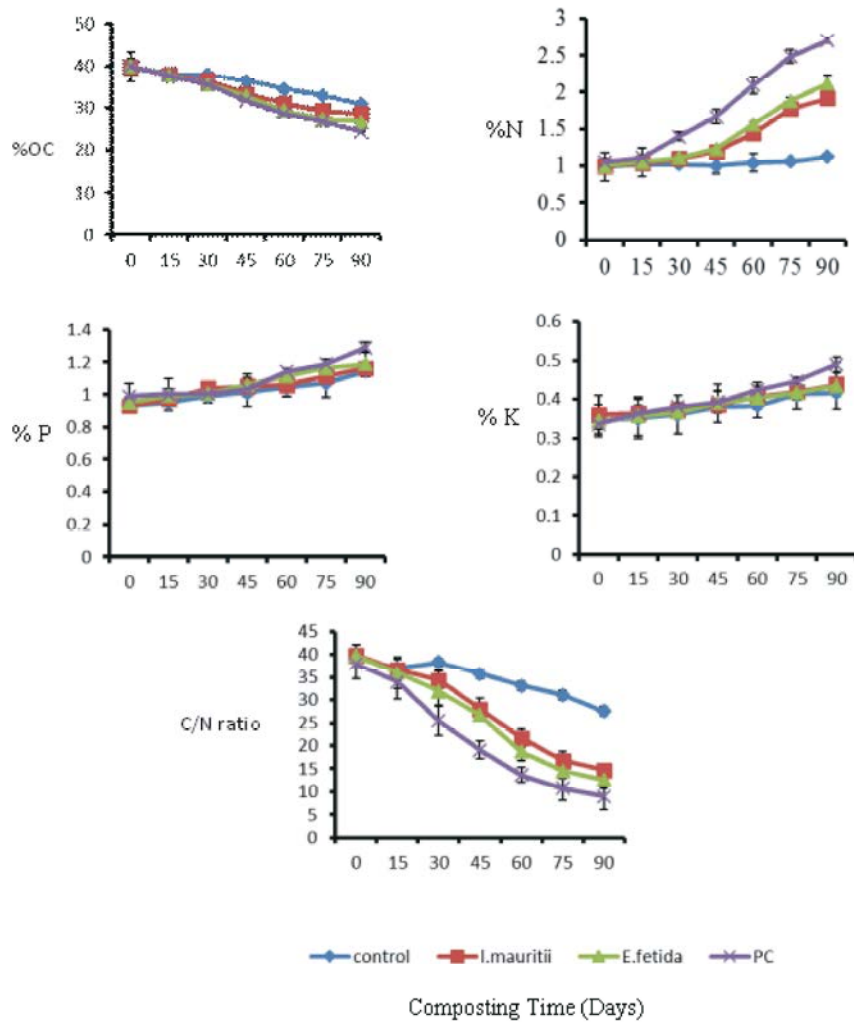


Fig. 1: Effects of *E.fetida* and *L. mauritii* inoculation on organic carbon, nitrogen, phosphorus, potassium and C/N ratio in monoculture and polyculture (PC) bedding material (LLM+ Cowdung). Each point represents mean ± SEM of three observations

Table 1: Vermicomposting coefficient (VC) of different soil nutrients in vermicbeds (n=3)

Vermibeds/parameters	VC _{OC}	VC _{TN}	VC _{C/N}	VC _P	VC _K
PC(E.F + L.M)	1.26	2.41	3.05	1.13	1.11
MC (E.F)	1.15	1.89	2.18	1.04	1.01
MC (L.M)	1.07	1.70	1.87	1.01	1.00

Table 2: Microbial count in different vermicbeds during organic waste decomposition

Days	Control (cfu/ml)		Mixed-culture bedding (<i>E.fetida</i> & <i>L.mauritii</i>) (cfu/ml)		Monoculture bedding (<i>L.mauritii</i>) (cfu/ml)		Monoculture bedding (<i>E.fetida</i>) (cfu/ml)	
	Bacteria	Yeast	Bacteria	Yeast	Bacteria	Yeast	Bacteria	Yeast
0	1.48×10 ⁵	10×10 ⁵	-	-	-	-	-	-
30	1.56×10 ⁵	1.5×10 ⁶	4.36×10 ⁵	4.3×10 ⁶	3.92×10 ⁵	3.8×10 ⁶	2.88×10 ⁵	1.8×10 ⁶
60	2.00×10 ⁵	2.0×10 ⁶	4.28×10 ⁷	4.9×10 ⁶	4.32×10 ⁵	4.1×10 ⁶	3.88×10 ⁵	2.4×10 ⁶
90	2.36×10 ⁵	2.3×10 ⁶	6.68×10 ⁷	5.5×10 ⁶	5.04×10 ⁶	4.3×10 ⁶	3.96×10 ⁵	2.7×10 ⁶

Table 3: Biological productivity of earthworm in different vermicomposting system, Each datum represents mean± SEM of thrice observations

Parameters	Polyculture reactor		Monoculture reactor	
	<i>E. fetida</i>	<i>L. mauritii</i>	<i>E. fetida</i>	<i>L. mauritii</i>
Individual mass (mg)				
Start	446.3±3.28	465.0±4.04	451.66±3.84	467.6±3.52
End	676.3±3.27	780.6±4.25	738.0**±2.66	844.3**±2.782
Individual max gain(mg)	230.0±1.47	3.15±2.49	286.33*±1.92	376.7**±1.79
Growth rate of individual (mg/day)	2.55±0.36	3.51±0.28	3.18*±0.23	4.18**±0.14
Total cocoon production	47.33±2.90	27.0±1.52	75.33 ^{NS} ±7.68	32.33 ^{NS} ±4.09
Reproduction rate (cocoon/worm/week)	2.36±0.35	1.35±0.54	3.76 ^{NS} ±1.22	1.62 ^{NS} ±0.73
Total no. of individual at end (only clitellate)	53.30±4.35	26.3±2.33	81.0*±3.46	31.3 ^{NS} ±2.03

Student t-test performed between monoculture and mixed-culture vermireactor *E. fetida* and *L. mauritii* * p<0.05, ** p<0.01, NS not significant

DISCUSSION

The MLL + CD (1:1) substrates subjected to monoculture and mixed- culture vermicomposting with *E. fetida* and *L. mauritii* for 90 days showed notable changes in nutrients. There was decrease in pH of vermibed with the passage time. It may be due to mineralization of N and P [22]. During vermicomposting, the organic carbon in all vermibeds declined. At the end of the experiment the highest loss of organic carbon (compared to its initial level) was observed for mixed-culture (39%) followed by monoculture of *E. fetida* (33%) and monoculture of *L. mauritii* (28%). Organic carbon exhibited significant differences over 90 days of vermicomposting as compared to initial day (P<0.001) in all vermibeds. Our data are in agreement to the report of Elvira *et al.*[14] and Sonowal *et al.* [23] who observed loss of organic carbon as CO₂ during vermicomposting of different organic wastes. The combined process (feeding of earthworms on organic matter and microbial degradation) brought about C loss from substrates and accelerated waste stabilization process [9, 12, 24, 25]. The total N content increased in all the vermibeds over 90 days of composting. In vermibeds the total N content was maximum in mixed culture (62%) followed by content in (*E. fetida*) (50.4%) and (*L. mauritii*) (45%) monoculture. The nitrogen content was not statistically significant in mixed- vermibeds (P>0.05), whereas it was significant (P<0.05) in other two monoculture beddings. In the processing of organic waste through composting, earthworms accelerated the nitrogen mineralization and subsequently N profile was higher in the end product. The final content of nitrogen in vermicomposting is dependent on initial nitrogen present in the waste and the extent of decomposition [26, 27]. In the present study it could be seen that available P and K increased in mixed-culture as compared to both monoculture vermibeds. The variation in the end product might be

associated with the differences in working and design of reactor type along with difference in niche structure or ecological functioning of epigeic and anecic worms used in monoculture and mixed-culture vermibeds. The C/N ratio of all vermibeds decreased significantly (P<0.05) over 90days of vermicomposting. Comparison of vermicomposting (experiment) with control was done by using vermicomposting coefficient (VC_c) and its highest value was recorded for mixed-culture vermibed (Table 1). The anecic (*L. mauritii*) species is capable of both organic waste consumption as well as of modifying the soil structure, whereas epigeic species (*E. fetida*) is capable to work hard to convert all the organic waste into manure and they are of no use in modifying the soil structure. In the present study, when both species were cultured together (mixed-culture) gave better results in terms of decomposition and mineralization as compared to monoculture of respective species.

Total microbial count was highest in mixed-culture vermibed (*E. fetida* and *L. mauritii*) as compared to other vermibed (Table 2). The order of degree of microbial load in different cultures was: mixed-culture (*E. fetida* and *L. mauritii*) > Monoculture (*L. mauritii*) > Monoculture (*E. fetida*). This indicates that the mixedculture vermireactor, especially designed by using at least one burrowing earthworm (anecic) along with epigeic worm, not only accelerated the mineralization but also enhanced the microbial activities in reactor. Bhatnagar [28] explained that anecic earthworms create vertical burrows in decomposing system and cement it with mucus and other body secretions which are rich in nitrogen. Therefore, burrowing earthworm attracted the decomposer community, especially bacteria associated with N mineralization due to its mucus rich wall.

Earthworms (*E. fetida* and *L. mauritii*) of different ecological groups exhibited remarkable differences in their reproductive potential in leaf- litter and cow dung beddings (Table 3). *L. mauritii* in monoculture vermibed

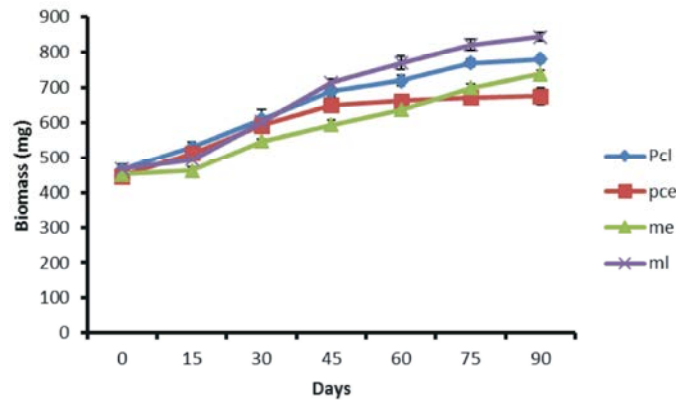


Fig. 2: Growth of *E. fetida* and *L. mauritii* in different vermiculture sets. Pcl- polyculture with *L. mauritii*, pce- polyculture with *E. fetida*; ml- monoculture with *L. mauritii*, me- monoculture with *E. fetida*

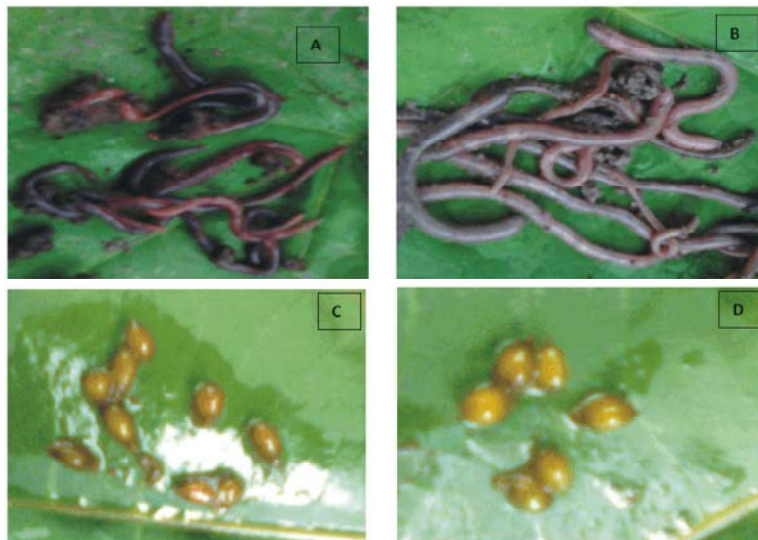


Fig. 3: Earthworm species used *E. fetida* (A) with cocoon (C) and *L. mauritii* (B) with cocoon(D) during the vermiculture.

showed highest ($P < 0.01$) mass gain and growth rate, as compared to mixed-culture. *L. mauritii* being an anecic worm forms a drilosphere (1-2 mm thick lining in burrow) which contains a great amount of bacteria [28]. However, it is well established that microbial population in vermicomposting system is of primary importance as they play important role in worm diet. So, better growth of *L. mauritii* might be related to of microbial growth and more availability of nutrients. Likewise, the epigeic species *E. fetida* when inoculated in monoculture bedding gave highest ($p < 0.05$) mass gain and growth rate in comparison to mixed-culture. But this value is less when compared with *L. mauritii* in monoculture. Differences in growth pattern of different vermicultures (monoculture and mixed-culture) may be assigned to species- specific difference in feeding behavior of epigeic and anecic earthworms [29].

Growth (biomass) of *L. mauritii* was significantly ($p < 0.05$) higher in both the vermibeds than the values obtained for *E. fetida*. A gradual increase in growth rate was observed from 15 to 60 days in monoculture and mixed-culture vermibeds. After 60 days of worm inoculation (both species) in mixed-culture the growth was more or less constant which may be attributed to interspecific competition between the two species for food (Fig. 2). The increase in mass has also been reported for *E. fetida* and *P. excavatus* on cattle dung [13], *L. mauritii* on cow-dung [30] *E. fetida* and *L. mauritii* on mixed- bedding [31].

Fig. 3 shows the species of earthworms used in cocoon production. The rate of cocoon production varied in mono- and mixed- cultures. In monoculture reactor *E. fetida* produced maximum number of cocoons that was not significant ($P > 0.05$) as compared to mixed-culture

vermireactor. Similarly, reproduction rate in monoculture vermibed of *E. fetida* was maximum and it was 37% more than those of mixed-culture. Whereas *L. mauritii* (monoculture) showed 16% more reproduction rate when compared with mixed-culture. This could be related to the preference of food which might influence the variability in cocoon number and reproduction rate between vermicultures. Hence, epigeic worm reproduced more as compared to anecic worm. They also avoided niche overlapping. Total number of clitellate worm varied significantly in both reactors for *E. fetida* and *L. mauritii*. Maximum number of adult worm of *E. fetida* was recorded in monoculture reactor. The number of *L. mauritii* also varied significantly in mixed-culture set. This supports the hypothesis that the earthworm production is influenced by quality and quantity of food [6, 32].

In mixed-culture vermibed there was marked difference in biological productivity as compared to both the monoculture vermireactors. It may be attributed to overlapping of the habitat which in turn leads to competition for food by the two different species. Earthworm mortality was also reported in mixed-culture vermireactor which may be due to niche overlapping and interspecific competition. Since little information is available in the current literature regarding mixed-culture of earthworm species, further study is required to establish relationship among different ecological groups of earthworms.

CONCLUSIONS

The epigeic (*E. fetida*) and anecic (*L. mauritii*) earthworms differed in their decomposition potential, growth and reproductive rate in different culture types (i.e., mono- and mixed- culture). It may be assigned to the differences in cellulose content of waste materials, microbial activity and enhanced water holding capacity. Due to overlapping of niche in mixed-culture both the species showed better growth and reproduction potential when cultured separately in monoculture vermibeds. Worm activity and multiplication also depended on carrying capacity of waste materials. The decomposition rate in vermireactor of both the species showed that mixed culture was efficient than monocultures in stabilizing organic waste and production of nutrient rich vermicompost.

ACKNOWLEDGEMENTS

Dr. Poonam Bhardwaj thanks the Department of Science and Technology, Government of India, New Delhi for providing Women Scientist (WOS-A) scheme

REFERENCES

1. Elvira, C., J. Dominguez, L. Sampedro and S. Mato, 1995. Vermicomposting for the paper-pulp industry. *Biocycle*, 36: 62-63.
2. Zucconi, F., A. Pera and M. De Bertoldi Forte, 1981. Evaluating toxicity of immature compost. *Biocycle*, 22: 54-57.
3. Bouche, M.B., 1979. Valorization des dechets organiques par les lombriciens. *Collection Recherche of Environment.*, 11: 384-394.
4. Divya, U.K., 2001. Relevance of vermiculture in sustainable agriculture. *Agriculture General- World July 2001*, pp: 9-11.
5. Munnoli, P.M., J.A. Silva and S. Bhosle, 2010. Dynamics of the soil earthworm- Plant Relationship: A Review In: *Dynamic soil, Dynamic Plant 4(special issue1)*, (pp: 1-21). Global Science Books.
6. Manivannan, S., P. Ramamoorthy, K. Parthasarathi and L.S. Ranganathan, 2004. Effect of sugar industrial wastes on the growth and reproduction of earthworms. *J. Exp. Zool. India*, 7(1): 29- 37.
7. Ranganathan, L.S. and K. Parthasarathi, 1999. Precocious development of *Lampito mauriti* (Kinberg) and *Eudrilus eugeniae* (Kinberg) reared in pressmud. *Pedobiologia*, 43: 904-908.
8. Chaudhuri, P.S., T.K. Pal, G. Bhattacharjee and S.K. Dey, 2002. "Rubber leaf litters (*Hevea brasiliensis*, var RRIM 600) as vermiculture substrate for epigeic earthworm, *Perionyx excavatus*, *Eudrilus eugeniae* and *Eisenia fetida*", *Pedobiologia*, 47: 796-800.
9. Tripathi, G. and P. Bhardwaj, 2004a. Comparative studies on biomass production, life cycles and composting efficiency of *Eisenia fetida* (Savigny) and *Lampito mauritii* (Kinberg), *Biores. Technol.*, 92: 275-283.
10. Gajalakshmi, S., E.V. Ramasamy and S.A. Abbasi, 2005. "Composting-vermicomposting of leaf litter ensuing from the trees of mango (*Mangifera indica*)", *Bioresour. Technol.*, 96: 1057-1061.
11. Yadav, A. and V.K. Garg, 2011. Recycling of organic wastes by employing *Eisenia fetida*, *Bioresour. Technol.*, 102: 2874-2880.
12. Tripathi, G. and P. Bhardwaj, 2004b. Decomposition of kitchen waste amended with cow manure using epigeic species (*Eisenia fetida*) and an anecic species (*Lampito mauritii*). *Biores. Technol.*, 92: 215-218.

13. Reinecke, A.J., S.A. Viljoen and R.J. Aayman, 1992. The suitability of *Eudrilus eugeniae*, *Perionyx excavatus* and *Eisenia fetida* (Oligochaeta) for vermicomposting in southern Africa in terms of their temperature requirements. *Soil. Biol. Biochem.*, 24(12): 1295-1307.
14. Elvira, C., L. Sampedro, E. Benitez and R. Nogales, 1998. "Vermicomposting of sludge from paper mill and dairy industries with *Eisenia andrei*: a pilot scale study". *Bioresour. Technol.*, 64: 205-211.
15. Kale, R.D., 1998. "Earthworms: Nature's gift for utilization of organic wastes". In: CA Edwards (Ed.), "Earthworm Ecology", 2nd ed., (pp: 355-373). CRC Press.
16. Dominguez, J., 2004. "State-of-the art and new perspectives on vermicomposting research". In: CA Edwards (Ed.), "Earthworm Ecology", second ed, (pp: 401-424). CRC Press.
17. Garg, V.K. and V. Kaushik, 2005. Vermistabilisation of solid textile mill sludge spiked with poultry droppings by an epigeic earthworm *Eisenia fetida*. *Bioresour. Technol.*, 96: 1063-1071.
18. Walkley, A. and C.A. Black, 1934 An estimation of the Digtjareff method for determining soil organic matter and proposed modification of chromic acid titration method. *Soil Sci.*, 37: 29-38.
19. Jackson, M.L., 1973. *Soil Chemical Analysis*, Prentice Hall of India Pvt. Ltd., New Delhi, India.
20. Olsen, S.R., C.V. Cole, F.S. Watanabe and L.A. Dean, 1954. Estimation of available phosphorous in soils by extraction with sodium bicarbonate. *Circ. US Dep. Agric.*, pp: 939.
21. Simard, R.R., 1993. Ammonium acetate extractable elements. In: Martin, R., Carter, S., (Eds), *Soil sampling and methods of analysis*. (PP. 39-43.) Lewis Publisher, Florida, USA.
22. Ndegwa, P.M., S.A. Thompson and K.C. Das, 2000. Effects of stocking density and feeding rate on vermicomposting of biosolids. *Bioresource Technol.*, 71: 5-12.
23. Sonowal, P., K. Dhamodharan, M. Khwairkham and S. Kalandhad, 2013. Feasibility of vermicomposting of dewatered sludge using *P. excavatus* earthworms. *European Journal of Environmental Sciences*, 3(1): 17-26.
24. Loh, T.C., Y.C. Lee, J.B. Liang and D. Tan, 2005. Vermicomposting of cattle and manures by *Eisenia fetida* and their growth and reproduction performance. *Bioresource Technol.*, 96: 111-114.
25. Bhardwaj, A., 2010. Management of kitchen waste material through vermicomposting. *Asian J. Exp. Biol. Sci.*, 1: 175-177.
26. Gaur, A.C. and G. Singh, 1995. Recycling of rural and urban waste through conventional and vermicomposting. In: Tandon, H.L.S. (Ed.), *Recycling of Crop, Animal Human and Industrial Waste in Agriculture*. Fertiliser Development and Consultation Organization, (pp: 31-49). New Delhi.
27. Kaviraj and S. Sharma, 2003. Municipal solid waste management through vermicomposting employing exotic and local species of earthworms. *Bioresource Technol.*, 90: 311-316.
28. Bhatnagar, T., 1975. "Lombriens et humification: un aspect nouveau de l'incorporation microbienne d'azote induite par les vers de terre, " in *Biodegradation et Humification*, (pp: 169-182). Pierron, Satreguemines: France.
29. Shuster, W.D., S. Subler and E.L. McCoy, 2001. Deep burrowing earthworm additions changed the distribution of soil organic carbon in a chisel-tilled soil. *Soil Biol. Biochem.*, 33: 983-996.
30. Kale, R.D. and K. Bano, 1992. Niche divergence- a limiting factor for recommendation of earthworms for biotechnology. *Proc. Nat. Sem. Org. Fmg.*, Bangalore, pp: 42-44.
31. Tripathi, G. and P. Bhardwaj, 2005. Management of organic wastes by earthworms *Eisenia fetida* and *Lampito mauritii* in arid environment. *J. Appl. Biosci.*, 32(2): 150-159.
32. Edwards, C.A., J. Dominguez and E.F. Neuhauser, 1998. "Growth and reproduction of *Perionyx excavatus* (Perr.) (Megascolecidae) as factors in organic waste management". *Biol. Fertil. Soils.*, 27: 155-161.