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Microbial and Nutrient Enhancement of *Gliricidia sepium* and *Leucaena leucocephala* Leaf Materials Using *Eisenia fetida*

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ABSTRACT

Pre-decomposed (15 days) leaves of *Gliricidia sepium* and *Leucaena leucocephala* were mixed with cowdung (1: 1: 2) and subjected to vermicomposting (60 days) using an exotic earthworm, *Eisenia fetida* Savigny. The same substrate was kept without earthworms as control. Worm-worked (vermicompost) and worm-unworked substrates were separately analyzed for electrical conductivity (EC), pH, organic carbon, NPK and colony-forming units (CFU) of bacteria, fungi and actinomycetes. Activity of *E. fetida* increased the EC, NPK and microbial CFU. The initial bacterial population of $188 \pm 1 \times 10^6$ CFU g^{-1} increased gradually during vermicomposting and it was $281.67 \pm 1.15 \times 10^6$ CFU g^{-1} on the 45th day. A similar trend was also observed for fungi and actinomycetes. The results reveal that the leaf leaves of *G. sepium* and *L. leucocephala* can be converted into microbial- and nutrient-rich vermicompost using *E. fetida*.

Keywords: exotic earthworm, microbial colony forming units, pre-decomposition, vermicomposting

Abbreviations: CFU, colony forming units; **EC**, electrical conductivity; **NPK**, nitrogen, phosphorus and potassium

INTRODUCTION

Vermicomposting is an ecofriendly and inexpensive technology in which earthworms are used as bioreacters to covert organic materials into valuable compost (Karmegam and Daniel 2000a). Earthworms live in a close relationship with microorganisms and together bring out composting and enhance soil fertility. The alimentary canal of earthworms possess a great number of microorganisms which also process organic wastes. Major sources of nutrients for earthworms are microorganisms present in the soil and in organic substrates (Nagarathinam *et al*. 2000). Also, earthworms promote microbial activity during the decomposition of organic matter (Szabo *et al* 1990; Daniel and Anderson 1992; Kristufek *et al.* 1993; Aira *et al*. 2007; Parthasarathi 2007). The present study was undertaken to find out the efficiency of *Eisenia fetida* Savigny, an exotic compost worm, in the decomposition of *Gliricidia sepium* (Fabaceae) and *Leucaena leucocephala* (Mimosae) leaves, which are widely available in this part of South India and also the changes in the microbial colony forming units (CFU) of bacteria, fungi and actinomycetes during vermicomposting. These two plants are also used as green manure by farmers in this area.

MATERIALS AND METHODS

The fresh leaves from *G. sepium* and *L. leucocephala* plants were collected from in and around Gandhigram Campus, the leaf samples were dried and individually subjected to pre-decomposition for 15 days in rectangular draining cement tanks by sprinkling water, regular mixing and turning of the leaf materials (Daniel and Karmegam 1999). *E. fetida* 8-9 weeks of age was mass multiplied in the Department of Biology, Gandhigram Rural University, Gandhigram and used for vermicomposting. Vermibeds were prepared in rectangular troughs of 45 cm × 35 cm × 15 cm size using pre-decomposed leaf materials along with cowdung (1: 1; equal portions of two species' leaves: cowdung). Water was sprinkled over the vermibeds to a moisture content of 70-80% and kept for 24 h for stabilization. The next day 30 pre-weighed *E. fetida* were introduced into each trough with four replicates. The same set-up without earthworms was maintained as the control. Moisture content of 70-85% was maintained throughout the period of study and all the set-ups were kept for 60 days at an average room temperature of 27 ± 2°C.

After 60 days the worm-un-worked (WUW) and worm-worked (WW) composts were analyzed for nutrient values such as electrical conductivity (EC), organic carbon (Walkley and Black method 1947), nitrogen (Microkjeldhal method), phosphorous (colorimetric method) and potassium (flame photometric method) and the total CFUs of bacteria, fungi and actinomycetes were enumerated using standard plate count method: bacteria (nutrient agar medium, $\times 10^{-6}$ g^{-1}), fungi (Martins Rose Bengal agar medium, $\times 10^{-4}$ g^{-1}) and actinomycetes (Kenknight agar medium, $\times 10^{-4}$) (Cappuccino 1999).

RESULTS AND DISCUSSION

EC showed a considerable increase in the WW compost when compared to the WUW compost (**Table 1**). The soluble salt level increased due to the mineralization activity of earthworm and microorganisms present in the organic substrate (Joshi and Kelkar 1952). The pH value showed a reduction in the WW vermicompost when compared to the WUW compost (**Table 1**). The reduction in pH towards neutrality is an important factor in retaining N, for it seems to promote nutrient availability for plants (Pramanik *et al*. 2007). The C/N ratio of the WW vermicompost showed highly significant reduction when compared to the WUW compost (**Table 1**). Because of the combined action of microorganisms and earthworms a large portion of the organic matter in the initial substrate was lost as CO_2 by the end of the vermicomposting period (Govindan 1988). The N content showed considerable increase in WW compost compared to the WUW compost (**Table 1**) due to the mineralization process by *E. fetida* along with microorganisms (Daniel and Karmegam 1999; Karmegam and Daniel 2000). The P and K value also increased in the WW vermicompost when compared to the WUW compost (**Table 1**). The rise in the level of P is probably due to the bacterial and fungal phosphatase activity of the earthworms (Krishnamoorthy

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Short Communication

Table 1 Physico-chemical characteristics of worm-un-worked and worm-worked composts from 0-60 days.

Day	Treatment	EC	pH	OC (%)	N (%)	P (%)	K (%)
0	Control	1.20	7.50	44.33	1.10	0.60	0.40
	Treatment	1.20	7.50	44.33	1.10	0.60	0.40
15	Control	1.28	7.49	44.20	1.12	0.63	0.42
	Treatment	1.50	7.30	44.00	1.81	0.67	0.46
30	Control	1.33	7.45	41.55	1.14	0.66	0.45
	Treatment	1.70	7.10	38.17	1.23	0.74	0.53
45	Control	1.43	7.44	39.51	1.16	0.69	0.48
	Treatment	1.90	7.00	34.12	1.29	0.82	0.61
60	Control	1.50	7.43	38.47	1.18	0.70	0.49
	Treatment	1.10	6.90	31.52	1.34	0.87	0.67

Table 2 Number of colony forming units of bacteria in worm unworked (WUW) and worm worked (WW) composts from 0-60 days (Values are mean ± standard deviation).

	Number of days (0-60 d)	Worm-un-worked compost (treatment)	Worm-worked compost (control)
	0	187.00 ± 1.00	188.00 ± 1.00
	15	201.00 ± 1.00	221.67 ± 1.53
	30	212.33 ± 0.58	252.33 ± 0.58
	45	221.67 ± 1.15	281.67 ± 1.15
	60	215.33 ± 0.58	264.33 ± 0.58
SED	T	D	TD
	0.26943	0.42601	0.60246
CD ($P < 0.05$)	0.56606	0.89502	1.26575
CD ($P < 0.01$)	0.77566	1.22643	1.73444

Values are mean ± standard error (n = 3); SD = Standard Deviation; T = treatment; D = days; TD = treatment and days.

Table 3 Number of colony forming units of fungi in worm unworked (WUW) and worm worked (WW) composts from 0-60 days (Values are mean ± standard deviation).

	Number of days (0-60 d)	Worm-un-worked compost (treatment)	Worm-worked compost (control)
	0	171.67 ± 1.15	172.33 ± 0.58
	15	181.67 ± 1.15	193.00 ± 1.00
	30	187.00 ± 1.00	231.67 ± 1.15
	45	202.00 ± 1.00	261.67 ± 1.15
	60	191.00 ± 1.00	242.00 ± 1.00
SED	T	D	TD
	0.34355	0.54320	0.76819
CD ($P < 0.05$)	0.72178	1.14123	1.61394
CD ($P < 0.01$)	0.98904	1.56381	2.21156

Values are mean ± standard error (n = 3); SD = Standard Deviation; T = treatment; D = days; TD = treatment and days.

1990). The increased levels of micro- and macronutrients in the vermicompost agree with the results of earlier studies by Parthasarathi (2007) and Daniel and Karmegam (1999).

Higher CFU values in WW compost than in WUW compost for all three microbes i.e. bacteria (**Table 2**), fungi (**Table 3**) and actinomycetes (**Table 4**), was observed on the 45th day. The bacterial flora in the vermibed increased 8.97, 15.94, 21.40 and 18.43% during 15th, 30th, 45th and 60th days of vermicomposting, respectively. A similar trend was also observed for fungal and actinomycete flora. The inter-relationship of microorganisms with earthworms by their presence inside or outside the body of earthworms and in the environment has been established already by Tiwari and Mishra (1993) and Heijnen and Marinissen (1995). Prakash *et al.* (2008) reported that the mineralization activity of the earthworm, *Perionyx ceylanensis* attributed to the increase of nutrients in vermicasts. The casts produced by *P. ceylanensis* harbor a higher fungal population and rich nutrient contents in leaf litter and cowdung mixture (1: 1) than the WUW control. The vermicompost could be a definite source of plant growth regulators produced by interactions between microorganisms and earthworms, which could contribute significantly to increased plant growth, flowering

Table 4 Number of colony forming units of actinomycetes in worm unworked (WUW) and worm worked (WW) composts from 0-60 days (Values are mean ± standard deviation).

	Number of days (0-60 d)	Worm-un-worked compost (treatment)	Worm-worked compost (control)
	0	181.00 ± 1.00	182.00 ± 1.00
	15	189.33 ± 0.58	211.67 ± 1.15
	30	201.67 ± 1.53	243.00 ± 1.00
	45	209.33 ± 0.58	271.33 ± 1.53
	60	188.67 ± 0.58	251.33 ± 1.53
SD	T	D	TD
	0.29565	0.46746	0.66109
CD ($P < 0.05$)	0.62114	0.98211	1.38891
CD ($P < 0.01$)	0.85114	1.34577	1.90321

Values are mean ± standard error (n = 3); SD = Standard Deviation; T = treatment; D = days; TD = treatment and days.

and yields (Arancon and Edwards 2009). The present study also reveals that the vermicompost derived from leaf materials of *G. sepium* and *L. leucocephala* were rich in nutrients and microbial contents than the WUW substrates. This clearly indicates that the activity of the earthworm, *E. fetida* improved the nutrient and microbial contents of the vermibed mixtures. Thus the vermicomposting of predecomposed leaf materials of *G. sepium* and *L. leucocephala* in combination with of cowdung can give microbial- and nutrient-rich vermicompost.

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