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RESEARCH ARTICLE

Comparative Study of Municipal Solid Waste Using by *Lampito Mauritii* and *Eudrilus Eugeniae* Earthworms Enhance for Microbial Enzyme Activity

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ABSTRACT

Background: Industries development and population growth, migration of people from villages to cities, which release 1000 tons of municipal solid wastes (MSW) every day. India enormous quantities of disposable organic waste materials like MSW. MSW cannot be eaten directly by earthworms due to its bad odor, harmful insects, heat generates thermopile bacteria to organic wastes, etc. Hence, the organic wastes such as cattle waste – cow dung (CD) and agroindustrial waste-pressmud with clay soil high nutritive content were mixed in equal ratio and used as bedding material (BM). The experimental BMs were prepared on dry weight basis by mixing the MSW + BM in the following percentage: T_1 – 20% BM + 80% MSW, T_2 – 40% BM + 60% MSW, T_3 – 60% BM + 40% MSW, and T_4 – 80% BM + 20% MSW, and C_1 control (BM alone) were also maintained separately. **Results:** The microbial enzyme activities such as cellulase, amylase, protease, and phosphatase were in the samples of (initial day), 15, 30, 45, and 60 days. The enzyme activity in the vermicompost has increased more than initial worm unworked compost. The maximum level of enzyme activity was observed in the vermicompost of T_7 and it could be due to adequate moisture, higher fungal biomass, species-specific activity of earthworm (*Eudrilus eugeniae*) in terms of higher palatability, selective predation of microbes, and suitable environment, in the gut of *E. eugeniae*. **Conclusion:** The vermicompost obtained from T_7 by *E. eugeniae*, on the basis of content of NPK, highest microbial population and enzyme activity was selected for the field application. The unutilized enormously available MSW can be vermicomposted along with any organic additives such as industrial Sludge's, CD, sheep dung, pig manure, kitchen wastes (vegetable wastes), flower waste, and agricultural waste to convert into the valuable organic manure. In addition to this, it may be recommended that the vermicompost from MSW is utilized for sustainable organic agriculture.

Keywords: Enzyme, cow dung, sludge, earthworm, agriculture

INTRODUCTION

The major constituents such as cellulose, hemicelluloses, lignin, starch, and different protein compounds present in waste are degraded by specific enzymes. The quantification of enzyme activity during composting can reflect the dynamics of composting process in terms of decomposition of organic matter and nitrogen transformation. In correlation with enzyme activity, the changes in microbial numbers and types also helpful in providing information about the maturity of the composted product.[1]

Various hydrolytic enzymes are believed to control the rate at which various substrates are degraded. It is necessary to determine the relationship between microbial population and enzymatic activity during organic matter decomposition; further, it is also important to quantify the amount of extracellular enzyme activity. Such knowledge could lead to a better understanding of how earthworms and

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microorganisms interact during organic matter decomposition.

Higher microbial population and higher dehydrogenase activity were observed in the vermicompost of fly ash with cow dung (CD) and leaf litter than the composts without earthworms.[2] Studies have shown that earthworm casts had enhanced carbohydrase, protease,[3] phosphatase,[4] dehydrogenase, and phosphatase.[5] According to the earthworm gut secretes various enzymes.[6] Although microorganisms are responsible for the biodegradation of organic waste, earthworms fragment and condition the substrate by increasing surface area for microbial activity.

Vermicomposting process during the passage through the worm's gut, organic matter undergoes physicochemical, and biochemical changes by the combined effect of earthworms and microbial activity.[7] Higher activity of cellulase, amylase, protease, peroxidase, urease, phosphatase, and dehydrogenase in the wormcasts has been reported.[8] The effects of vermicompost aging, especially changes in microbial biomass and enzyme activities, which can control the quality of the resulting vermicompost.[9] Vermicast contains enzymes such as amylase, lipase, cellulase, and chitinase, which continues to break down organic matter in the soil (to release the nutrients and make it available to the plant's roots) even after they have been excreted.[10] Most studies seem to indicate that the microbial compost of earthworm gut reflects that of ingested soil or plant residues possess a distinctive gut microflora.[11]

Decomposition and humification of biodegradable organic waste materials are predominantly carried out by microorganisms in the soil, but the recent study had shown that earthworms too have roles in humification.[12] Earthworms transform organic waste constituents into more useful forms by grinding and digesting with the help of aerobic and anaerobic microflora.[13]

The enhanced level of amylase activity on addition to the organic amendments promoted the recycling of nutrients in the soil ecosystem.[14] During vermicomposting of municipal solid waste (MSW), Paul *et al*.[15] reported that the organic substrates could initiate the proliferation of the microorganisms and the earthworm species and acted as a medium for the rapid microbial colonization whereby increases the microbial activity.

There is no detailed reports are available on the comparative aspect of MSW vermicomposting using *Lampito mauritii* and *Eudrilus eugeniae.* Hence, investigation is needed to identify the potentiality of earthworms in relation to the enzymatic activity during vermicomposting of MSW with various combinations of bedding material (BM). Hence, it was intended to establish the activity of enzymes such as cellulase, amylase, protease, and phosphatase in the vermicompost of MSW during different days.

MATERIALS AND METHODS

Collection and maintenance of *L. mauritii*

L. mauritii were collected from the agricultural fields in Vaiyur, nearby village to Annamalai University. The worms were stocked in cement tank and CD was used as substrate to maintain the earthworms. Moisture content of 60–70% was continuously maintained by sprinkling the water. This stock culture was covered with moisture gunny bag and maintained at room temperature (27 ± 2°C) inside the animal house, Department of Zoology-DDE Wing, Annamalai University.

Collection of organic waste

MSW

MSW was collected from Sethiathope town Panchayat, Cuddalore District, Tamil Nadu. After removing the plastics, polythene, metal scraps, and glass pieces MSW was dried and brought using jute bags to the laboratory.

CD

CD is deemed as highly suitable natural feed for earthworms.[16] Hence, CD is selected for the present study for the biodegradation of MSW. Urine and straw free CD were collected from dairy yard at

the Faculty of Agriculture, Annamalai University. It was sun dried, powdered, and stored in jute bags.

Pressmud (PM)

The PM was collected from MRK Co-operative Sugar Mill, Sethiathope. The collected PM was cured for a month to remove the odor. Then, it was used for the preparation of BM.

Preparation of BM

The CD and 1-month-old cured PM were used for the preparation of BM and they were equally mixed on dry weight basis and kept as such for 15 days and used for the preparation of substrate for vermiculture.

Preparation of different experimental media – with BM and MSW

Combinations of BM and MSW in four proportions were prepared in the following order given below:

S. No.	Experimental proportions of BM+MSW	Weight of BM+MSW
C_1+C_2	BM alone (Control)	500 g CD+500 g PM+200 g soil
T_1 and T_5	20%+80% (BM+MSW)	200 g BM+800 g MSW+200 g soil
T_2 and T_6	40%+60% (BM+MSW)	400 g BM+600 g MSW+200 g soil
T_3 and T_7	60%+40% (BM+MSW)	600 g BM+400 g MSW+200 g soil
T_4 and T_8	80%+20% (BM+MSW)	800 g BM+200 g MSW+200 g soil

C_1, T_1– T_4–: Substrates used for *Lampito mauritii*, C2, T_5– T_8–: Substrates used for *Eudrilus eugeniae*, MSW: Municipal solid wastes, BM: Bedding material, CD: Cow dung

After the preparation of substrates in the above different proportions, water was sprinkled and kept as such for thermophilic composting for 15 days.

Inoculation of earthworms

After the completion of thermophilic composting 15 g of sexually mature, clitellate *L. mauritii* (approx. 65 days) were introduced in plastic troughs separately; containing 1 kg substrate + 200 g of soil. BM alone was used as control, separately for *L. mauritii* as C_1, respectively. Six replications in each experimental treatment have been maintained for 60 days.

Enzymes analysis

Cellulase and amylase enzyme activity methods were followed by Galstyan,[17] protease activity method was followed by Hoffmann and Teicher,[18] phosphatase activity.[19]

Statistical analysis

Cellulase, amylase, protease, and phosphatase activities in the substrates were tested statistically significant using the method of analysis of variance at 0.05% level.

RESULTS AND DISCUSSION

The enzyme activities in earthworm compost especially cellulase, amylase, protease, and phosphatase were significantly higher than the initial compost. The activities of enzymes are presented in Tables 1-8.

Cellulase activity

L. mauritii

Vermicompost of *L. mauritii* on the basis of the experiment shows gradually increased cellulase enzyme activities. Initially (0-day), the cellulase activities were recorded as 4.23 ± 0.30 (C_1), 3.57 ± 0.25 (T_1), 3.82 ± 0.40 (T_2), 4.10 ± 0.29 (T_3), and 4.19 ± 0.23 (T_4), respectively.

The maximum enzyme activity was observed in T_4 and it was, followed by C_1, T_3, T_2, and T_1 on 15th and 30th day. Enzyme activity increased progressively up to 60 days. On 60th day, the highest percentage of cellulase activity was observed in T_4 (48.69%) and it was followed by others and they could be marked in the following order C_1 (34.28%), T_3 (33.66%), T_2 (29.84%), and T_1 (29.41%), respectively. In the present study, the activities of cellulase enzyme were rich in the *L. mauritii*.

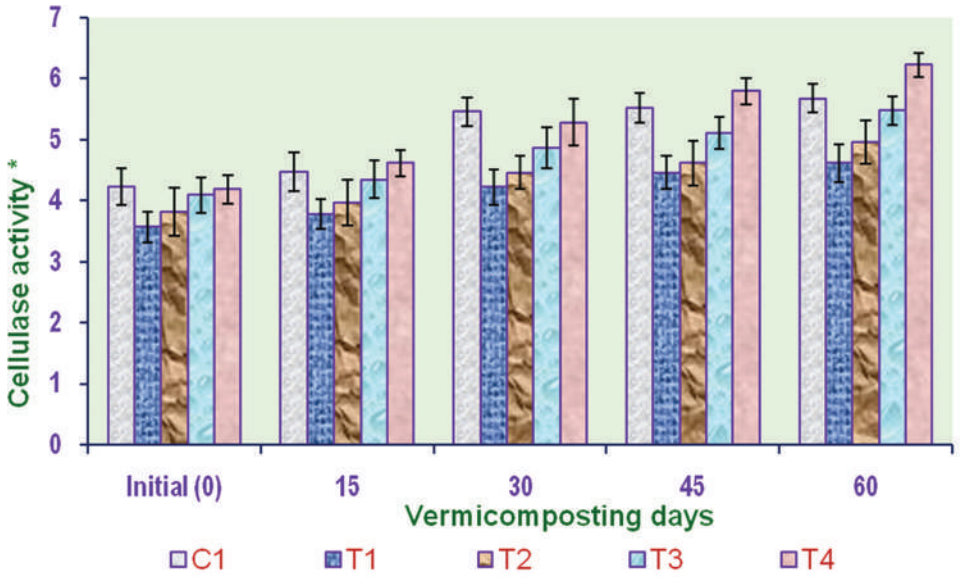
E. eugeniae

Initially, the cellulase enzyme activities are same as that of *L. mauritii*. Among the treatments (T_5 to T_8), the highest activity was observed in T_7 and followed by others. During vermicomposting from initial to

Table 1: Cellulase activity* in the vermicompost from MSW made by *L. mauritii* ($P<0.05$)

Substrate proportions	*L. mauritii*				
	Vermicomposting days				
	0 (Initial)	15	30	45	60
C_1	4.23±0.30	4.47±0.32	5.46±0.24	5.53±0.24	5.68±0.23 (34.28)
T_1	3.57±0.25	3.78±0.24	4.23±0.29	4.46±0.27	4.62±0.31 (29.41)
T_2	3.82±0.40	3.97±0.38	4.46±0.27	4.62±0.37	4.96±0.36 (29.84)
T_3	4.10±0.29	4.35±0.31	4.87±0.34	5.11±0.26	5.48±0.24 (33.66)
T_4	4.19±0.23	4.62±0.21	5.29±0.39	5.80±0.22	6.23±0.19 (48.69)
Analysis of variance	**Sum of square**	**Df**	**Mean of square**	**F-value**	***P* value**
Rows	4.28088	4	1.07022	32.92985	1.54E-07
Columns	6.99392	4	1.74848	53.79938	4.44E-09
Error	0.52	16	0.0325		

mg glucose/g ovendry substrates for 24 h incubation. C_1: Control (BM alone), T_1: (20% BM+80% MSW), T_2: (40% BM+60% MSW), T_3: (60% BM+40% MSW), T_4: (80% BM+20% MSW). Initial (0): Worm unworked substrates, mean±standard deviation of six observations. (±): Percent change of increase or decrease over the initial. Cellulase activity in the vermicompost from MSW made by *L. mauritii* ($P<0.05$). MSW: Municipal solid wastes, BM: Bedding material, *L. mauritii*: *Lampito mauritii*



*mg glucose/g ovendry substrates for 24 h incubation

60th day, the cellulase activity gradually increased. The highest cellulase activity was recorded on 60th day and the values were 5.88 ± 0.27 (C_2), 4.69 ± 0.31 (T_5), 5.11 ± 0.34 (T_6), 6.29 ± 0.36 (T_7), and 5.65 ± 0.22 (T_8), respectively. The cellulase activity of *E. eugeniae* could be ranked in the following way: $T_7 > C_2 > T_8 > T_6 > T_5$.

In the present study, the activities of cellulase enzyme were rich in *E. eugeniae* than the *L. mauritii*.

Amylase activity

Like cellulase activity, the activity of amylase in the vermicompost of *L. mauritii* and *E. eugeniae* increased up to 60th day.

L. mauritii

The amylase activity in the substrate given to *L. mauritii* was low on the initial day and they were in C_1 (3.98 ± 0.21), T_1 (3.34 ± 0.31), T_2 (3.57 ± 0.36), T_3 (3.74 ± 0.21), and T_4 (3.86 ± 0.19), respectively. The higher value of amylase activity was recorded in T_4 (6.11 ± 0.37), followed by C_1 (5.82 ± 0.19), T_3 (5.34 ± 0.27), T_2 (4.89 ± 0.24), and T_1 (4.43 ± 0.26) on 60th day. The above results revealed that the highest value of amylase activity was found in T_4 and followed by others.

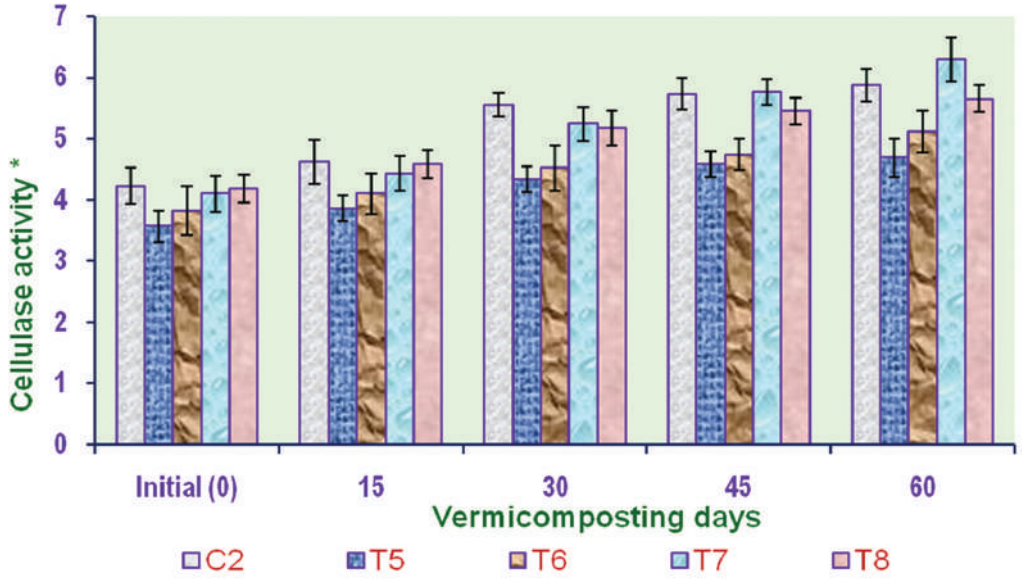
E. eugeniae

In *E. eugeniae* vermicompost, the amylase activity was rich in T_7. Amylase activity was low in initial

Table 2: Cellulase activity* in the vermicompost from MSW made by *E. eugeniae* ($P<0.05$)

Substrate proportions	*E. eugeniae*				
	Vermicomposting days				
	0 (Initial)	15	30	45	60
C_2	4.23±0.30	4.63±0.36	5.56±0.19	5.73±0.25	5.88±0.27 (39.01)
T_5	3.57±0.25	3.86±0.24	4.34±0.21	4.58±0.21	4.69±0.31 (31.37)
T_6	3.82±0.40	4.10±0.34	4.52±0.37	4.74±0.26	5.11±0.34 (33.77)
T_7	4.10±0.29	4.43±0.29	5.24±0.27	5.76±0.21	6.29±0.36 (53.41)
T_8	4.19±0.23	4.58±0.23	5.17±0.29	5.45±0.22	5.65±0.22 (34.85)
Analysis of variance	**Sum of square**	**Df**	**Mean of square**	**F-value**	***P* value**
Rows	4.038184	4	1.009546	26.54884	6.87E-07
Columns	8.275864	4	2.068966	54.40925	4.09E-09
Error	0.608416	16	0.038026		

mg glucose/g ovendry substrates for 24 h incubation. C_2: Control (BM alone), T_5: (20% BM+80% MSW), T_6: (40% BM+60% MSW), T_7: (60% BM+40% MSW), T_8: (80% BM+20% MSW). Initial (0): Worm unworked substrates, mean±standard deviation of six observations. (±): Percent change of increase or decrease over the initial. Cellulase activity in the vermicompost from MSW made by *E. eugeniae* ($P<0.05$). MSW: Municipal solid wastes, BM: Bedding material, *E. eugeniae*: *Eudrilus eugeniae*

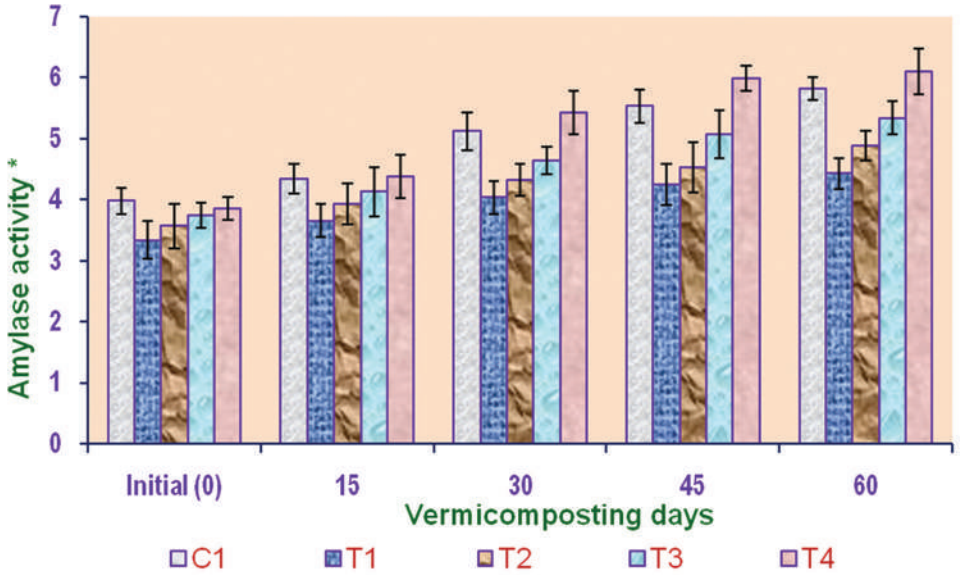


*mg glucose/g ovendry substrates for 24 h incubation

Table 3: Amylase activity* in the vermicompost from MSW made by *L. mauritii* ($P<0.05$)

Substrate proportions	*L. mauritii*				
	Vermicomposting days				
	0 (Initial)	15	30	45	60
C_1	3.98±0.21	4.34±0.24	5.13±0.31	5.54±0.27	5.82±0.19 (46.23)
T_1	3.34±0.31	3.66±0.28	4.04±0.27	4.25±0.34	4.43±0.26 (32.63)
T_2	3.57±0.36	3.93±0.34	4.32±0.26	4.53±0.41	4.89±0.24 (36.98)
T_3	3.74±0.21	4.13±0.40	4.64±0.22	5.08±0.39	5.34±0.27 (42.78)
T_4	3.86±0.19	4.38±0.36	5.43±0.35	5.99±0.21	6.11±0.37 (58.29)
Analysis of variance	**Sum of square**	**Df**	**Mean of square**	**F-value**	***P* value**
Rows	4.950744	4	1.237686	2 3.48394	1.58E-06
Columns	9.150264	4	2.287566	43.40444	2.14E-08
Error	0.843256	16	0.052704		

mg glucose/g ovendry substrates for 24 h incubation. Amylase activity in the vermicompost from MSW made by *L. mauritii* ($P<0.05$). MSW: Municipal solid wastes, *L. mauritii*: *Lampito mauritii*

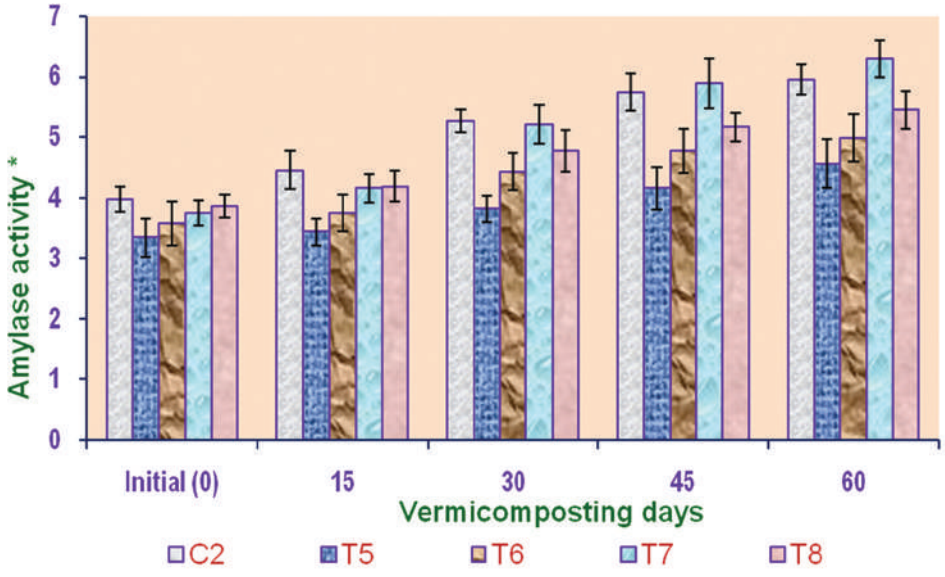


*mg glucose/g ovendry substrates for 24 h incubation

Table 4: Amylase activity* in the vermicompost from MSW made by *E. eugeniae* ($P<0.05$)

Substrate proportions	*E. eugeniae*				
	Vermicomposting days				
	0 (Initial)	15	30	45	60
C_2	3.98±0.21	4.46±0.31	5.27±0.19	5.74±0.31	5.95±0.25 (49.50)
T_5	3.34±0.31	3.44±0.22	3.82±0.22	4.16±0.35	4.56±0.40 (36.53)
T_6	3.57±0.36	3.75±0.31	4.43±0.31	4.77±0.36	4.99±0.39 (39.78)
T_7	3.74±0.21	4.16±0.24	5.21±0.33	5.89±0.41	6.29±0.31 (68.18)
T_8	3.86±0.19	4.19±0.26	4.78±0.34	5.17±0.24	5.45±0.32 (41.19)
Analysis of variance	**Sum of square**	**Df**	**Mean of square**	**F-value**	***P* value**
Rows	5.393784	4	1.348446	23.29486	1.67E-06
Columns	11.0061	4	2.751526	47.53353	1.1E-08
Error	0.926176	16	0.057886		

mg glucose/g ovendry substrates for 24 h incubation. Amylase activity in the vermicompost from MSW made by *E. eugeniae* ($P<0.05$). MSW: Municipal solid wastes, *E. eugeniae*: *Eudrilus eugeniae*

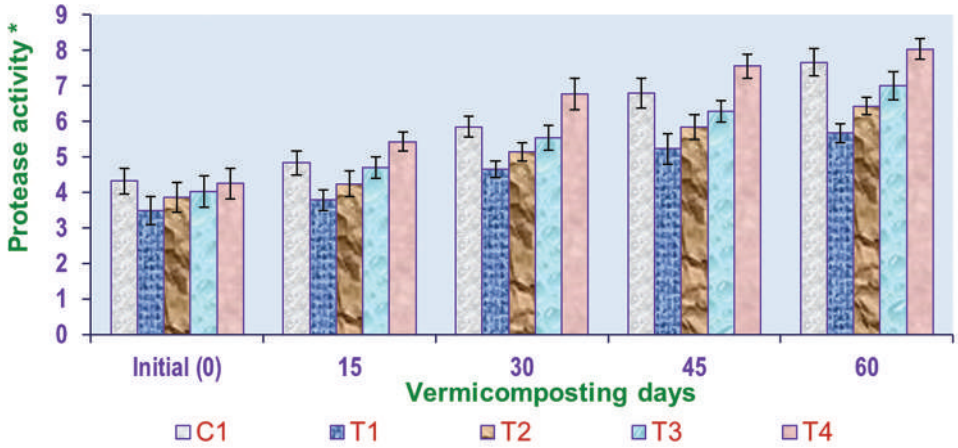


*mg glucose/g ovendry substrates for 24 h incubation

Table 5: Protease activity* in the vermicompost from MSW made by *L. mauritii* ($P<0.05$)

Substrate proportions	*L. mauritii*				
	Vermicomposting days				
	0 (Initial)	15	30	45	60
C_1	4.32±0.36	4.83±0.34	5.85±0.29	6.79±0.42	7.67±0.39 (77.55)
T_1	3.49±0.39	3.78±0.29	4.65±0.24	5.23±0.43	5.67±0.27 (62.46)
T_2	3.85±0.42	4.24±0.36	5.15±0.26	5.83±0.35	6.43±0.24 (67.01)
T_3	4.02±0.45	4.70±0.31	5.54±0.34	6.28±0.31	7.01±0.40 (74.38)
T_4	4.25±0.43	5.43±0.27	6.77±0.45	7.56±0.34	8.04±0.29 (89.18)
Analysis of variance	**Sum of square**	**Df**	**Mean of square**	**F-value**	***P* value**
Rows	10.08922	4	2.522306	14.0549	4.19E-05
Columns	28.20082	4	7.050206	39.28545	4.39E-08
Error	2.871376	16	0.179461		

mg glutamic acid/g ovendry substrates for 24 h incubation. Protease activity in the vermicompost from MSW made by *L. mauritii* ($P<0.05$). MSW: Municipal solid wastes, *L. mauritii*: *Lampito mauritii*



*mg glutamic acid/g oven dry substrates for 24 h incubation

compost, i.e., C_2 (3.98 ± 0.21), T_5 (3.34 ± 0.31), T_6 (3.57 ± 0.36), T_7 (3.74 ± 0.21), and T_8 (3.86 ± 0.19), respectively. The amylase activity gradually increased from initial day to 60th day. At the end of the experiment, the highest amylase activity was observed in T_7 (68.18% increase over initial) and the lowest activity was observed in T_5 (36.53% increase over initial) on 60th day.

Protease activity

Tables 5 and 6 represent protease activity in the vermicompost of *L. mauritii* and *E. eugeniae* in different experimental media.

The protease activity of *L. mauritii* was lowest in the initial day, having the values of 4.32 ± 0.36 (C_1), 3.49 ± 0.39 (T_1), 3.85 ± 0.42 (T_2), 4.02 ± 0.45 (T_3). and 4.25 ± 0.43 (T_4) and increased thereafter. From initial day to 60th day, the protease activity increased in all the treatments and control. During the experimental period, the maximum change in the protease activity was recorded on 60th day in T_4 (89.18%) followed by C_1 (77.55%), T_3 (74.38%), T_2 (67.01%), and T_1 (62.46%), respectively.

Phosphatase activity

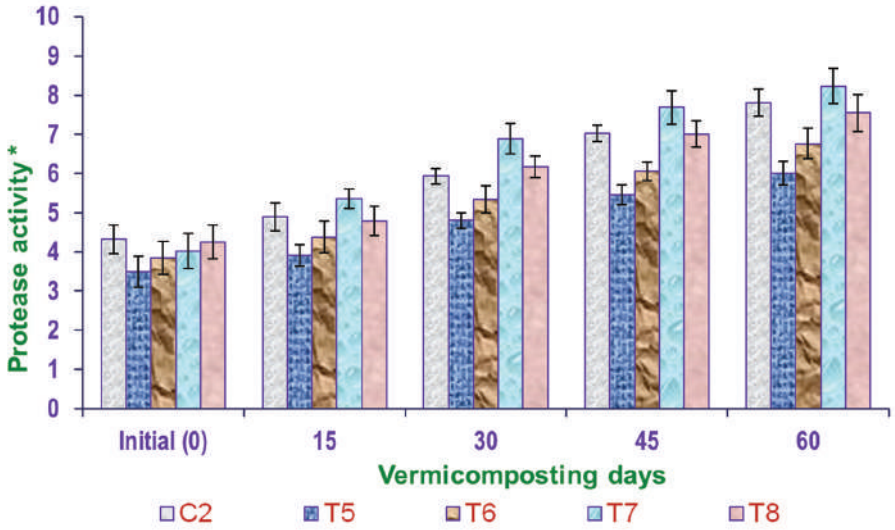
Tables 7 and 8 represent phosphatase activity in the vermicompost of *L. mauritii* and *E. eugeniae.*

The vermicompost of *L. mauritii* exhibited more activity of enzyme than the worm unworked (initial) substrates. In the vermicompost of *L. mauritii*, the protease activities were gradually increased from initial day to 60th day. On initial day, the values recorded are 2.67 ± 0.17 (C_1), 2.35 ± 0.15 (T_1), 2.41 ± 0.12 (T_2), 2.48 ± 0.14 (T_3), and 2.58 ± 0.21 (T_5), respectively. Among the following treatments, i.e., C_1, T_1, T_2, T_3, and T_4,

Table 6: Protease activity* in the vermicompost from MSW made by *E. eugeniae* (P<0.05)

Substrate proportions	*E. eugeniae*				
	Vermicomposting days				
	0 (Initial)	15	30	45	60
C_2	4.32±0.36	4.89±0.36	5.93±0.20	7.03±0.21	7.81±0.34 (80.79)
T_5	3.49±0.39	3.91±0.27	4.80±0.20	5.46±0.25	6.01±0.31 (72.21)
T_6	3.85±0.42	4.38±0.41	5.34±0.34	6.06±0.24	6.76±0.39 (75.58)
T_7	4.02±0.45	5.35±0.25	6.89±0.39	7.69±0.43	8.23±0.45 (104.73)
T_8	4.25±0.43	4.79±0.37	6.17±0.28	7.01±0.33	7.55±0.47 (77.65)
Analysis of variance	**Sum of square**	**df**	**Mean of square**	**F-value**	***P* value**
Rows	9.014936	4	2.253734	25.25362	9.66E-07
Columns	36.99286	4	9.248214	103.6284	3.17E-11
Error	1.427904	16	0.089244		

mg glutamic acid/g ovendry substrates for 24 h incubation. Protease activity in the vermicompost from MSW made by *E. eugeniae* (P<0.05). MSW: Municipal solid wastes, *E. eugeniae*: *Eudrilus eugeniae*



*mg glutamic acid/g ovendry substrates for 24 h incubation

the highest percentage change of phosphatase was recorded in T_4 (53.88% increased over initial) and the lowest phosphatase activity was observed in T_1 (35.75% increased over initial) on 60th day. The vermicompost, i.e., (*L. mauritii*), exhibited more enzyme activities, the highest enzyme activity was found in the vermicompost of the *L. mauritii*.

DISCUSSION

The present investigation shows higher enzyme activities (cellulase, amylase, protease, and phosphatase) in the worm worked vermicompost of both earthworms (*L. mauritii* and *E. eugeniae*) than the initial substrates (control). The worm casts have been shown to exhibit more enzymatic activity, microbial, and NPK contents in the PM vermicasts.[20]

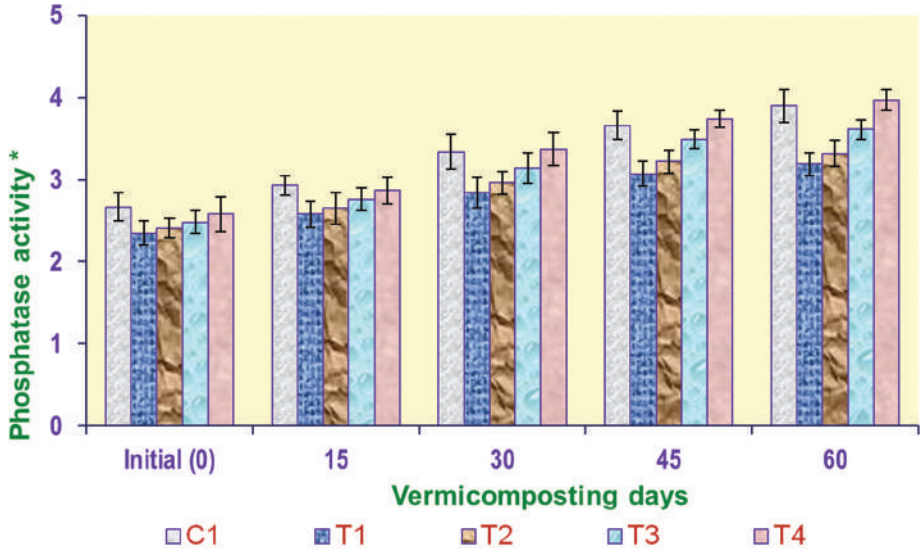
Several enzymatic activities have been measured to describe organic matter decomposition in two microbial driven processes, composting and vermicomposting.[21] Goyal *et al.*[22] have reported that the chemical and biological changes vary during composting of various organic wastes with particular reference to microflora and enzyme activities.

Aira *et al.*[23] suggested that the earthworms played a major role in enzyme activities: (i) Increase the availability of substrate and (ii) activate the microbial metabolism. Dominguez[24] pointed out that the extracellular enzyme activity increases during vermicomposting. Benitez *et al.*[25] revealed that there is a high correlation between protease and microbial biomass. Falling in line with the above observations in the present study, the activities of amylase, cellulase, protease, and phosphatase

Table 7: Phosphatase activity* in the vermicompost from MSW made by *L. mauritii* ($P<0.05$)

Substrate proportions	*L. mauritii*				
	Vermicomposting days				
	0 (Initial)	15	30	45	60
C_1	2.67±0.17	2.93±0.12	3.34±0.21	3.66±0.17	3.90±0.20 (46.07)
T_1	2.35±0.15	2.58±0.16	2.84±0.19	3.07±0.15	3.19±0.14 (35.75)
T_2	2.41±0.12	265±0.19	2.96±0.14	3.22±0.14	3.32±0.16 (37.76)
T_3	2.48±0.14	2.76±0.14	3.14±0.19	3.49±0.11	3.61±0.12 (45.56)
T_4	2.58±0.21	2.87±0.16	3.37±0.20	3.74±0.10	3.97±0.13 (53.88)
Analysis of variance	**Sum of square**	**df**	**Mean of square**	**F-value**	***P* value**
Rows	1.01476	4	0.25369	26.81004	6.43E-07
Columns	4.19944	4	1.04986	110.9495	1.88E-11
Error	0.1514	16	0.009462		

mg phenol/g ovendry substrates for 24 h incubation. Phosphatase activity in the vermicompost from MSW made by *L. mauritii* ($P<0.05$). MSW: Municipal solid wastes, *L. mauritii*: *Lampito mauritii*



*mg phenol/g ovendry substrates for 24 h incubation

are more in the vermicasts of *L. mauritii* and *E. eugeniae*, might be due to the higher microbial population in vermicasts than initial substrate.

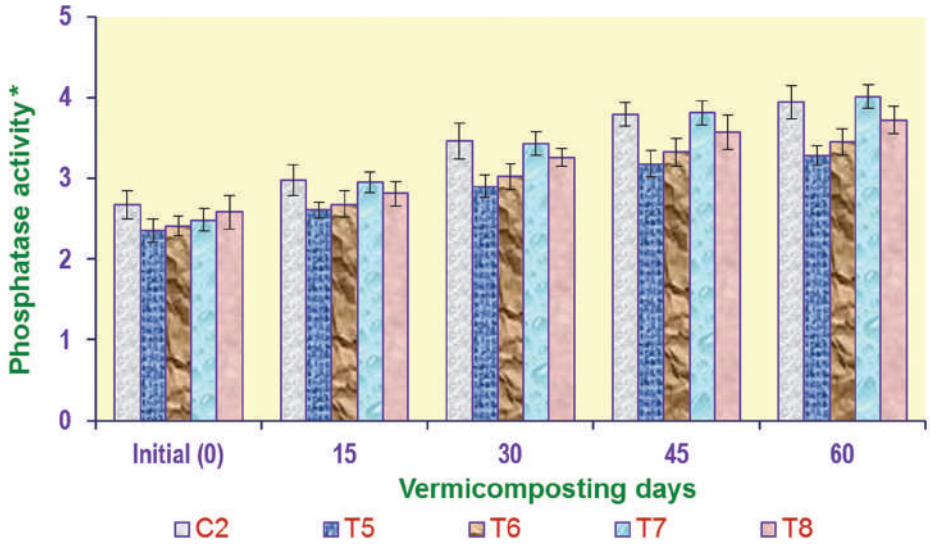
The microbes enter into worm guts, consume nitrogenous compounds the mucus, which increase their activity, which in turn enable them to contribute enzymes.[26] Earthworm bioreactors have an in-house supply of enzymes such as amylase, cellulase, nitrate reductase, and acid and alkaline phosphatases. The following enzymes such as amylase, cellulase, nitrate reductase, and acid and alkaline phosphatases *viz*., are biodegrade the complex biomolecules into simple compounds. The digestive enzymes of earthworms are responsible for the decomposition and humification of organic matter.[27]

Various hydrolytic enzymes are believed to control the rate at which various substrates are degraded. Important enzymes involved in the composting process include cellulases, which depolymerize cellulose and β-glucosidases, which hydrolyze glucoside and urease involved in N-mineralization, phosphatases, and arylsulphatase that remove phosphate and sulfate groups from organic compounds.[28] Cellulase activity was increased in the vermicasts of T_4 and T_7 made by *L. mauritii* and *E. eugeniae*. The activities were found more on 60th day. Cellulase has a major role in decomposition process.[29] It has been reported that the casts of *Martiodrilus* sp. had higher cellulolytic activity than the initial substrates.[30]

Table 8: Phosphatase activity* in the vermicompost from MSW made by *E. eugeniae* ($P<0.05$)

Substrate proportions	*E. eugeniae*				
	Vermicomposting days				
	0 (Initial)	15	30	45	60
C_2	2.67±0.17	2.98±0.19	3.46±0.22	3.79±0.14	3.94±0.20 (47.57)
T_5	2.35±0.15	2.61±0.10	2.90±0.14	3.18±0.16	3.28±0.12 (39.57)
T_6	2.41±0.12	2.68±0.16	3.02±0.16	3.32±0.17	3.45±0.16 (43.15)
T_7	2.48±0.14	2.95±0.13	3.43±0.14	3.81±0.15	4.01±0.14 (61.69)
T_8	2.58±0.21	2.81±0.15	3.26±0.11	3.57±0.21	3.72±0.17 (44.19)
Analysis of variance	**Sum of square**	**df**	**Mean of square**	**F-value**	***P* value**
Rows	0.977856	4	0.244464	27.09043	5.98E-07
Columns	4.879136	4	1.219784	135.1711	4.08E-12
Error	0.144384	16	0.009024		

mg phenol/g ovendry substrates for 24 h incubation. Phosphatase activity in the vermicompost from MSW made by *E. eugeniae* ($P<0.05$). MSW: Municipal solid wastes, *E. eugeniae*: *Eudrilus eugeniae*



*mg phenol/g ovendry substrates for 24 h incubation

The extracellular enzyme activity was increased in vermicasts with the rate of pig slurry application indicates the substrate availability for their action. Falling in line with the above study, in the present investigation also the cellulase activity increased with increased percentage of BM, but in T_7 the highest activity was found due to the species-specific action of earthworms.

Amylase activity was found in the vermicompost of *Paulownia elongata,* which is able to degrade starch, a root substrate, and up to glucose.[31] Enzyme activity was enhanced in the PM vermicompost of *E. eugeniae* than worm unworked PM.[32] In support of the above studies, in the present study also it was observed that the less percentage of MSW was found to have higher enzyme activity.

Protease is the enzyme which catalyzes the dissociation of proteins. Protease activity is linked to N cycle because it catalyzes the hydrolysis of protein N which can increase the pool of available dissolved organic N.[33] The increase in protease activity in the vermicompost of *E. eugeniae* than *L. mauritii* may be due to casting as described.[4] Moreover, the substrate availability increases the activity of enzymes.[9] Tiwari *et al*.[4] suggested that higher enzyme activity in earthworm casts was due to increased bacterial and fungal biomass. Earthworms processed pig manure was found to have increased microbial biomass as well as higher protease activity.[34]

Phosphatase activity measurement provides an index for the availability of phosphate in the soil.

Satchell and Martein[35] suggested that increased phosphatase activities in the vermicompost are due to higher microbial population. Jayakumar *et al*.[36] observed higher amylase, cellulase, invertase, phosphatase, and protease activity in the vermicasts of *E. eugeniae* than activity found in the vermicasts of *L. mauritii* and *Perionyx ceylanensis.*

Pramanik *et al*.[26] found greater phosphatase activity in the vermicompost from CD and suggested that the substrate had higher N which in turn resulted the higher microbial activity. In the same way in our study, the increased enzyme activity was found in the substrates with higher percentage of BM which enhances the microbial population than BM due to the palatability of earthworm and suitability for microbial propagation. The activity of amylase was high in the treatment where glucose and acetic acid were available.[37] Glucose is a ready energy resource for the microorganisms. In the same way during biodegradation of MSW, the breakdown of organic material occurs which increases the glucose level which in turn increases the biomass of microorganisms. The production of enzymes was depended on microbial biomass.[38]

Comparative studies on the enzymes in gut of earthworms *E. eugeniae* and *Eisenia fetida* were made[39] and found that the activity of amylase and cellobiose was more in the casts of *E. eugeniae* as we found in our study. They added that the activity of cellulase and phosphatase was more in the vermicasts of *E. fetida*. On the other hand, Jayakumar *et al*.[36] observed the activities of amylase, cellulase, invertase, phosphatase, and protease in the vermicompost of *E. eugeniae, L. mauritii*, and *P. ceylanensis*. They reported higher activities of all enzymes in the casts of *E. eugeniae*. This recent observation supports our present findings.

The survey of the above-mentioned four enzymes seems to be more in the casts of *E. eugeniae* than *L. mauritii* due to higher feeding rate and prolific breeding ability of *E. eugeniae* as suggested.[40] Due to the variation in feeding activity of the earthworms on PM, there occurred variation in the change of activities of enzymes. Especially in case of *E. eugeniae*, its voracious feeding habit and gut transit time, it shows maximum activity of the enzymes in the present study.

CONCLUSION

The unutilized enormously available MSW can be vermicomposted along with any organic additives such as industrial Sludge's, CD, sheep dung, pig manure, kitchen wastes (vegetable wastes), flower waste, and agricultural waste to convert into the valuable organic manure. In addition to this, it may be recommended that the vermicompost from MSW is utilized for sustainable organic agriculture.

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