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Research Article

SCREENING OF MICROBIAL POPULATION FROM *EISENIA FETIDA* AND *LAMPITO MAURITII* VERMICOMPOST

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ABSTRACT

Microorganisms are essential part of biodiversity and play a significant role in structuring and functioning of the ecosystem on the environment. An attempt was made for vermicomposting of Municipal solid waste (MSW) was mixed with Elephant dung (ED) using *Eisenia fetida* and *Lampito mauritii* and analyzed microbial population such as bacterial, fungal and actinomycetes in the vermicomposts. In the present examines the high number of microbial populations found in T_2 and control than other treatment. This T_2 shows suitable medium for microbial population.

Keywords: *Eisenia fetida, Lampito mauritii*, Municipal solid waste, Elephant dung, Microbial population.

INTRODUCTION

Municipal solid waste management is one of the most important environmental problems of Indian cities. But in most of cities, MSW is generated by human and animal activities that are discarded as useless or unwanted waste. Organic matters are outstanding resource of plant that have available nutrients and adding this to soil could maintain increasing microbial populations and high microbial activities (Norman and Clive, 2005). Earthworms which are known to improve the soil structure and fertility are reported to harbour a cocktail of micro organisms (Marinissen and Bok, 1988). There are various types of micro organisms in the vermicompost. Among these types of microorganisms, bacteria, actinomycetes and fungi are predominant in vermicompost. Large number of earthworms in an organic soil not only helps to decompose organic material in the soil by ingestion, disintegration and transport, but their waste products may also stimulate microbial decomposition. Fungi play an important role in the degradation of organic wastes. Most fungi are saprophytes and active producers of various hydrolytic enzymes. Further, fungi have the ability to predominate over the bacteria. Fungi require less nitrogen than bacteria per unit mass of protoplasm. They continue the decomposition process after bacteria and actinomycetes essentially end up the process. Actinomycetes possess both the characters of fungi and bacteria and they are of great importance in the decomposition of organic matter and the liberation of nutrients from them. They also reduce even the more resistant compounds such as cellulose, chitin and phospholipids to simpler form. Various researchers by comparative analysis between earthworm casts and soils have reported that passage of soil or any other organic matter through the worm's gut usually resulted in increased level of microbial populations (Dash *et al.*, 1979; Tiwari *et al.*, 1989) microbial biomass (Lavelle and Martin, 1992) microbial activity (Mulongoyn, 1986) microbial respiration and nitrification (Parle, 1963).

The vermicomposts are coated with muco-polysaccharides and are enriched with nutrients which act as important substrate for free living beneficial microbes. So, celluloytic, nitrifying and nitrogen fixing micro

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organisms are found to establish on wormcast (Satchell, 1983; Kale *et al.,* 1988). During the composts, total microbial activity and biomass, total number of cultural actinomycetes and the presence of other microorganisms have been recommended to improve the suppression of a variety of plant diseases (Noble and Coventry, 2005; Perez-Piqueres *et al*., 2006). The extent of the diversity of microorganisms in soil is seen to be critical to the maintenance of soil health and quality, as a wide range of microorganisms are involved in important soil functions (Hedrick *et al*., 2000).

Haritha Devi *et al.* (2009) reported that the enhanced microbial activity accelerated the decomposition process leading to humification, thus oxidizing unstable organic matter to stable form. *Streptomyces* are abundant in soil and help in the degradation of complex molecules to simple molecules for plant growth and development (Petrosyan *et al.,* 2003; Ding *et al.,* 2004). Hence it can be safely assumed that soil material associated with earthworm burrows may provide a substantially different environment to soil microflora. Casting of earthworms has been shown to have enhanced nutrients status and microbial composition and activity with respect to the present soil (Aira *et al.,* 2003). Earthworms act as a bio reactor and promote the growth of microorganisms (Curry and Schmidt, 2007). In contrast to numerous studies that have analyzed the microbial population of the composting processes, but the microbiological characterization of MSW and ED vermicompost is still in its infancy. The main aim of the present study is to examine and compare microbial characterization in MSW mixed with ED wastes and their respective compost and vermicomposts across different time intervals (at 0, 15^{th}, 30^{th}, 45^{th} and 60 days) for a period of 60^{th} days.

MATERIALS AND METHODS

Earthworm collection and maintenance

E. fetida and L. mauritii were obtained from the stock culture maintained in the Department of Zoology, Annamalai University, Tamil Nadu, India stocked in plastic containers and cow dung was used as substrate to maintain the adult earthworms.

Collection of organic wastes

Municipal solid waste

Municipal solid waste collected from Sirkali Municipality, Nagapattinam (District), Tamil Nadu, India, after removing polythene covers, glass pieces, scraps, clothes and metals. MSW was air dried and brought by using jute bags to the vermi-biotechnology lab.

Collection of Elephant Dung

Elephant Dung collected from Sri Abirami Temple Thirukadayure Nagapattinam district, Tamil Nadu, India, after the waste was dried and used to bedding materials before 10 days precomposting

Preparation of the experimental media

In the present study, 10 proportions and controls of MSW mixed with ED were prepared in the following order below:

Table 1. Preparation of the experimental media.

Treatment	MSW+ED Proportion	Weight of MSW+ED/ Kg
C1,C2	100% ED	1000g
T1,T6	10%+90%	200g +800g
T2,T7	20% + 80%	300g +700g
T3,T8	30% + 70%	400g +600g
T4,T9	40% + 60%	500g +500g
T5,T10	50% + 50%	600g +400g

Inoculation of earthworm

The preclitellate *E. fetida* worms were weighed and inoculated at the rate of 15 g per Kg of each mixture after pre decomposition. The plastic troughs were covered with nylon mesh and maintained at the room temperature $27^{o}C \pm 2^{o}C$ with 60-70% of moisture, the medium without MSW were treated as control. Six replicates were maintained in the each combination. The substrate named as C_1, T_1 to T_5 were inoculated with *E. fetida* and Substrate C_2, T_6, to T_{10} for *L. mauritii*.

Quantitative analysis of microbes

For the purpose of quantitative analysis of microbes, were estimated using the serial dilution and standard pour plate methods. The number of colony forming Units (CUF) was expressed as CFU g^1. The following samples were collected from controls and treatments. The microbial populations (bacteria, fungi and actinomycetes) were enumerated in the samples of 0, 15^{th}, 30^{th}, 45^{th} and 60 days by the following methods.

Statistical analysis

Microbial population of the all data is calculated standard deviation (SD), percentage increase or decrease over initial to final. Further, the data were analyzed statistically (significance of difference of 0.05 levels) by using two-way analysis of variance (ANOVA).

RESULTS

The total microbial populations (bacteria, fungi and actinomycetes) in different MSW and ED mixtures of worm unworked (compost) and worm worked (vermicomposts) of *E. fetida and L. mauritii* at different time intervals (0, 15^{th}, 30^{th}, 45^{th} and 60^{th} day) are presented in Tables. 2, 3, 4.

Total number of bacterial population (CFU × 10^6g-1)

The present investigation examines the bacterial population was found to be increased significantly (p<0.05) in controls and other treatments. Among these treatments the bacterial population high in T_2 and T_7 treatments. The bacterial population gradually increased in all treatments and controls (Table 2).

Eisenia fetida

Table 2 shows the bacterial population of *E. fetida* vermicompost was increased gradually from 0–60^{th} days. The maximum number of bacterial population was found in the vermicomposts obtained in T_2 (749.93±1.83 in CFU × 10^6) and it was followed by C_1 (648.34±2.33 in CFU × 10^6), T_1 (603.63±2.08 in CFU × 10^6), T_3 (519.57±1.95 in CFU × 10^6), T_4 (501.67±1.92 in CFU × 10^6) and T_5 (418.95±1.88 in CFU × 10^6) on 60^{th} day. The percent changes over the initial in bacterial population recorded on 60^{th} day were T_2 (92.05%), C_1 (55.39%), T_1 (53.43%), T_3 (49.45%), T_4 (42.29%) and T_5 (36.00%) respectively.

Lampito mauritii

In *L. mauritii* vermicompost, the bacterial population was increased up to 60 days. On 60^{th} day T_7 (593.60±2.05 in CFU×10^6) showed highest bacterial count and lowest count was observed in T_{10} (392.72±2.04 in CFU×10^6)

Total number of fungal population (CFU × 10^4 g-1)

The present investigation examines the number of Fungal population was increased significantly (p<0.05) in T_2 and T_7 which have more ED. The fungal population gradually increased in all treatments and represented in Table 3.

Eisenia fetida

The highest fungal colonies were observed in the T_2 vermicompost and the other treatments follow the T_2. The percent change in the population of fungi collected on 60^{th} days are ranked as (98.11%) in T_2, (92.70%) in C_1, (88.54%) in T_1, (79.52%) in T_3, (71.30%) in T_4 and (63.10%) in T_5.

Lampito mauritii

Similar results were observed in *L. mauritii* vermicompost. The T_7 Showed increased fungal population 81.60% on 60^{th} day vermicompost, it was followed by T_6, C_2, T_8, T_9 and T_{10} produced from different MSW mixture.

Total number of Actinomycetes (CFU × 10^5g-1)

Table 4 shows the actinomycetes population in worm-unwoked (initial) and worm – worked (vermicomposts) produced from MSW mixed with ED used by *E. fetida* and *L. mauritii*

Eisenia fetida

The maximum number of actinomycetes was observed in the vermicompost T_2 (37.42± 1.82 in CFU × 10^5) followed by C_1 (27.92±1.76 in CFU × 10^5), T_1 (26.59±2.28 in CFU × 10^5), T_3 (24.78±1.83 in CFU × 10^5), T_4 (20.81±1.22 in CFU × 10^5) and T_5 (17.93±1.30 in CFU × 10^5) on 60^{th} day. The T_2 treatment shows the maximum (80.2%) percentage change over the initial on 60^{th} day.

Lampito mauritii

In different MSW mixture, the actinomycetes population was maximum in T_7 (34.76±1.58 in CFU×10^5) the efficiency of other treatments were found to be ranked in the following order i.e,) C_2 (32.43±2.28 in CFU×10^5)>T_6 (31.23±2.05 in CFU×10^5) T_8 (24.28±2.23 in CFU×10^5)>T_9 (22.1±1.87 in CFU×10^5)> T_{10} (20.26±1.73 in CFU×10^5) on 60^{th} day vermicomposts.

Table 2. Bacterial population (CFU × 10^6 g^{-1}) in the vermicompost from MSW mixed with ED by *E. fetida and L. mauritii* ($p< 0.05$).

Substrate Proportions	*E. fetida*					Substrate proportions	*L. mauritii*				
	Vermicomposting days						Vermicomposting days				
	0	15	30	45	60		0	15	30	45	60
C_1	417.46±1.77	468.48±2.12	486.74±2.21	520.78±2.25	648.34±2.33 (55.39)	C_2	338.55±2.09	431.64±2.12	476.92±2.42	499.63±2.16	518.71±2.19 (53.25)
T_1	393.77±2.53	456.64±2.12	479.58±2.17	566.59±2.48	603.63±2.08 (53.43)	T_6	331.73±2.07	415.46±2.17	442.71±2.12	491.80±2.20	510.83±1.76 (54.07)
T_2	390.42±2.32	464.97±1.91	599.71±2.16	632.87±2.16	749.93±1.83 (92.37)	T_7	321.87±1.76	458.60±2.05	481.20±1.83	524.19±2.41	593.60±2.05 (84.73)
T_3	361.11±11.84	457.61±2.10	466.93±1.99	472.51±2.02	519.57±1.95 (49.45)	T_8	312.51±2.03	341.90±2.12	371.63±2.04	405.51±1.87	468.61±1.87 (50.54)
T_4	351.07±2.22	421.58±1.98	437.38±1.78	481.66±2.09	501.67±1.92 (42.29)	T_9	292.86±2.17	329.09±1.89	368.89±2.22	395.88±2.19	414.42±2.32 (41.78)
T_5	307.50±1.95	331.84±2.11	366.80±1.78	406.57±1.96	418.95±1.88 (36.70)	T_{10}	287.69±1.88	297.19±2.06	314.88±1.83	352.00±1.82	392.72±2.04 (36.48)

Note: C_1 & C_2 – Control, T_1 & T_6 – (10% MSW + 90% ED), T_2 & T_7 – (20% MSW + 80% ED), T_3 & T_8 – (30% MSW + 70% ED), T_4 & T_9 – (40% MSW + 60% ED), T_5 & T_{10} – (50% MSW + 50% ED), Initial (0) – Worm unworked substrates, Mean ± SD of six observations, (+/–) – Percent change of increase or decrease over the initial.

Table 3. Fungal population (CFU × 10^4 g^{-1}) in the vermicompost from MSW mixed with ED by *E. fetida and L. mauritii* ($p< 0.05$).

Substrate proportions	*E. fetida*					Substrate proportions	*L. mauritii*				
	Vermicomposting days						Vermicomposting days				
	0	15	30	45	60		0	15	30	45	60
C_1	145.17±2.35	156.23±1.86	242.48±1.74	256.94±2.16	279.75±1.96 (92.70)	C_2	138.17±2.35	166.64±1.76	175.18±2.26	184.04±1.34	214.85±1.84 (55.68)
T_1	131.43±1.43	172.48±1.58	191.69±1.19	214.30±1.09	247.58±1.27 (88.37)	T_6	128.47±1.43	159.42±1.88	172.29±2.48	196.91±2.09	228.40±1.82 (77.78)
T_2	133.14±2.26	207.67±2.06	238.05±1.04	258.23±1.28	312.30±2.43 (98.1)	T_7	132.32±1.26	197.21±1.02	206.44±1.45	228.48±1.46	239.54±1.51 (81.60)
T_3	127.27±1.18	153.81±2.23	177.97±1.76	206.37±2.34	228.43±1.25 (79.52)	T_8	127.25±1.56	139.86±2.28	153.93±1.62	166.22±2.22	190.87±1.72 (49.60)
T_4	115.56±2.10	135.12±1.35	143.28±1.82	165.01±2.58	197.29±2.14 (71.30)	T_9	106.53±2.15	118.37±2.23	137.61±1.66	149.65±1.86	155.08±2.25 (46.26)
T_5	103.19±2.40	113.31±1.42	127.46±1.38	161.62±1.60	168.86±1.58 (63.10)	T_{10}	101.92±2.40	106.56±1.66	118.34±1.92	121.83±2.31	136.78±2.16 (34.65)

Note: C_1 & C_2 – Control, T_1 & T_6 – (10% MSW + 90% ED), T_2 & T_7 – (20% MSW + 80% ED), T_3 & T_8 – (30% MSW + 70% ED), T_4 & T_9 – (40% MSW + 60% ED), T_5 & T_{10} – (50% MSW + 50% ED), Initial (0) – Worm unworked substrates, Mean ± SD of six observations, (+/–) – Percent change of increase or decrease over the initial.

Table 4. Actinomycetes (CFU × 10^5 g^{-1}) in the vermicompost from MSW mixed with ED by *E. fetida and L. mauritii*.

Substrate proportions	*E. fetida*					Substrate proportions	*L. mauritii*				
	Vermicomposting days						Vermicomposting days				
	0	15	30	45	60		0	15	30	45	60
C_1	14.15±1.75	18.37±2.12	21.48±1.83	24.23±2.35	27.92±1.76 (92.85)	C_2	20.18±1.70	22.24±2.14	25.46±2.24	29.95±1.59	32.43±2.28 (60.5)
T_1	15.54±2.03	16.98±1.87	19.22±1.83	21.46±2.12	26.59±2.28 (73.33)	T_6	21.56±1.87	23.83±1.93	27.28±2.24	28.55±1.81	31.23±2.05 (55.76)
T_2	17.31±1.73	21.66±2.20	26.72±2.28	29.38±2.42	37.42±1.82 (94.11)	T_7	20.34±1.73	24.68±2.22	29.09±1.82	31.84±2.27	34.76±1.58 (70.08)
T_3	14.84±2.38	17.56±1.87	19.19±2.17	22.52±1.54	24.78±1.83 (71.42)	T_8	16.86±2.12	18.42±1.64	21.65±1.63	23.72±2.03	24.28±2.32 (50.13)
T_4	12.68±2.29	15.11±1.70	17.95±1.83	19.13±2.05	20.81±2.22 (66.00)	T_9	15.69±2.29	17.23±1.72	19.07±2.12	21.48±1.65	22.15±1.87 (46.66)
T_5	11.78±2.32	13.42±1.82	16.05±2.17	16.99±1.63	17.93±2.30 (54.54)	T_{10}	19.44±2.04	15.36±2.27	17.82±1.79	19.68±2.12	20.26±1.73 (42.85)

C_1 & C_2 – Control, T_1 & T_6 – (10% MSW + 90% ED), T_2 & T_7 – (20% MSW + 80% ED), T_3 & T_8 – (30% MSW + 70% ED), T_4 & T_9 – (40% MSW + 60% ED), T_5 & T_{10} – (50% MSW + 50% ED), Initial (0) – Worm unworked substrates, Mean ± SD of six observations, (+/–) – Percent change of increase or decrease over the initial.

DISCUSSION

The enhanced microbial population was observed in all treatments and controls vermicomposts over the initial. The highest microbial population was observed in the vermicomposts of T_2 and T_7 it may be due to the availability of optimum minerals for the multiplication of microbial groups and the low mineral from MSW concentration was preferable suited for earthworm and microbes significant growth was observed. Microorganism provided a source of nutrition for earthworms, of which fungi and protozoa consitiute important compounds. During vermicomposting process, when organic matter passes through the worm's gut, it undergoes physical, chemical and biochemical changes by the combined effect of earthworms and microbial activities. In the present study the changes in the microbial communities of MSW are in consistence with that of earlier reports. Kumar and Singh (2001) reported that there was a significant increase in the population of bacteria in vermicompost by the 2^{nd} week and maximum numbers was found between 45 to 60 days. Organic matter changes in the soil resulted from the incursion of earthworms powerfully modify the microbial communities (Bohlen *et al.*, 2004). The present study shows that the bacteria, fungi and actinomycetes were more in the vermicompost of T_2 it may be due to the availability of optimum minerals for the multiplication of microbial groups. In the finding of Pizl and Novakova (2004) the density of microfungi was higher in the earthworm gut and vermicompost than in fresh substrate. Similar to our present findings Rajesh Banu *et al.* (2005) reported that the bacterial population gradually increased up to the 30^{th} day in 20, 50 and 75 percent concentration of petrochemical sludge, whereas in 100 percent petrochemical sludge decline the population of microbes was observed from the 15^{th} day and confirmed the fact that at 100 percent concentration survival rate of earthworm was very low and it was due to the higher concentrations of petrochemical sludge which is to be toxic to the earthworm.

Earthworm has been found to cause changes in the population of microbes in different organic wastes during vermicomposting (Lores *et al.*, 2006) likewise these microbes have been found to be more efficient metabolically (Aira *et al.*, 2007). The present investigation is similar to the above results in improved populations of microbes in the vermicompost of *E. fetida* is also comparable with the reports of Parthasarathi *et al.* (2007) the improvement of microbial population, microbial activity and nutrient contents in the vermicompost at 31˚C and 60 to 70 percent moisture during vermicomposting of sugar industrial wastes. Kavitha *et al.* (2011) reported that the total number of microbial population and microbial activity were found to have increased in the vermicomposts obtained from banana waste and cow dung than worm unworked natural composts. According to Gomez - Brandon *et al.* (2013) reported that the impact of the worm *E. fetida* on the microbial biomass, structure and microbial activity through vermicompost, using continuous feeding vertical reactors that they are designed to processing the higher quantity of waste. Yang *et al.* (2014) results showed the increase soil microbial communities like bacteria, fungi and actinomycetes in food waste vermicompost. According to Ravindran *et al.* (2015) shows, that the microbial population and activity was increase to significant levels in vermicompost product derived from tannery fermented waste mixed with cowdung and leaf litter compared to control mixture by the earthworm *E. eugeniae* can utilize these waste mixture through the gut and can digest it with enzyme activity to produce a nutrient rich manure. In the present study vermicomposts of *E. fetida* is rich in microbial communities and diversity, particularly bacteria, fungi and actinomycetes in different concentrations of MSW and ED mixture. From the forgoing discussion It can be concluded that the vermicompost of *E. fetida* possess higher microbial communities in all MSW with ED treatments than vermicompost of *L. mauritii.* This difference may be due to the type of worms castings.

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