Growth Assessment of Microorganisms in Vermicomposting of Municipal Wastes Materials in Different Days

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Abstract— *In India over population, migrate village to town, various industries development, agricultural and municipal wastes have release to dumping of waste materials caused a serious causes to the environment. India enormous quantities of disposable organic wastes materials like municipal solid waste (MSW) rich in plant nutrients were presented.*

The macro and micro nutrients are available more in MSW, it is not properly decomposed (or) vermicomposting. MSW can't be eaten directly by earthworms due to it bad smell, heavy metals, insects; water leachate to organic wastes etc. So the organic wastes such as dairy farm waste – cowdung (CD) and sugar industrial waste - pressmud (PM) with clay soil high nutritive content were mixed in equal ratio and used as bedding material (BM). The experimental bedding materials were prepared on dry weight basis by mixing the MSW + BM in the following percentage: T1 – 20% BM + 80% MSW, T2 – 40% BM + 60% MSW, T3 – 60% BM + 40% MSW, T4 – 80% BM + 20% MSW, C1 Control (BM alone) were also maintained separately. The microbial populations (bacteria, fungi and actinomycetes) were enumerated in the samples of 0 (Initial day), 15, 30, 45 and 60 days. The quantity of microbial population in the worm worked compost (vermicomposts) has significantly increased than worm unworked mixture. Microbial population was observed more in the vermicomposts of E. eugeniae than the L. mauritii. It could be due to the higher feeding rate, prolific breeding ability, suitable environment and multiplication of microbes while passing through the gut of worms and optimal moisture and activity of microbes.

Keywords— Bedding materials, Municipal solid waste, Lampito mauritii, Eudrilus eugeniae, Microorganisms.

I. INTRODUCTION

Vermicomposting process, when organic matter passes through the worms gut it undergoes physical, chemical and biochemical changes by the combined effect of earthworm and microbial enzymatic activities. The role of microbial activity in the gut as well as in the casts is very essential for the degradation of organic waste and release of nutrients to plants (James, 1991). Vermicomposts have large particulate surface areas that provide many micro sites for microbial activity and for the strong retention of nutrients (Shi-wei and Fu-Zhen, 1991).

Vermicomposts are rich in microbial populations and diversity, particularly fungi, bacteria and actinomycetes (Edwards, 1998). Garcia-Gil et al. (2000) reported that the increase of microbial biomass in this long-term experiment with the organic amendments is mainly due to the microbial biomass contained in the organic residues and the addition of substrate-C, which stimulates the indigenous soil micro-biota, as confirmed by present analysis.

Dominguez (2004) suggested that vermicomposting is a combined operation of earthworm and microorganisms in which the earthworm fragments and homogenize the ingested material through muscular action of their foregut and also adds mucus and enzymes to ingested material and thereby increases the surface area for microbial action while, microorganisms perform the biochemical degradation of waste material providing some extra – cellular enzymes required for organic waste decomposition with the worm's gut. Moreover, this biological mutuality caused C loss in the form of CO2 from the substrates during the decomposition of organic waste.

Tripathi and Bhardwaj (2004b) reported that various organisms are involved in the composting process which includes microorganisms like bacteria, fungi and worms.Suthar (2008b) stated that the microbial population increases during vermicomposting of cowdung.

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. During the passage through the gut of earthworms the surviving microorganisms are voided along with cast. Unfortunately, the comparative potentiality of Lampito mauritii and Eudrilus eugeniae for municipal solid waste (MSW) management has not been carried out. Many aspects of earthworm – microbial interaction require further study including selection, multiplication, feeding (Review: Curry and Schmidt, 2007). The objective of the present study was to test the role of L. mauritii and E. eugeniae on the population kinetics of bacteria, fungi and actinomycetes during vermicomposting of MSW.

II. MATERIALS AND METHODS

Collection and maintenance of *Lampito mauritii* and *Eudrilus eugeniae*

Lampito mauritii were collected from the agricultural fields in Sivapuri, nearby village to Annamalai University. The worms were stocked in cement tank and cow dung 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± 2^0c) inside the animal house, Department of Zoology - DDE Wing, Annamalai University.

The breeding stock of *Eudrilus eugeniae* was obtained from the culture maintained Department of Zoology Wing- DDE, Annamalai University. *Eudrilus eugeniae* were maintained in a separate cement tank. Cowdung (CD) was used as substrate to maintain the adult worms; moisture content of 60 – 70% was continuously maintained by sprinkling water on the stock culture of the cement tank. This stock culture in the cement tank were covered with gunny bag and maintained at room temperature (27± 2°) inside the animal house.

Collection of organic waste

Municipal solid 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 by using jute bags to the laboratory.

Cowdung

Cowdung is deemed as highly suitable natural feed for earthworms (Hatanaka *et al.*, 1983; Lee, 1985). Hence, cowdung (CD) is selected for the present study for the biodegradation of municipal solid waste (MSW). Urine and straw free cowdung was collected from dairy yard at the Faculty of Agriculture, Annamalai University. It was sun dried, powdered and stored in jute bags.

Pressmud

The pressmud was collected from M.R.K Co-operative Sugar Mill, Sethiathope. The collected pressmud was cured for a month to remove the odour. Then it was used for the preparation of Bedding Material (BM).

Preparation of Bedding Material (BM)

The cow dung and one month old cured pressmud was used for the preparation of bedding material 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.

Combinations of bedding material (BM) and municipal solid waste (MSW) in four proportions were prepared in the following order given below:

PREPARATION OF DIFFERENT EXPERIMENTAL MEDIA – WITH BEDDING MATERIAL (BM) AND MUNICIPAL SOLID WASTE (MSW)

S. No.	Experimental Proportions of Bedding Material (BM) + Municipal Solid Waste (MSW)	Weight of Bedding Material (BM) + Municipal Solid Waste (MSW)
$C_1 + C_2$	BM alone (Control)	500g CD + 500g PM + 200g soil
T_1 & T_5	20% + 80% (BM + MSW)	200g BM + 800g MSW + 200g soil
T_2 & T_6	40% + 60% (BM + MSW)	400g BM + 600g MSW + 200g soil
T_3 & T_7	60% + 40% (BM + MSW)	600g BM +400g MSW+ 200g soil
T_4 & T_8	80% + 20% (BM + MSW)	800g BM + 200g MSW+ 200g soil

C_1, T_1 – T_4 – Substrates used for *Lampito mauritii*
C2, T_5 – T_8 – Substrates used for *Eudrilus eugeniae*

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 fifteen grams of sexually mature, clitellate Eudrilus eugeniae (approx. 38 days old) and Lampito mauritii (approx. 65 days) were introduced in plastic troughs separately; containing 1 Kg substrate + 200 g of soil. Bedding material alone was used as control, separately for L. mauritii and E. eugeniae as C1 & C2 respectively. Six replications in each experimental treatment have been maintained for 60 days.

Quantitative analysis of microbes

For the purpose of quantitative analysis of microbes, the following samples were collected from controls and treatments (T1-T8). The microbial populations (bacteria, fungi and actinomycetes) were enumerated in the samples of 0, 15, 30, 45 and 60 days by the following methods.

III. STATISTICAL ANALYSIS

The data on various samples were computed and mean values with standard deviation (SD) were obtained and recorded. The statistical significance between treatments were analysed by using two- way analysis of variance (ANOVA).

IV. RESULTS AND DISCUSSION

The total microbial population (bacteria, fungi and actinomycetes) in different ratio of bedding material (BM) and municipal solid waste (MSW) mixtures of worm unworked compost (initial) and worm worked compost (vermicasts) of L. mauritii and E. eugeniae at different time intervals (0, 15, 30, 45 and 60 days) are represented in the Tables - 1 to 3.

TABLE: 1
BACTERIAL POPULATION (CFU × 10^6 G^{-1}) IN THE VERMICOMPOSTS FROM MSW MADE BY L. MAURITII AND E. EUGENIAE (P<0.05)

Substrate Proportions	*L. mauritii*					Substrate Proportions	*E. eugeniae*				
	Vermicomposting Days						Vermicomposting Days				
	0 (Initial)	15	30	45	60		0 (Initial)	15	30	45	60
C_1	353.4± 12.15	374.6± 11.69	412.4± 14.01	446.3±1 2.50	470.4± 13.54 (33.11)	C_2	353.4± 12.15	378.2± 12.04	417.7± 13.90	449.5± 12.52	470.7± 12.97 (33.19)
T_1	330.3± 14.29	354.4± 11.91	381.1± 12.01	403.6± 12.75	418.2± 12.58 (26.61)	T_5	330.3± 14.29	356.7± 12.13	385.7± 12.00	408.0± 12.71	425.8± 12.52 (28.91)
T_2	330.9± 13.24	356.8± 12.36	388.3± 12.90	404.9± 13.04	421.4± 13.97 (27.34)	T_6	330.9± 13.24	358.4± 11.93	392.2± 12.75	413.5± 13.64	432.7± 13.54 (30.76)
T_3	334.4 ± 11.09	364.2± 13.71	399.7± 13.25	416.1± 13.72	435.7± 12.98 (30.29)	T_7	334.4± 11.09	365.8± 13.5	409.3± 13.15	445.9± 13.72	477.1± 12.69 (42.67)
T_4	342.5 ± 12.01	378.± 12.51	418.1± 13.60	455.3± 12.79	475.7± 12.54 (38.89)	T_8	342.5± 12.01	370.4± 12.11	409.6± 13.54	435.0± 12.39	455.0± 12.47 (32.85)

C_1 & C_2 – Control (BM alone), T_1 & T_5 – (20% BM + 80% MSW), T_2 & T_6 – (40% BM + 60% MSW), T_3 & T_7 – (60% BM + 40% MSW), T_4 & T_8– (80% BM + 20% MSW)
Initial (0) – Worm unworked substrates, Mean ± SD of six observations.
(+/–) – Percent change of increase or decrease over the initial.
CFU – Colony forming unit

Bacterial population

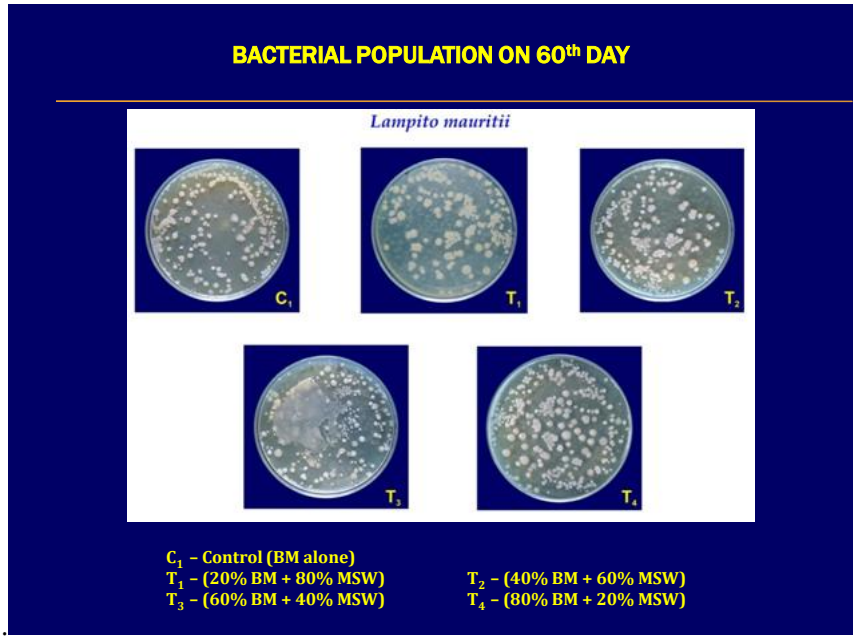
In the present analysis more bacterial population was found in control on 0 – day next to that the population was high in T_4 which had more BM. Among the treatments the initial content of bacterial population was least in T_1 which has most quantity of MSW. The population of bacteria increased in all the treatments and control.

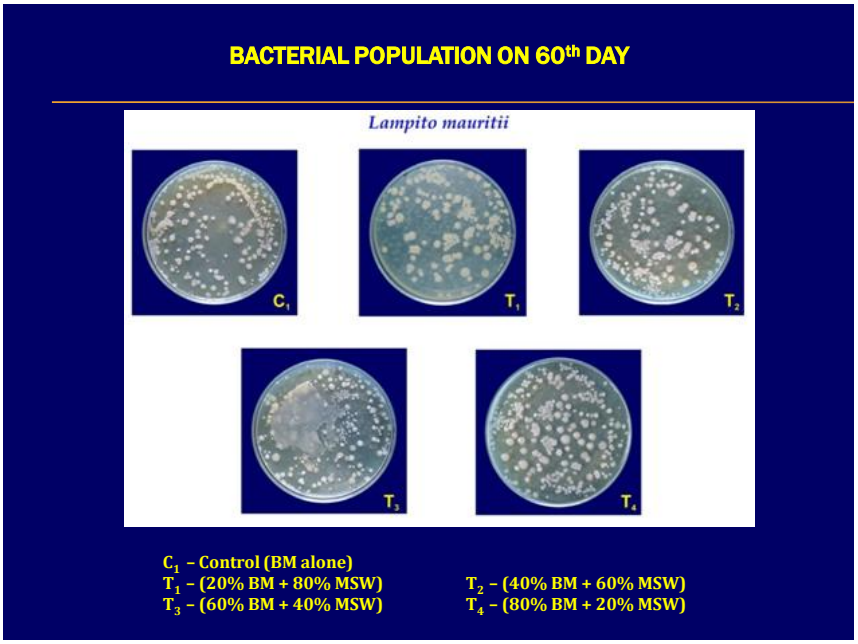
L. mauritii

The bacterial population was found most in the vermicomposts obtained in T_4 (475.7 ± 12.54 CFU × 10^6). As that of the nutrient content of vermicomposts the bacterial content can also be ranked as $T_4 > C_1 > T_3 > T_2 > T_1$. Highest percent change over the initial in the population was found in T_4 (38.89%). The percent changes in the population of bacteria over the initial were as follows: 38.89 in T_4, 33.11 in C_1, 30.29 in T_3, 27.34 in T_2 and 26.61 in T_1.

E. eugeniae

Among the *E. eugeniae* worked compost the bacterial population was highest in T_7 (477.1 ± 12.69 CFU × 10^6) and it was followed by C_2 (470.7 ± 12.97 CFU × 10^6), T_8 (455.0 ± 12.47 CFU × 10^6), T_6 (432.7 ± 13.54 CFU × 10^6) and T_5 (425.8 ± 12.52 CFU × 10^6) on 60th day. On the other days bacterial population was significantly increased (*i.e.*, 15, 30 and 45th day). The percent changes over the initial in bacterial population recorded on 60th day were T_7 in 42.67%, C_2 in 33.19%, T_8 in 32.85%, T_6 in 30.76% and T_5 in 28.91%.





Fungal population

On initial (0) – day highest fungal population was found in control whereas the least value was observed in T_1.

L. mauritii

Most number of fungal colonies were observed in the vermicompost obtained in T_4 treatment. Other vermicomposts made by *L. mauritii* show lesser population than the T_4. The population of fungi in the vermicomposts collected on 60th day are ranged as 221.3 ± 3.94 CFU × 10^4 in T_4, 217.1 ± 3.60 CFU × 10^4 in C_1, 196.6 ± 3.80 CFU × 10^4 in T_3, 184.3 ± 3.70 CFU × 10^4 in T_2 and 173.4 ± 3.40 CFU × 10^4 in T_1. The highest percentage change over the initial in the population of fungi could be ranked as $T_4 > C_1 > T_3 > T_2 > T_1$.

E. eugeniae

On the contrary highest fungal population in the vermicomposts made by *E. eu*geniae was found in T_7 (227.2 ± 3.81 CFU × 10^4). Secondly the more colonies of fungi were found in C_2 (221.6 ± 3.64 CFU × 10^4). These two (T_7 and C_2) were followed by T_8, T_6 and T_5. On the basis of fungal population the vermicomposts may be ranked as $T_7 > C_2 > T_8 > T_6 > T_5$.

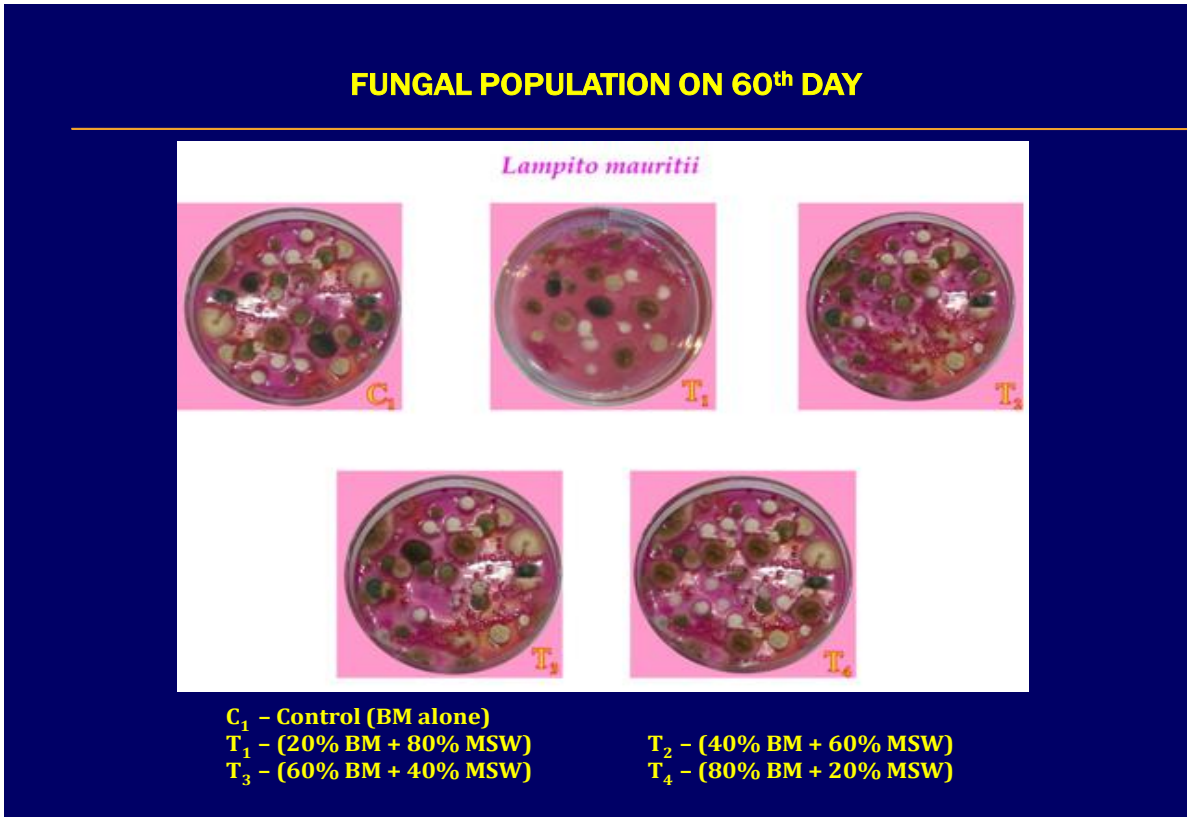
TABLE: 2 FUNGAL POPULATION (CFU × 10^4 G^{-1}) IN THE VERMICOMPOSTS FROM MSW MADE BY L. MAURITII AND E. EUGENIAE ($P<0.05$)

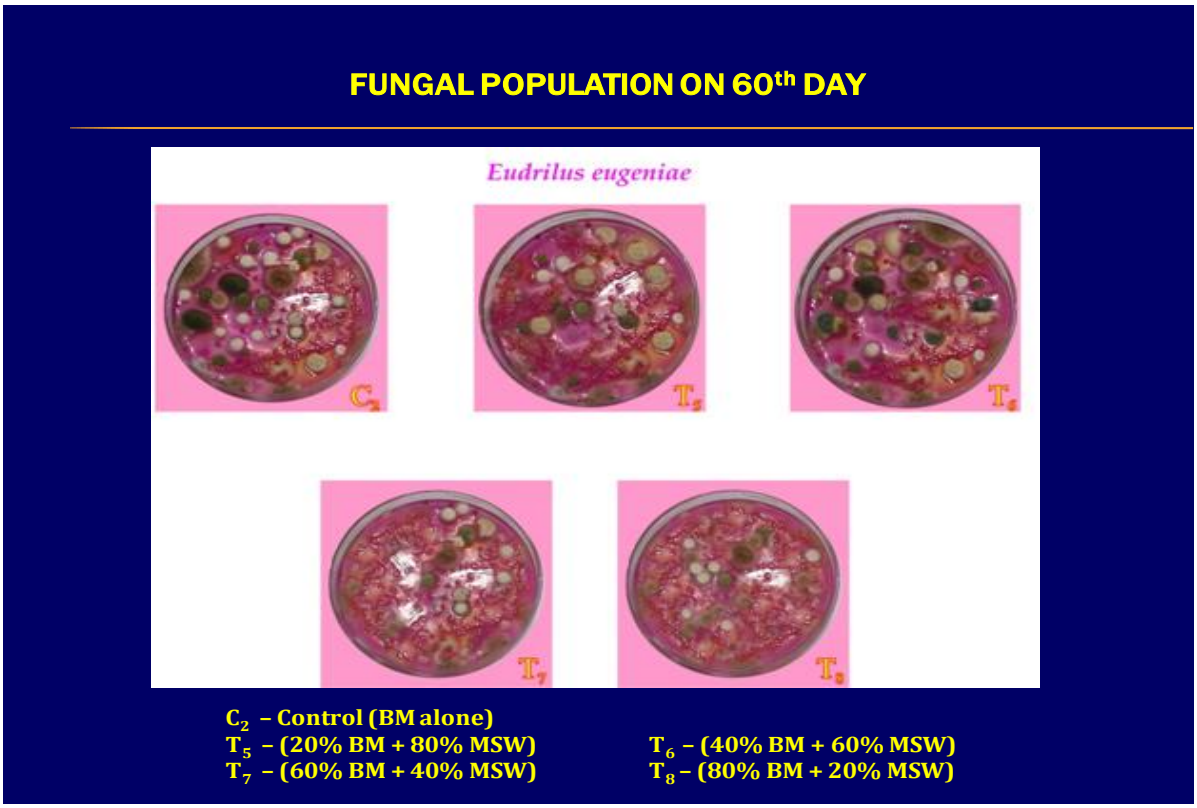
Substrate Proportions	*L. mauritii*					Substrate Proportions	*E. eugeniae*				
	Vermicomposting Days						Vermicomposting Days				
	0 (Initial)	15	30	45	60		0 (Initial)	15	30	45	60
C_1	135.2± 3.14	152.4±3 .26	183.6±4 .00	206.2±3 .31	217.1± 3.60 (60.58)	C_2	135.2± 3.14	155.3±3. 21	187.4± 3.71	212.2± 3.36	221.6±3. 64 (63.91)
T_1	118.8± 3.25	132.2±3 .21	154.1±2 .96	167.3±3 .10	173.4± 3.40 (45.96)	T_5	118.8± 3.25	133.4±3. 24	155.1± 3.87	169.6± 3.35	177.9±3. 24 (49.80)
T_2	120.6± 2.95	136.6±2 .97	162.3±3 .00	176.5±2 .61	184.3± 3.70 (52.82)	T_6	120.6± 2.95	139.1±2. 98	166.1± 3.41	177.8± 2.79	186.1±3. 79 (54.31)
T_3	125.6± 2.75	143.4±3 .28	173.0±3 .54	189.7±2 .97	196.6± 3.80 (56.53)	T_7	125.6± 2.75	144.7±3. 25	179.2± 2.78	205.6± 2.97	227.2±3. 81 (80.90)
T_4	131.7± 3.47	150.9±3 .90	183.2±3 .25	207.7±2 .78	221.3± 3.94 (68.03)	T_8	131.7± 3.47	146.2±3. 69	177.9± 2.98	193.4± 2.45	203.4±3. 24 (54.44)

C_1 & C_2 – Control (BM alone), T_1 & T_5 – (20% BM + 80% MSW), T_2 & T_6 – (40% BM + 60% MSW), T_3 & T_7 – (60% BM + 40% MSW), T_4 & T_8– (80% BM + 20% MSW)

Initial (0) – Worm unworked substrates, Mean ± SD of six observations.

(+/–) – Percent change of increase or decrease over the initial. CFU – Colony forming unitc





Actinomycetes population

The estimation of actinomycetes population in the worm worked compost made by *L. mauritii* and *E. eugeniae* substrates with BM and MSW different mixtures are presented in Table - 3.

L. mauritii

After the introduction of earthworm the actinomycetes population steadily increased upto 60th day of the experimental period. On 60th day highest population of actinomycetes was noted in T_4 (31.1 ± 0.78 CFU × 10^5) followed by C_1 (29.7 ± 0.77 CFU × 10^5), T_3 (28.0 ± 0.89 CFU × 10^5), T_2 (26.3 ± 0.99 CFU × 10^5) and T_1 (24.1 ± 0.97 CFU × 10^5).

On the other days (*i.e.*, 15, 30 and 45th day) actinomycetes population gradually increased. The percentage change over the initial on 60th day were T_4 in 35.80, C_1 in 28.02, T_3 in 26.71, T_2 in 25.24 and T_1 in 24.23 respectively.

E. eugeniae

Similar to the vermicomposts of *L. mauritii* the vermicomposts of *E. eugeniae*, show increasing trend in the population of actinomycetes in all treatments. Maximum number of actinomycetes colonies were found in the vermicomposts of T_7 (32.3 ± 0.56 CFU × 10^5). Next to that the higher population was found in C_2 (30.6 ± 0.76 CFU × 10^5). Thirdly the actinomycetes population was found in T_8 (29.7 ± 0.67 CFU × 10^5). Fourth place was attained by T_6 (26.9 ± 0.97 CFU × 10^5). Least population of actinomycetes was found in T_5 (24.8 ± 0.84 CFU × 10^5).

TABLE: 3

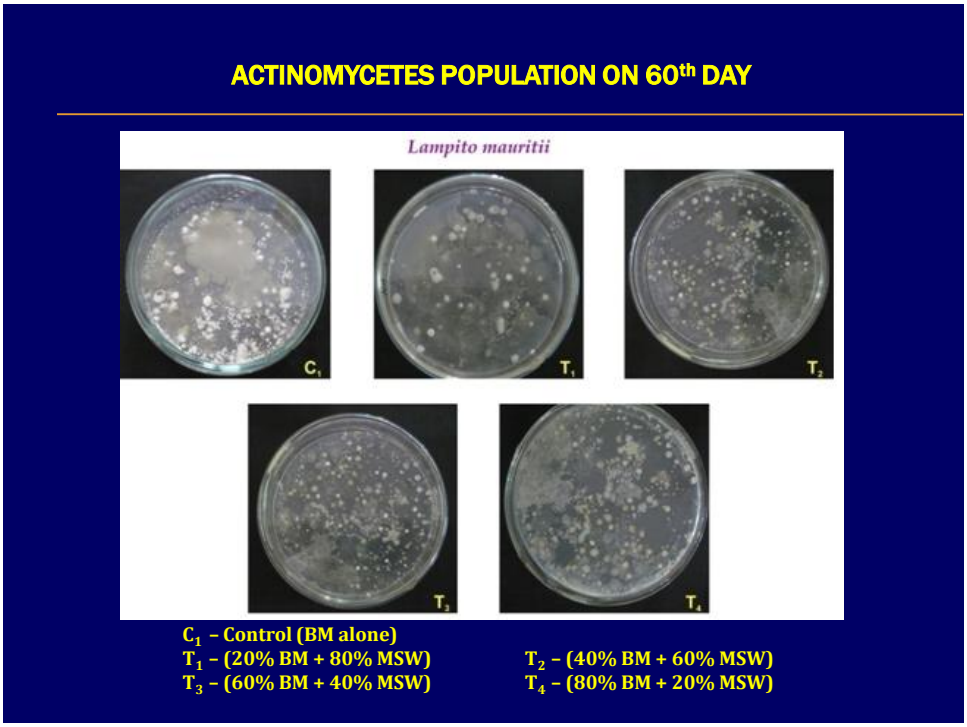
ACTINOMYCETES POPULATION (CFU × 10^5 g^{-1}) IN THE VERMICOMPOSTS FROM MSW MADE BY L. MAURITII AND E. EUGENIAE (P<0.05)

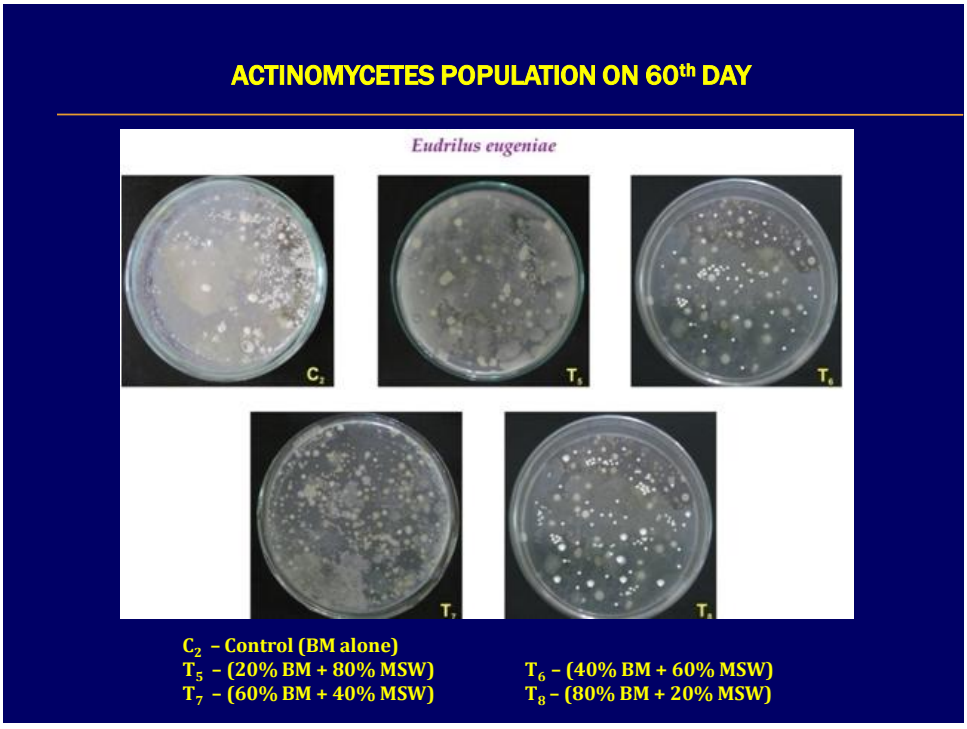
Substrate Proportions	*L. mauritii*					Substrate Proportions	*E. eugeniae*				
	Vermicomposting Days						Vermicomposting Days				
	0 (Initial)	15	30	45	60		0 (Initial)	15	30	45	60
C_1	23.2± 0.84	24.9± 0.99	26.8± 1.02	28.6± 0.94	29.7± 0.77 (28.02)	C_2	23.2± 0.81	25.2±0.87	27.9±1.01	29.6± 0.91	30.6± 0.76 (31.91)
T_1	19.4± 0.85	20.7± 0.97	22.5± 1.06	22.9± 0.87	24.1± 0.97 (24.23)	T_5	19.4± 0.87	21.1±0.65	23.2±1.04	24.1± 0.86	24.8± 0.84 (27.84)
T_2	21.0± 1.04	22.7± 1.07	24.2± 1.01	25.3± 0.74	26.3± 0.99 (25.24)	T_6	21.0± 1.03	22.6±0.94	24.9±1.08	26.1± 0.91	26.9± 0.97 (28.11)
T_3	22.1± 0.98	24.1± 0.93	26.0± 0.87	27.3± 0.89	28.0±0.89 (26.71)	T_7	22.1± 1.21	23.5±0.93	26.6±0.78	29.5± 0.98	32.3± 0.56 (46.15)
T_4	22.9± 1.12	25.1± 0.67	27.4± 0.65	29.3± 0.61	31.1± 0.78 (35.80)	T_8	22.9± 1.04	24.8±0.74	27.1±0.87	28.6± 0.63	29.7± 0.67 (26.69)

C_1 & C_2 – Control (BM alone), T_1 & T_5 – (20% BM + 80% MSW), T_2 & T_6 – (40% BM + 60% MSW), T_3 & T_7 – (60% BM + 40% MSW), T_4 & T_8– (80% BM + 20% MSW)

Initial (0) – Worm unworked substrates, Mean ± SD of six observations.

(+/–) – Percent change of increase or decrease over the initial. CFU – Colony forming unit





The vermicomposts of L. mauritii show highest population of bacteria, fungi actinomycetes in T4 whereas the maximum numbers of colonies of microorganisms (bacteria, fungi and actinomycetes) were found T7 (vermicompost made by E. eugeniae). The control, vermicomposts of both earthworms show second place in the population of microbes.

The increased microbial population was observed in all experimental and control vermicomposts more than initial (0-day). The maximum microbial population was noted in the worm worked composts of T4 (L. mauritii) and T7 (E. Eugeniae).

Vermicomposting process, when organic matter passes through the worms gut it undergoes physical, chemical and biochemical changes by the combined effect of earthworm and microbial enzymatic activities. The role of microbial activity in the gut as well as in the casts is very essential for the degradation of organic waste and release of nutrients to plants (James, 1991). The size of microbial population in worm casts is mainly depends on the type and quality of ingested soil and plant materials (Edwards and Bohlen, 1996).

Earthworms are mainly responsible for fragmentation and conditioning of the substrate, increasing surface area for microbial activity and significantly altering biological activity of the process (Dominguez, 2004). Munnoli et al. (2010) reported that the earthworms are considered as natural bioreactors which proliferate along with other microorganisms and provide required conditions for the biodegradation of wastes.

In the present study the population of bacteria, fungi and actinomycetes are found to increase from the initial, but highest increase among the vermicomposts of L. mauritii was found in T4 and in the vermicomposts of E. eugeniae was found in T7. It might be due to the optimal moisture, nutrient rich substrate concentration ideally suited for better feeding and multiplication of microbes as suggested by Parthasarathi (2002). John Paul et al. (2011); Ananthakrishnasamy (2013) mentioned that the higher CFU of microbes in the vermicomposts from MSW are due to higher proportion of cowdung which is supporting our present investigation in which we have observed higher microbial population in T4 by L. mauritii whereas in T7 by E. eugeniae due to the difference in palatability, gut enzymes and worm gut micro flora.

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