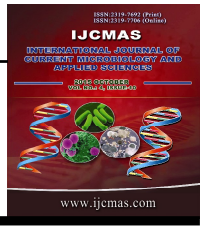
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 10 (2015) pp. 497-506**
http://www.ijcmas.com



Original Research Article

Microbial Population and Activity on Vermicompost of *Eudrilus eugeniae* and *Eisenia fetida* in Different Concentrations of Tea Waste with Cow Dung and Kitchen Waste Mixture

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Keywords

Vermicast, Bacteria, Fungi, Actinomycetes, TW, CD, KW, *Eudrilus eugeniae*, *Eisenia fetida*

A B S T R A C T

Microorganisms are essential part of biodiversity and play a significant role in structuring and functioning of the ecosystem on the environment. In the present investigation an attempt was analysed in the vermicompost microbial population such as Bacterial, fungal and Actionomycetes and its activities. From the present work was found to the total microbial population of vermicompost of *Eudrilus eugeniae* and *Eisenia fetida* from T1-T4 Among the different treatment T4 and T3 treatments were found to have significantly ($P<0.05$) higher microbial population than T2 and T1 treatments. In the present analysis the microbial activity of vermicompost obtained from all the treatments T1-T4 were increased significantly ($P<0.05$) and especially in T4 of *E. eugeniae* (7.32 ± 0.31) and *E. fetida* (6.92 ± 0.59) and T3 of *E. eugeniae* (6.60 ±0.05) and *E. fetida* (5.95 ± 0.63) treatments were found to be significantly ($P<0.05$) higher than T2, T1 treatments. The present study also the fungal population is found to be significantly higher in the fresh vermicast obtained from treatments T1-T4. The bacterial population was found to be significantly greater in the fresh vermicast obtained from the treatments T1-T4. The actinomycetes population was found to be significantly greater in the fresh casts obtained from the treatments T4.

Introduction

Microorganisms are essential part of biodiversity and play significant role in structuring and functioning of the ecosystem on the environment. The microorganisms (mainly bacteria, fungi, actinomycetes) are the primary decomposer of organic wastes. The microorganisms not only mineralize complex substances (organic waste) into plant available form but also can synthesis whole series of biologically active substances (Pramanik *et al.*, 2007). Microbes are responsible for the biochemical degradation of the organic matter. Earthworms are the important drivers of the process, conducting the substrate (organic wastes), producing congential conditions for the activities of microbes and altering biological activity (Aira *et al.*, 2002).

Earthworms prime the symbiotic gut microflora with secreted mucus and water to increase their degradation of ingested organic matter and the release of assimilable metabolites (Pramanik *et al.,* 2007). Thus the micro-organisms and earthworms act symbiotically to accelerate and enhance the decomposition of organic matter and as a consequence, mineralization and humification takes place resulting in the availability of nutrients for plants (Lee, 1985; Edwards and Bohlen, 1996; Chaioui *et al.,* 2003).

Many earthworm species have been found to predominantly utilize soil bacteria (Pedersen and Hendriksen, 1993) and soil fungi (Cooke and Luxton, 1980; Edwards and Bohlen, 1996). The total microbial load in the different regions of the gut of worms has also shown more intense colonization of microbes in the anterior part of the intestine than the other region. The presence of fungal propagules in the earthworm gut, and in cast material has been known for some time (Parle, 1963) and earthworm have been implicated in both the reduction and dispersal of soil borne animal and plant fungal disease and the spread of beneficial group such as mycorrhizal fungi (Gange, 1993).

The bacteria isolated from vermicasts and earthworm skin were endospore- forming Gram Bacilli (Munnoli, 2007). The bacterial counts in gut/vermicompost was higher than the surrounding soil (Edwards and Lofty, 1977; Edwards and Bohlen, 1996; Munnoli, 2000, 2007; Suthar, 2008; Nechitaylo *et al.*, 2010) and as the organic matter ingested passes through the gut, it undergoes biochemical changes effected by gut-inhabiting bacteria (Munnoli, 2007).

Fungi and bacteria are the sources of protein rich food for earthworms. Many earthworm species have been found predominantly to utilize soil fungi and bacteria as the food (Edwards and Bohlen, 1996; Ranganathan and Parthasarathi, 1999). Bacterial and fungal feeding by earthworms have been reported by many investigators (Parle, 1963; Dash *et al.,* 1979; Cook and Luxton, 1980; Parthasarathi *et al.,* 1997; Parthasarathi and Ranganathan, 1999; Ramalingam, 2004). In this way, the aims of present investigations have been attempt microbial biomass and activity are usually enhanced the determination of total microbial population (bacteria, fungi and actinomycetes), from the vermicast fresh samples were collected of earthworm.

Preparation of experimental media

In the present study, four preparation of industrial tea waste, cow dung and kitchen waste mixture were prepared in following manner.

T1 - 100% soil
T2 - 400(g) TW + 200(g) CD + 400(g) KW
T3 - 500(g) TW + 100(g) CD + 400(g) KW
T4 - 600(g) TW + 100(g) CD + 300(g) KW

The vermicomposting experiments were performed for 90 days. Tea Waste (TW), kitchen waste (KW) and Cow dung (CD) were weighed (dry weight) in specific concentration and mixed using well water, so as to have 60–70% moisture. The feed mixtures were transferred to separate plastic troughs (35 diameter x 12 cm depths). Since initial decomposition was found to improve food acceptability by worms feed substrates in the troughs were allowed 15 days for initial decomposition.

Determination of microbial population in vermicompost and control compost

The exotic earthworm *Eudrilus eugeniae*

and *Eisenia fetida* has been selected for the present study. The determination of total microbial population (bacteria, fungi and actinomycetes), the fresh samples were collected from control (WU) (i.e. 90 days maintained worm unworked compost) and vermicompost (WW) (i.e. 90 days wormworked compost) produced from different concentrations of TW + CD + KW feed mixtures (vide section 3.2.2.1).

Culture and determination of total microbial population

The total number of bacteria, fungi and actinomycetes were estimated by "serial dilution plate method" (Allen, 1953). It is assumed that each developing colony in the plate is coming up from a single cell or spore or hypae. 10 grams of manure sample was transferred to a 250 ml conical flask with 100 ml of deionized water and thoroughly shaken in a rotary shaker for 10 minutes. 10 ml of the mixture was pipetted out and transferred to water blanks with 90ml of sterile water. The serial dilutions of each mixture were made by using sterile deionised water and dilution of the manure sample viz. 10^{-4}, 10^{-5}, and 10^{-6} were prepared.

Appropriate dilution viz., 10^{-4} for fungi, 10^{-5} for actinomycetes and 10^{-6} for bacteria were chosen for respective organisms. An aliquot of 1ml of the respective dilution were spread in sterile petriplate aseptically and dispersed with respective media viz., Rose Bengal Agar medium for fungi (Emmon *et al.*, 1970), Nutrient Agar medium for bacteria (Anonymous, 1977), and Kenknight's Agar medium for actinomycetes (Emmon *et al.*, 1970). Plates were rotated gently three times in clockwise and anticlock wise direction to ensure uniform distribution of the manure mixture. The petriplates were incubated at room temperature (28°C). The colonies were developed. The colonies were counted on 3rd, 5th, and 11th day for bacteria, fungi, actinomycetes respectively using colony counter and expressed the population per gram of oven dried sample.

Determination of microbial activity (dehydrogenase activity)

To determine the microbial activity (in terms of dehydrogenase activity), samples were collected from initial substrate, worm unworked natural compost (control) and vermicompost prepared with *E. eugeniae and E. fetida* of all the treatments (T1-T4). Dehydrogenase activity was determined according to the method described by Stevenson (1959).

Quality analysis of microbes

The qualitative analysis of microbes the samples were collected from initial, worm unworked natural compost (control), and the vermicompost of (*E. eugeniae and E. fetida)* T_4 treatment. The T_4 treatment was chosen in this experiment because this treatment was found to be more suitable for growth and reproduction of the earthworms, higher nutrient contents and more population of microbes than other treatments (T_1, T_2, T_3 and T_4).

Species of fungus, bacteria and actinomycetes isolation from test samples were done according to the method described by Mackie and McCartney (1989).

Statistical analysis

The estimated microbial populations were expressed as the mean ± S.E. Percent changes over control values were also calculated. The difference in the mean values of control (WU) and experimental (WW) microbial population were tested for their statistical significance using one way analysis of variance (ANOVA).

Results and Discussion

Total microbial population

The total microbial population of vermicompost of *E. eugeniae* and *E. fetida* ranged from 3.38 ± 0.21 to 3.89 ± 0.3 and from 3.31 ± 0.21 to 3.84 ± 0.28 respectively in all the treatment (T1-T4). Among the different treatment T4 and T3 treatments were found to have significantly ($P<0.05$) higher microbial population than T2 and T1 treatments (Table 1).

Microbial activity

In the present analysis the microbial activity of vermicompost obtained from all the treatments T1-T4 were increased significantly ($P<0.05$) and especially in T4 of *E. eugeniae* (7.32 ± 0.31) and *E. fetida* (6.92 ± 0.59) and T3 of *E. eugeniae* (6.60 ±0.05) and *E. fetida* (5.95 ± 0.63) treatments were found to be significantly ($P<0.05$) higher than T2, T1 treatments (Table 2).

Quantitative analysis of microbes

Fungal isolation

The list of fungal flora isolated from the vermicompost of *E. eugeniae* and *E. fetida* read in industrial tea waste, cow dung and kitchen waste mixtures are regarded in table 3 which shows a total of 10 fungal species belonging to 7 genera. This includes two phytocomycetes (*Rhizopus nigricans*, NUCO plumbers sps) only one Ascomycetes (Chaetomium globossum) Seven Deutromycetes (*Aspergillus flavus, A. niger A. nidulans, Cladosporium herbarium, Fusarium oxysporum, F. moniliforne, Penicillium citrinum.* Of these six fungal species such as *Aspergillus flavus, A. nidulans, Cladosporium herbarium, Fusarium moniliforme, Chaetomium globossom, Mucor plumbeus* were identified in worm unworked natural compost (Control) and 8 species in vermicompost of *E. eugeniae* and 5 species in vermicompost of *E. fetida* were identified (Table 3, Fig. 1).

Bacterial isolation

The isolation of bacteria from worm unworked natural compost found to contain six species such as *Pseudomonas aeruginosa, Enterobacter acrogens, Proteus vulgaris, Escherichia coli, Citrobactor diversus* and *Enterococcus faecivm*. In vermicompost of *E. eugeniae* nine species such as *Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter aerogenes, Morganella morganii, Proteus vulgaris, Escherichia coli, Enterococcus faecium, Bacillus subtilis* and *Bacillus cereus* and in vermicompost of *E. fetida* five species such as *Klebsiella Pneumoniae, Pseudomonas aeruginosa, Enterobacter acrogens, Citrobactor diversus* and *Bacillus subtilis.* Totally eleven bacterial species were identified of these eight gram negative bacteria and three gram positive bacterium (Table 4, Fig. 2).

Isolation of Actinomycetes

Actinomycetes such as *Streptomyces albus, S. griseus*, are identified in worm unworked natural compost. *Streptomyces albus* and *Nocardia caviae* present in vermicompost of *E. eugeniae* and only one species *Streptomyces albus* in vermicompost of *E. fetida* were identified (Table 4, Fig. 3).

Microorganisms are the primary decomposers of organic matter. The role of microbial activity in the vermicomposting of earthworms, in the casts and in the soil is very essential for the degradation of organic waste and release of nutrients in available form to plants (Syers *et al.*, 1979).

Table.1 Total microbial population in different treatment of tea waste+ cow dung + kitchen waste mixture and vermicompost

S.NO.	Total Microbial Population CFU x $10^6 g^{-1}$				
	Treatment	**Initial Substrate**	**Natural**	***E. eugeniae***	***E. fetida***
1	T1 control soil (1000g soil)	2.87	3.10	3.38	3.31
2	T2 (400g TW + 200g CD + 400g KW)	3.10	3.28	3.56	3.51
3	T3(500g TW + 100g CD + 400g KW)	3.23	3.47	3.67	3.62
4	T4 (600g TW + 100g CD + 300g KW)	3.32	3.58	3.89	3.84

Table.2 Microbial Activity in initial, worm unworked natural compost, vermicompost produced by two earthworm species

Treatment	**Initial**	**WUW natural compost**	**Vermicompost**	
			E. eugenia	***E. fetida***
T1	3.11±0.16	4.17±0.65	5.72±0.61	4.50±0.11
T2	3.21±0.17	4.27±0.67	6.12±0.27	5.46±0.29
T3	3.27±0.23	4.46±0.28	6.60±0.15	5.95±0.63
T4	3.31±0.51	4.82±0.43	7.32±0.31	6.92±0.59

Table.3 Fungal flora isolated from the vermicompost of industrial tea waste, cow dung and kitchen waste mixtures

S.No	**Fungal Species**	**Worm unworked compost**	**Vermicompost**	
			E. eugeniae	***E. fetida***
1	*Aspergillus flavus*	+	+	-
2	*Aspergillus niger*	-	+	+
3	*Aspergillus nidulans*	+	+	-
4	*Cladosporium herbarium*	+	+	-
5	*Fusarium oxysporum*	-	+	+
6	*Fusarium moniliforme*	+	+	+
7	*Chaetomium globossum*	+	-	+
8	*Penicillium citrinum*	-	+	-
9	*Rhizopus nigricans*	-	+	+
10	*Mucor plumbers*	+	-	-

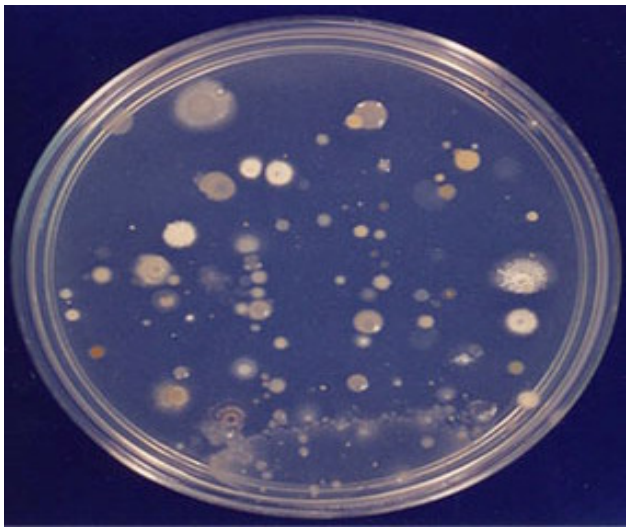
Table.4 Bacteria species and Actinomycetes isolated from the vermicompost of industrial tea waste, cow dung and kitchen waste mixtures

S.No	Bacterial Species	Worm unworked compost	Vermicompost	
			E.eugeniae	***E.fetida***
1	*Klebsiella pneumoniae* G+ve	-	+	+
2	*Pseudomonas aeruginosa* G –Ve	+	+	+
3	*Enterobacter aerogenes* G –ve	+	+	+
4	*Enterobacter cloacae* G –ve	-	-	-
5	*Morganella morgarii* G –ve	-	+	-
6	*Proteus vulgaris* G –ve	+	+	-
7	*Escherichia coli* G –ve	+	+	-
8	*Citrobactor diversus* G –ve	+	-	+
9	*Enterococcus faecium* G+ve	+	+	-
10	*Bacillus subtilis* G+ve	-	+	-
11	*Bacillus cereus* G+ve	-	+	-
Actinomycetes				
1	*Streptomyces albus*	+	+	+
2	*Sr. griseus*	+	-	-
3	*Nocardia cariae*	-	+	-

Fig. 1 Culture of fungus

Fig.2 Culture of bacteria

Fig.3 Culture of actinomycete



Earthworm gut harbours specific symbiotic microflora (Lavelle, 1983; Wall work, 1983). During vermicomposting process, the organic matter passes through the worms gut undergoes physical, chemical, and biochemical changes by the combined effect of earthworms and microbial activities. Earthworms not only help the proliferation of microbes by speeding up physical degradation process of organic matter when it passes through the gut but also stimulate other free living aerobic microbial activities in the casts favouring further decomposition (Kale *et al.*, 1991). Organic matter that passes through the gut of earthworms released as vermicast results in an increased level of microbial population, microbial activity, microbial respiration, enzyme activity and NPK enrichment, production of polysaccharide gum by bacteria, establishment of lignocellulolytic, nitrifying and nitrogen fixing microorganisms etc.

Hendrikson (1990) recorded high bacterial

population in the earthworm cast. In accordance with the above reports in the present study the bacterial population was found to be significantly greater in the fresh vermicast obtained from the treatments T1-T4 (especially TW+60%+CD10%+KW30% T3 & T2). The high population of bacteria may be due to bacterial growth during transit through the gut of earthworms.

The increased occurrence of fungal population in the cast is attributed to high rate of proliferation of fungi even though earthworm selectively feed fungi (Scheu, 1987; Tiunov and Scheu, 2000). Dash *et al.* (1986) reported that fungal population had increased in fresh casts of *L. mauritii* and *D. calebi*. Parthasarathi *et al.* (1997) found that fresh casts of *L. mauritii* and *E. eugeniae* reared on press mud contain high number of fungi population. Vinotha (1999) also found that fresh casts of *P. excavatus* and *E. eugeniae* reared on press mud contain greater numbers of fungi. In conformity with the above reported results in the present study also the fungal population is found to be significantly higher in the fresh vermicast obtained from treatments T1-T4. The enhancement of fungal population may be due to the multiplication of fungi during their transit through the worms gut. Further, from the results it could be also suggested that the tea waste and other organic wastes highly support the growth and multiplication of fungi than actinomycetes. Many earlier researchers have reported high actinomycetes population in the vermicast of earthworms (Jambhekar, 1992; Indra *et al.*, 1996; Pramanik *et al.*, 2007). The actinomycetes population was found to be significantly greater in the fresh casts obtained from the treatments T4. The enhancement of actinomycetes population in the present study may be due to multiplication of actinomycetes during their transit through the worm's gut.

In conclusion, vermicompost has higher economic value compared to compost derived from traditional methods. The earthworm derived nutrients from the decomposing organic matter and also from the proliferating microorganisms.

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