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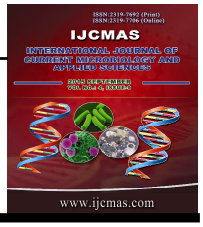

Original Research Article

Microbial diversity of Vermicompost and Veriwash prepared from *Eudrilus euginae*

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Keywords

Vermicompost, Vermiwash, microflora, *Vigna mungo*

A B S T R A C T

The aim of the study was to enumerate the diversity of microorganisms in different leaf litter vermicompost and vermiwash. To assess the microbial load of vermicompost and vermiwash before and after composting was analysed. The results revealed that the beneficial microflora such as bacteria, fungi and actinomycetes population in vermicompost and vermiwash was prepared from *Vigna mungo* leaf waste (E4) was significantly higher.

Introduction

Earthworms are popularly known as the "farmer's friend" or "nature's plowman". Earthworm influences microbial community, physical and chemical properties of soil. The primary decomposers of organic matter are microorganisms. Microbial activity in the earthworms gut, cast and soil is very essential for the breakdown and release of nutrients in available form to plants. The microorganisms and earthworms act symbiotically to accelerate and enhance the decomposition of organic matter.

Vermicomposting is a suitable system for studying microbe earthworm interactions Aira *et al*., 2006c. Microbial activity is stimulated by favourable conditions like moisture content, pH and high concentration of mucus in the anterior part of the gut, in the midgut this enhanced microbial activity results in the digestion of soil organic matter and the digestion are partially absorbed in the posterior part of the gut. Epigeic species which consume considerable amounts of raw organic matter have a range of enzymatic activities, probably mainly originating from ingested microflora. For instance the presence of fungal endophytes substantially increased the nutritional value of grass leaves for *E. fetida* (Humphries *et al*, 2001).

Soil, is the soul of infinite life that promotes diverse microflora. Soil bacteria viz., Bacillus, Pseudomonas and Streptomyces etc., are prolific producers of secondary

metabolites which act against numerous co-existing phytopathogeic fungi and human pathogenic bacteria (Pathma *et al*. 2011b). Soil, the major reservoir of microbes, meets the food requirement of earthworms and this has necessitated the establishment of different kinds of relationship between earthworms and microbes. They are : (1) microbes form a part of food for earthworm, (2) microbes are proliferated in the gut and vermicomposts, (3) earthworm help in the distribution of microbes and (4) together with earthworm microbes mineralise, humifies organic matter etc facilitates chelation (Lavelle *et al*., 1998; Parthasarathi and Ranganathan, 1998; Canellas *et al*., 2002).

Microbial biomass in the worm casts was found to be high and their activity was essential for release of nutrients into the medium so as to be taken by the plants (James, 1991). Enhanced nutrients (N, P, K, S, Ca, Mg, Mn, Fe, Zn) in the casts of earthworm, compared to the surrounding soil, was shown to be due to mineralization taking place in the gut as well as in the casts (Elvira *et al*., 1998; Parthasarathi *et al*, 2007).

Decomposition and humification of biodegradable organic waste materials is predominantly carried out by microorganisms in the soil but the few recent studies have shown that earthworms too have roles in humification (Edwards and Bohlen, 1996; Kadalli *et al*., 2000; Manivannan *et al*., 2004; Ranganathan and Parthasarathi, 2005). The composition of micro flora in the earthworm gut varies depending on the earthworm species (Kristufek *et al.*, 1993). The main objective of the present study was to assess the microorganisms of substrates that the earthworms ingest.

Materials and Methods

Microbial Culture

For the estimation of microbial populations such as bacteria, actinomycetes and fungi samples from various treatments under vermicomposting were collected.

1.Initial organic waste combinations (ie., before the commencement of vermicomposting)
2.Control and Experimental (ie., 60 days vermicimposts) (Daniel and Karmegam, 1999).

Experimental design

C-Cowdung vermicompost & vermiwash
E1-*Mangifera indica* leaf waste vermicompost & vermiwash
E2-*Syzygium cumini* leaf waste vermicompost & vermiwash
E-*Vigna radiate* leaf waste vermicompost & vermiwash
E4-*Vigna mungo* leaf waste vermicompost & vermiwash

Above samples were cultured to determine bacterial actinomycetes and fungal population using the following methods.
Culture media used

1.Modified nutrient agar (NA) (Rangaswami,1966) (Bacteria)
2 Ken-Knights agar (KKA) (Allen, 1953) (Actinomycetes)
3.Martins Rose Bengal agar (RBA) (Martin.,1950) (Fungi)

The total number of bacteria, actinomycetes and fungi were estimated by 'Serial dilution plate method' (Allen,1953;Kannan, 1996). It is assumed that each developing colony in the plate from a single cell or spore or hyphae.

One gram of each sample was taken in sterile conical flask containing nine ml of distilled water and shaken invortex mixer for 30 minutes. From this stock dilutions were prepared 10^5 with sterile distilled water as described by Kannan (1996).

To develop the microbial colonies from the sample suspension "pour plate method" was followed. For each dilution of each group of micro organism five replicates were maintained. The petridishes were poured with appropriate agar media ie NA KKA and RBA respectively for bacteria, actinomycetes and fungi. The medium was allowed to set and the plates were incubated in inverted position at room temperature (27+2ºC) for the following periods : for bacteria one day, for actinomycetes seven days and for fungi three days (Subba Rao,2000; Kannan, 1996).

Enumeration of microbes

The colonies on the plates were counted with the help of colony counter on first day, seventh day and third days of incubation for bacteria, actinomycetes and fungi respectively. The average number of colonies for three plates (bacteria, actinomycetes and fungi), kept as five replicates, were worked out.

Calculation

Microbial population present =

average number of colonies and dilution factor
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per gram oven dried sample Moisture factor

The total number of micro organisms were expressed per gram of oven dried sample and the changes in the total number of microbes in experimental over control were calculated.

Statistical Analysis

The estimated microbial populations were expressed as the Mean ±S.E. The difference in the mean values of control and experimental (E) microbial populations were tested for their statistical significance using t' test described by Gosset (Snedecor and Cochran,1968; Gupta and Kapoor,1998).

Result and Discussion

The microbial count observed in the initial and final day of vermicomposting is given in the Table - 1 & 2. In the microbial count observed during initial day of vermicomposting, maximum bacterial count was noticed in sample (E4). The bacterial Population was $79.9\pm1.45\times10^8$CFU/g. The observed values were (E1) $68.6\pm1.16\times10^8$CFU/g, (E3) $60.2\pm2.33\times10^8$CFU/g and (E2) $55.7\pm2.6\times10^8$CFU/g, (C) $54.3\pm2.31\times10^8$CFU/g respectively. The maximum fungal count was recorded in the sample (E4) $70.4\pm5.21\times10^5$CFU/g. The observed values are (E1) $62.1\pm3.16\times10^5$CFU/g, (E3)$54.5\pm2.2\times10^5$CFU/g (E2) $46.2\pm5.58\times10^5$CFU/g when compared to control (C) $38.3\pm2.15\times10^5$CFU/g.

The maximum actinomycetes population was found in (E4) $21.7\pm0.81\times10^4$CFU/g. The observed values are (E3) $20.1\pm0.78\times10^4$CFU/g, (E2) $17.5\pm0.73\times10^4$CFU/g (E1) $12.4\pm0.52\times10^4$CFU/g when compared to control (C) $10.5\pm0.92\times10^4$ CFU/g. The bacterial, fungal and actinomycetes counts were to be increasing from initial day to final (60^{th} day) vermi composts. In the present study the observation of bacterial,fungal and actionmycetes count was found to be increased in 60^{th} day of vermicomposting.

Table.1 Microbial count of *E.eugeniae* treated with different leaf litter wastes

Treatments	Initial			60th day of vermicomposting		
	Bacteria $\times 10^8$/g	Fungi $\times 10^5$/g	Actinomycetes$\times$ 10^4/g	Bacteria $\times 10^8$/g	Fungi $\times 10^5$/g	Actinomycetes $\times 10^4$/g
Control	54.3±2.31	58.2±1.87	10.5±0.92	254.6±14.30	38.3±2.15	12.8±1.29
E1	68.6±1.16	75.5±4.55	12.4±0.52	277.5±16.62	62.1±3.16	17.4±0.90
E2	55.7±2.6	60.25±2.10	17.5±0.73	214.12±10.60	46.2±5.58	21.5±0.77
E3	60.2±2.33	66.7±3.20	20.1±0.78	323.6±16.12	54.5±2.2	32.8±0.87
E4	79.9±1.45	98.4±5.50	21.7±0.81	401.2±12.20	70.4±5.21	37.6±0.2

Table.2 Microbial count of *E.eugeniae* treated with different leaf litter wastes vermiwash

Treatments	24 hrs extracted vermiwash			48 hrs extracted vermiwash		
	Bacteria $\times 10^8$/g	Fungi $\times 10^5$/g	Actinomycetes $\times 10^4$/g	Bacteria $\times 10^8$/g	Fungi $\times 10^5$/g	Actinomycetes $\times 10^4$/g
Control	185.3±0.72	45.5±0.89	11.2±0.61	215.3±0.29	22.3±0.82	18.6±0.77
E1	212.4±0.66	56.7±0.71	16.6±0.79	253.0±0.03	20.5±0.86	19.4±0.62
E2	240.9±0.61	58.2±0.92	14.4±0.92	302.8±0.64	18.5±0.92	20.2±0.33
E3	242.3±0.69	52.9±0.61	18.5±0.84	346.9±0.69	20.7±0.79	22.4±0.56
E4	248.8±0.84	59.6±0.76	20.7±0.88	382.2±0.71	32.3±0.63	24.8±0.52

Fig.1 Total Bacterial Population of *E.eugeniae* treated with different leaf litter vermicompost

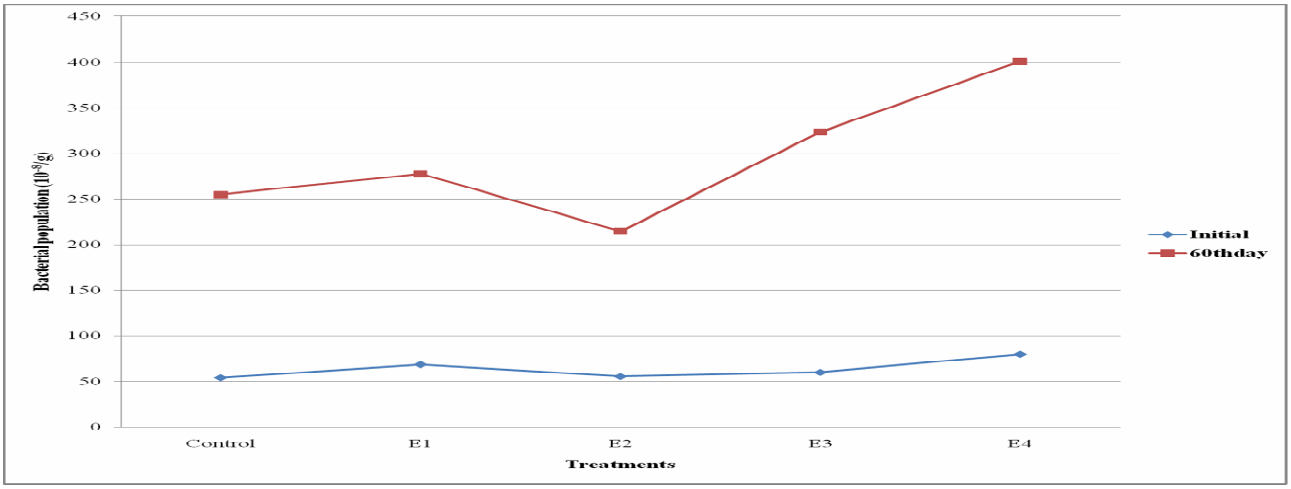


Fig.2 Total Fungal population of *E.eugeniae* treated with different leaf litter vermicompost

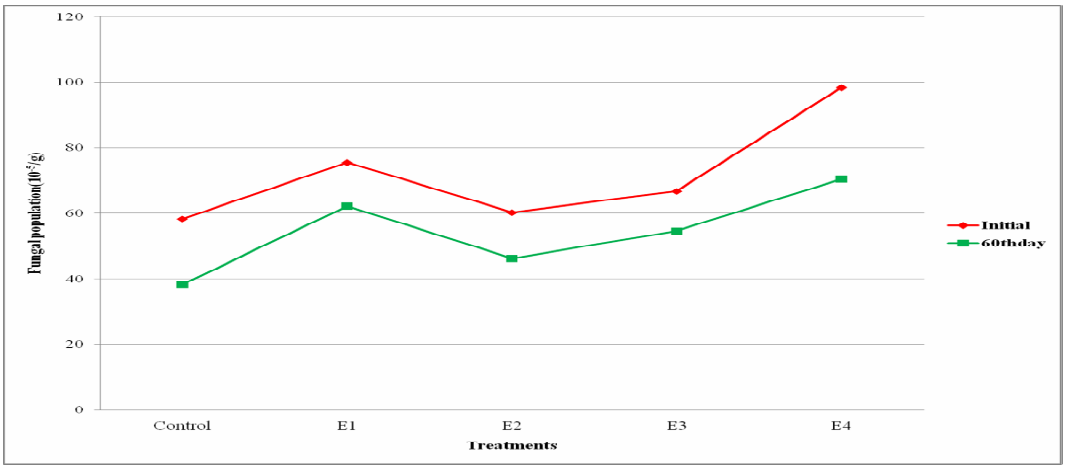


Fig.3 Total Actinomycetes count of *E.eugeniae* treated with different leaf litter vermicompost

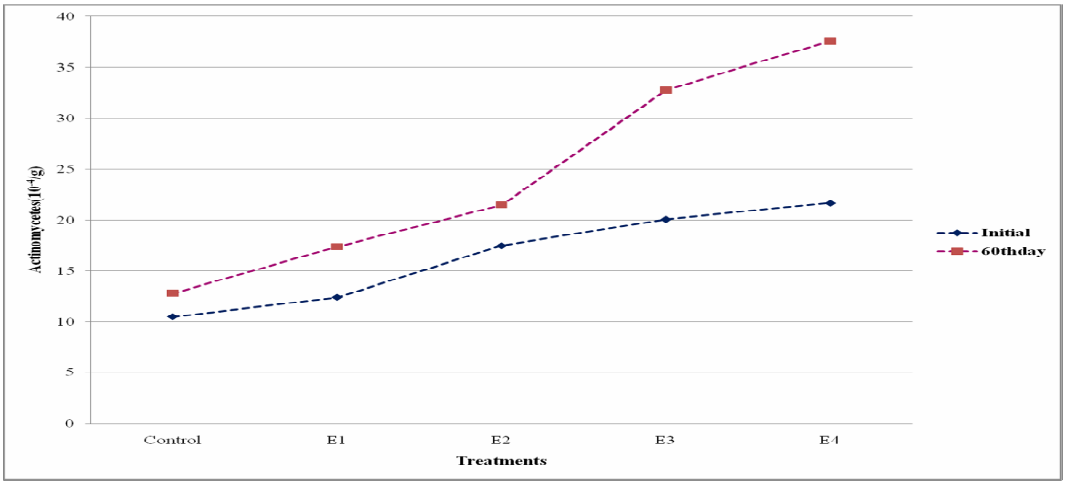


Fig.4 Total Bacterial Population of *E.eugeniae* treated with different leaf litter vermiwash

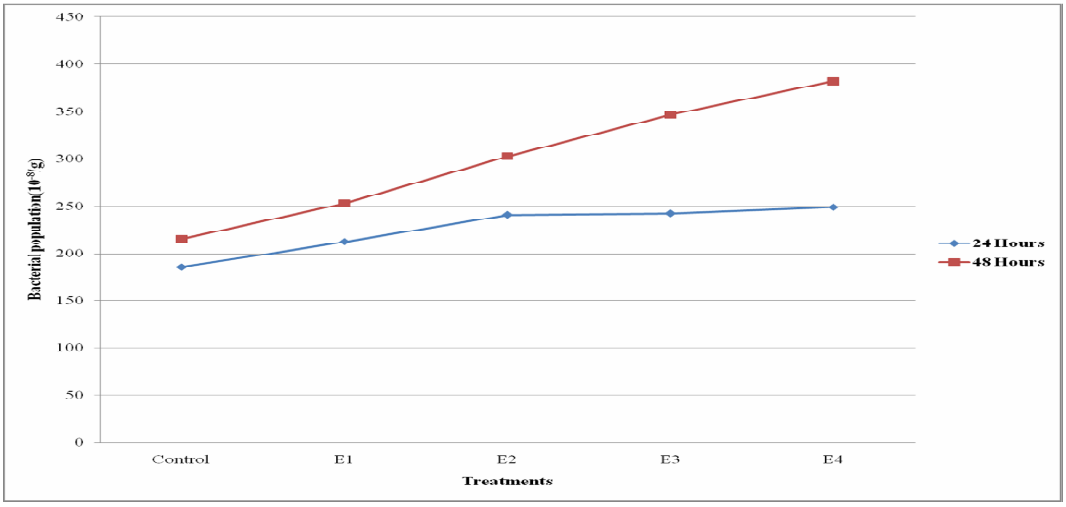


Fig.5 Total fungal population of *E.eugeniae* treated with different leaf litter vermiwash

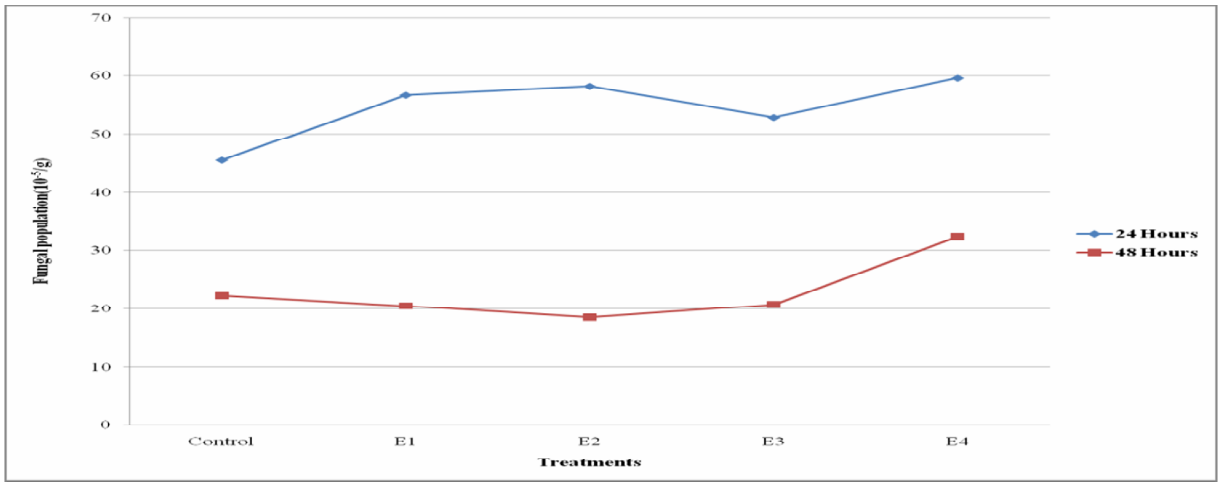
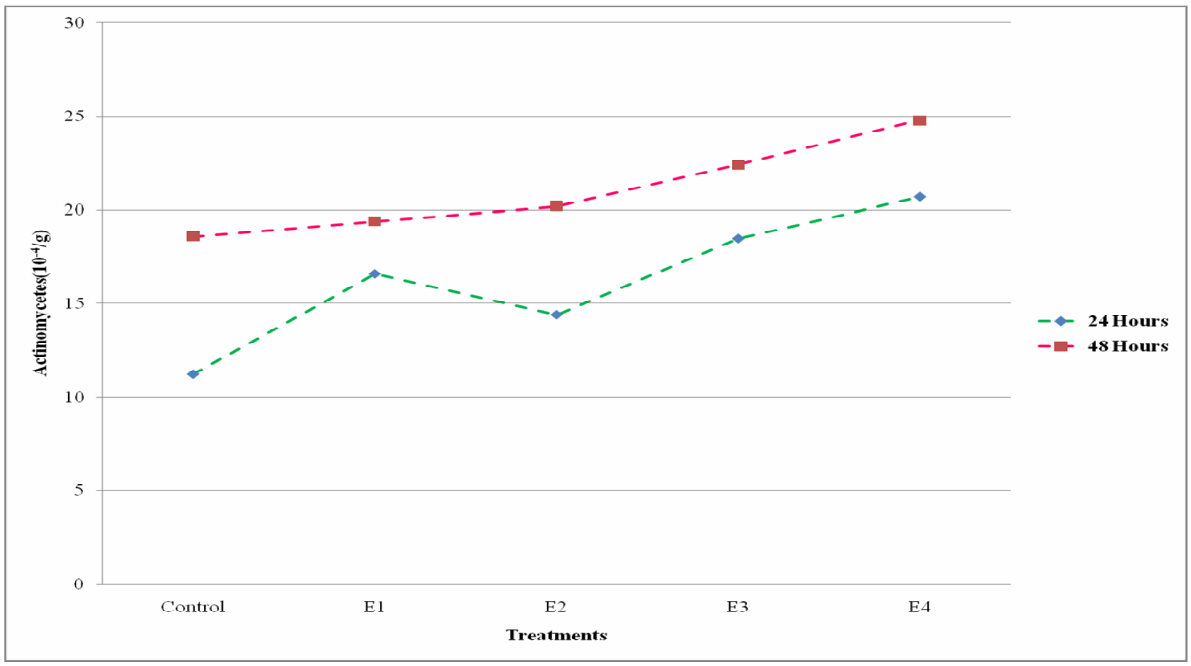


Fig.6 Total Actinomycetes Population of *E.eugeniae* treated with different leaf litter vermiwash



The microbial count increased by the end of the experiment (Table - 1). The bacterial populations were higher in the midgut region than in the foregut and hindgut region.

The microbial population (Bacteria, fungi and actinomycetes) increased from initial upto 60 days. An increased number of bacteria, fungi and actinomycetes in the treatments compared to the control were observed (Table - 1&2). Thus *E.eugeniae* contributed to the increase of the microbes of the organic matter. Similar increases were also observed in other vermicomposts (Karmegam and Daniel, 2009a; Prakash *et al*., 2009). This observation parallels that of Parthasarathi (2006) who reported increased

microbial population, microbial activity and N, P and K content in the vermicompost at 31ºC and 60–70% moisture during vermicomposting of sugar industrial wastes.

It is well known that the capacity to decompose complex organic matter varies with microbial community (Campbell, 1983),Nutrient enriched earthworm cast are good media for supporting the microbial growth (Lee,1985), Parle (1963) reported that population of yeast and fungi did not proliferate during passage through the gut, although actinomycetes and bacteria did. From the above discussion it is clearly known that the presence of bedding material is very essential for the composting and it helps in the growth of earthworm which is indirectly responsible for the increase in microbial population, nutrients and reduction of organic carbon in the compost (Nikita, *et al* 2007).

As indicated in Table 1&2 in the present study the bacterial population was found to be higher in the vermicompost than the control. Among the various treatments in all the 5 types of vermicomposts showed a significant increase over control. Therefore earthworm activity increases the population of plant growth-promoting rhizobacteria (PGPR) (Sinha *et al.* 2010). This specific group of bacteria stimulates plant growth directly by solubilization of nutrients (Ayyadurai *et al*. 2007; Ravindra et al. 2008 and Pathma and Sakthive,l 2012). The microorganisms must have been digested during transit through the earthworm intestine. In general the number of yeasts and fungi are little changed during passage through the earthworm gut, bacteria and actinomycetes increase exponentially from foregut to hindgut. Bacterial population were also found to be high in cast (Tiwari et al, 1989), The population of bacteria in the casts formed by Lampito mauritii and Eudrilus eugeniae were more than that of soil (Parthasarathi and Renganathan,1998). Parle,1959 found that numbers of bacteria and actinomycetes found in ingested material increased up to 1000 fold while passing through the earthworm gut.

Kale, 1988 recorded increase in numbers of actinomycetes and bacteria in *Eudrilus eugeniae* and *P. excavatus* worked organic wastes mixed soil. The greater the organic matter content the larger will be the microbial population. Ponomavera, 1962 reported that the number of bacteria in earthworm faeces was thirteen times higher than in the surrounding soil. In the present study the maximum bacterial population was found in casts of *Eudrilus eugeniae*. The study indicates that the action of earthworm *Eudrilus eugeniae* could increase the microbial colony forming units during vermicomposting of those organic substrates which in turn can be used for increasing the microbial population of the soil.

The number of fungal population between initial and vermicomposted wastes is shown in Table1&2 from which it is clear that in all the wastes the number of fungi was higher in worm worked compost whereas it was uniform lower than that of control. The increased fungal population might be due to the availability of nutrient rich organic wastes and partly due to increased surface area of the ingested wastes caused by the mechanical action of earthworm's gizzards for the proliferation of microbes (Edwards *et al* 1985). Earthworms live in close relationship with soil microorganisms. The alimentary canals of the earthworms itself possess a large number of bacteria, fungi and actinomycetes. This provides considerably enhanced surface area for microbial decomposition (Gajalakshmi, 2001). It was noticed in the present study that there was decrease in the fungal

population. the same result was observed by Esakkiammal and LakshmiBai, 2013. The decrease in fungal hyphae in these cases may be due to the selective feeding and digesting of fungi by some species of earthworms (Kale, 1998).

From the above discussion it can be concluded that E4, E3, E2,E1 and C because of their nutritional superiority could support the enormous growth and proliferation of microorganisms. So vermicompost not only provides mineralogical fertility factors to the soil but also contributes to the biological fertility factors to the soil. Organic substrates were stabilized by action of microorganisms in the presence of earthworms during vermicomposting (Edwards and Fletcher, 1988). Epigeic earthworms are generally used for organic waste decomposition and they consume microorganisms specially fungi to satisfy their nitrogen requirement. Pramanik and Chung (2011) also found similar results during vermicomposting of fly ash. This increase in microbial biomass indicated that vermicomposting facilitates microbial proliferation in final stabilized product.

The number of actinomycetes found in fresh, composted and vermicomposted wastes is shown in Table-1 from which it is clear that in all the wastes the number of actinomycetes in the vermicompost (E) was higher than the control (C). Among the vermicomposts derived from various treatments showed significant increase in actinomycetes population over control. Ponomarvera 1950 reported that there was an increase in numbers of actinomycetes and bacteria after passing through the earthworm intestine. The microorganism are stimulated while passing through the gut of earthworm ultimately resulting in an enhanced number of microbes (Shaw & Pawluk, 1986; Szabo *et al*., 1990; Daniel& Anderson, 1992, Kristufek et al. 1993). The increased number of actinomycetes and bacterial population in the present study gains support from the observation of (Esakkiammal and Lakshmi Bai, 2013) who reported that there was a great increase in total numbers of bacteria and actinomycetes in the earthworm gut compared to the soil.

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