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Enumeration of Microbial Content of Vermicasts of *Eudrilus Eugeniae* (Kinberg) and *Eisenia Fetida* (Savigny)

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Abstract:
In the present study the vermicompost was prepared from leaf materials such as Gliricidia sepium Jacq, Leucaena leucocephala (Lam.) De Wit and Cassia auriculata Linn andearthworms, Eudrilus eugeniae (Kinberg) and Eisenia fetida (Savigny) along with cowdung in four Treatments. Fortnightly analyses (0, 15, 30, 45 and 60 d) ofthe colony forming units (CFU) of bacteria, fungi and actinomycetes in vermicasts collected from T1, T2, T3 and T4 showed that the CFU of bacteria (231.00±1.00), fungi (160.67±0.58) and actinomycetes (240.67±2.52) were higher in T3 i.e vermicompost prepared using C.auriculata + L.leucocephala + Cowdung mixture with E.eugeniae is the best of all four treatments on 60th day compared to all other treatments i.e T1 [bacteria (190.00±5.00), fungi (151.00±1.00) and actinomycetes (200.33±5.03)], T2 [bacteria (185.00±1.00), fungi (145.33±0.58) and actinomycetes (195.00±2.00)] and in T4 [bacteria (225.67±0.58), fungi (155.00±1.00) and actinomycetes (235.00±1.00)]

Keywords: *Earthworms, vermicast, colony forming units*

1. Introduction

Soil is a Physical entity that is inhabited by millions of organisms which play an important role in the modification of the soil ecosystem. Among the soil organisms, earthworms form one of the wiggling wonders that perform a biocatalytic function in soil metabolism. The earthworms belong to the Phylum Annelida, Class Chaetopoda and Order Oligochaeta. Of more than 4,200 species of known Oligochaetes in the world, about 3,200 are Megadrili (earthworms) (Julka, 1993). The earthworms are a group of soil macrofauna well known for their considerable contribution in organic matter recycling. The worms have long been associated with productive soils. They modify soil structure, improve plant growth and are important in sustaining productivity (Buckerfield, 1998). The beneficial effects of earthworms in increasing soil fertility is well documented since the time of Darwin (1881). They have therefore rightly been called the "nature's miniature factories" and also as "biogold". Earthworms may be used as soil improver, pollution bioindicator, pollution controller, detoxifer, compost manufacturer, soil stabilizer, land reclaimer, medicine producer and as bait. People call them as environmental monitoring tool because they accumulate heavy metals, agricultural chemicals and their residues.

Earthworms play a major role in the breakdown of organic matter and release and recycle nutrients. They enhance decomposition by mechanical breakdown and stimulation of microbial activity. Earthworms remove partially decomposed plant litter and crop residues from the soil surface, ingest and fragment it and then transport it to the subsurface layers. Earthworms are omnivorous animals but they are often selective in their food habits. They derive their nutrition from organic materials, bacteria, fungi, diatoms, algae, protozoans, nematodes and decomposing animals. The key role of earthworms in improving soil fertility is well known since long. Earthworms can consume large quantities of organic wastes rapidly and process them through gizzard and excrete vermicasts. The nutrients present in the vermicast are rapidly soluble in water and are a rich source of macro and micronutrients, vitamins, enzymes, antibiotics, growth hormones and immobilized microflora (Sudha and Chandini, 2003). Thus, it is clear that treatment of wastes by vermitechnology not only reduces pollution, eliminating stench and disease but also produces something of immense value to agriculture. Hence, vermitechnology is called "wealth from waste".

The earthworms fragment the organic waste substances, stimulate microbial activity greatly and increase rates of mineralization, rapidly converting the wastes into humus-like substances with a finer structure than composts but processing a greater and more diverse microbial activity, commonly referred to as vermicomposts. Microorganisms are able to perform many chemical transformation during the decomposition of organic materials but their activity is highly dependent on other macro organisms with whom they are constantly in contact. Free soil microorganisms find suitable conditions for their activity in the anterior part of the

earthworm gut as well as in worm casts. Ingestion and passage through the intestine of earthworms also affect microorganisms that are associated with plant material. It has also been suggested that microorganisms provide a source of nutrients for earthworms, with fungi as a major and bacteria as a minor source (Edwards and Fletcher, 1988). Filamentous fungi have been shown to be digested by earthworms, although report from other studies showed that the numbers of fungi in the cast after passage through the intestine (Kristufek *et al*., 1992) were increased.

Vermicast is the excreta of earthworms and it is an extremely helpful tool in neutralizing soil pH (Karmegam and Daniel, 2009). They effectively harness the beneficial microflora, and has vitamins, enzymes, minerals, antibiotics and growth hormones (Anitha and Prema, 2003). Earthworm casts contain more water stable aggregates than non-cast soil, and part of this may be due to polysaccharide gums produced by the bacteria of the intestine and by the proliferation of fungal hyphae on the surface of the casts (Satchell, 1958). Earthworm casts have been shown to possess increased microbial and enzyme activity, and enriched macro and micronutrients (Karmegam and Daniel, 2009). Microbial activity plays an important role in regulating soil fertility.

2. Methods

2.1. Vermicompost Preparation

For the present study epigeic earthworms, *Eudrilus eugeniae* (Kinberg) and *Eisenia fetida*(Savigny) were collected from the breeding stock of the Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram, Tamilnadu, India and leaf materials of *Gliricidia sepium* Jacq,*Leucaena lucocephala* (Lam.) De Witand *Cassia auriculata* Linn were collected from Gandhigram campus. The leaf materials were separately subjected to predigestion for 15 days by sprinkling water on the heap and covering it with gunny bag and turning it periodically in order to release out the initial heat produced during decomposition of organic material. The changes in temperature was observed every three days. The vermibeds were prepared in plastic containers of 45x35x15 cm size and the substrate was moistened to hold 60-80 percent moisture and kept for 24 hours stabilization. 20 numbers of healthy clitellate *E. eugeniae* and 30 numbers of *E. fetida* were separately introduced in the vermibeds. The vermicomposting trials were carried out in the rearing room with the relative humidity and the temperature of 75-85 percent and 26-28^0 C respectively. The substrate was turned (mixed) once in a week and maintained up to 60 days. The experiment was carried out with three replicates for each substrate with proper control. (Daniel and Karmegam, 2000).

2.2. Microbial Study

The total colony forming units (CFU) of bacteria, fungi and actinomycetes in the vermicasts and worm-unworked substrate i.e., control were determined every 15 days (0, 15, 30, 45 and 60 d) using standard plate count method (Parthasarathi and Ranganathan, 1998; Nagarathinam *et al*., 2000 and Subba Rao, N.S., 1995). One gram of each sample was taken in a sterile conical flask containing nine ml of distilled water and shaken in a vortex mixer for 30 minutes. From this stock, various dilutions were prepared from 10-1 to 10-7 with sterile distilled water. One ml of the diluted sample was poured into petriplates containing nutrient agar, Martin's Rose Bengal agar and Kenknight's media for bacteria, fungi and actinomycetes respectively. Three replicates were maintained for each observation. The initial microbial CFU and the final microbial CFU (wormworked and worm-unworked) in the vermibed substrates were subjected to statistical analysis.

2.3. Statistical Analysis

The following statistical tools were used for the analyses and interpretation of the data. The experimental results are presented in the form of tables using Microsoft Excel (Version 2003 and 2007). Mean and Standard Deviation were also calculated with the help of the same tool. One-way ANOVA methods were used for the analyses using MS-DOS based software DMRT-AGRESS (Version 7.01) 1994, PASCAL-Intel Software Solutions. The data input was done manually and computed. The output results obtained from the software indicate whether the differences between the treatments are significant (at $P<0.05$ and $P<0.01$) or insignificant.

3. Results and Discussion

The present study conducted on microbial CFU of bacteria, fungi and actinomycetes showed a higher microbial load in vermicasts.Among the four treatments the microbial CFU of bacteria (231.00±1.00), fungi (160.67±0.58) and actinomycetes (240.67±2.52) invermicast was significantly higher in T3 i.e. *C.auriculata* + *L.leucocephala* + cowdung + *E.eugeniae*on 60th day compared to T1 bacteria (190.00 ±5.00), fungi (151.00±1.00)and actinomycets (200.33±5.03), T2 bacteria(185.00±1.00), fungi (145.33±0.58) and actinomycets (195.00±2.00) and T4 bacteria (225.67±0.58), fungi (155.00±1.00) and actinomycets (235.00±1.00),(Tables 1, 2 and 3). The results of the statistical analysis also proved the same. Among the CFU, the actinomycetes load was higher followed by the bacteria and the fungi. Earthworms feed on large quantities of organic matter and produce cast, finely fragmented and processed organic wastes. Microbial numbers are generally higher in casts than in the surrounding soil (Bohlen and Edwards, 1995). Casts compared with upper 6-cm horizon contained more cellulolytic aerobes, hemolytic, amylolytic and nitrifying bacteria and less denitrifying bacteria. Earthworm and their casts stimulated soil fertility by increasing soil aggregate stability via bacterial polysaccharides and by enhancing the rate of organic matter breakdown via microflora. The interaction between the microorganisms and earthworm is essential for decomposition and mineralization of organic wastes into useful manure. The worm cast is the granular aggregate, the stability of which is due to the coating of mucoplysaccharides of microbes. (Anand *et al*., 1995 and Kumar *et al*., 2010). Bacteria namely *Staphylococcus aureus*, *Proteus mirabiis*, *Klebsiella* species and *E.coli* and fungi namely

Aspergillus flavus, *Aspergillus terrus*, *Aspergillus niger*, *Alternaria* species and *Penicillium* species are present in the gut and casts of the earthworm.

The microbial CFU of bacteria (80.00±1.73), fungi (71.00±1.00) and actinomycetes (91.00±2.00) in vermicast was significantly higher in T3 i.e. *C.auriculata* + *L.leucocephala* + cowdung + *E.eugeniae*on 0^{th} day compared to T1 bacteria (70.33±1.53), fungi (61.00±1.00) and actinomycets (80.67±2.52), T2 bacteria (70.33±1.53), fungi (61.00±1.00) and actinomycets (80.67±2.52) and T4 bacteria (80.00±1.73), fungi (71.00±1.00) and actinomycets (91.00±2.00),(Tables 1, 2 and 3). Considerable work has been carried out on the use of earthworms in composting various organic materials and it has been established that epigeic varieties of earthworms can hasten the composting process to a significant extent, with production of a better quality of compost as compared with compost prepared through traditional methods. Earthworms are voracious feeders on organic wastes and, while utilizing, only a small portion is used for their body synthesis and they excrete a large part of the consumed waste materials in a digested form, as vermicasts (Ghosh *et al*., 1999). Microbial metabolites particularly growth regulatory substances are involved in the biological effects of worm casts (Aira *et al*., 2003). Many free living nitrogen fixing bacteria are good producers of growth regulating substances. Microorganisms mineralize complex substances, releasing into soil available nutrients for plants and on the other hand, synthesize as a consequence of their secondary metabolism, a whole series of substances, many of which are biologically active.

The microbial CFU of bacteria (131.00±1.00), fungi (100.67±1.15) and actinomycetes (140.00±1.00) in vermicast was significantly higher in T3 i.e. *C. auriculata* + *L. leucocephala* + cowdung + *E. eugeniae* on 15^{th} day compared to T1 bacteria (110.00±2.00), fungi (91.00±2.00) and actinomycets (120.67±2.52), T2 bacteria (105.00±1.00), fungi (84.67±0.58) and actinomycets (115.00±2.00) and T4 bacteria (125.33±2.52), fungi (95.00±1.00) and actinomycets (135.00±1.00), (Tables 1, 2 and 3). Studies on the microbial colony forming units have established the superiority of the vermicast over the worm-unworked compost by their presence in higher counts indicating their symbiotic association with the earthworms, which is essential for the biodegradation of organic wastes. The inter-relationship of microorganisms with macroorganisms by their presence inside or outside the body of macroorganism and in their environment has been well established (Lavelle *et al*., 1995 and Parthasarathi and Ranganathan, 1998). The microbial activities in earthworm casts have an important effect on soil crumb structure, by increasing the stability of the wormcast in soil relative to that of surrounding soil. Earthworm casts contain more water stable aggregates than non-cast soil, and part of this may be due to polysaccharide gums, produced by the bacteria of the intestine and by the proliferation of fungal hyphae on the surface of the casts (Marinissen and Dexter, 1990).

The microbial CFU of bacteria (180.33±0.58), fungi (131.00±1.00) and actinomycetes (190.67±1.53) in vermicast was significantly higher in T3 i.e. *C. auriculata* + *L. leucocephala* + cowdung + *E. eugeniae*on 30^{th} day compared to T1 bacteria (150.33±5.03), fungi (121.00±1.00)and actinomycets (160.67±2.52), T2 bacteria (145.00±4.00), fungi (114.67±0.58) and actinomycets (154.33±0.58) and T4 bacteria (175.00±1.00), fungi (125.00±1.00) and actinomycets (185.00±1.73),(Tables 1, 2and 3). The earthworm casts usually have greater populations of bacteria, fungi and actinomycetes (Lee, 1985 and Karmegam and Daniel, 2000-a). Alexander (1976) observed that the microbial activities are directly related to the availability of energy sources and inorganic nutrients required for their growth. Distinct interaction between the bacteria and the fungi during leaf litter decomposition was observed by Dilly *et al* (2001), where the fungal communities appeared to play a predominant role in litter breakdown at the early stages while the bacteria completed the mineralization at the later phase. Fungi such as *Absidia*, *Fusarium*, *Penicillium*, *Trichoderma*, *Cladosporium*, *Gliocladium*, *Didiodendron* and yeasts like *Candido laurentii*, *C.humicola*, *C.curvata*, *Debaryomyes hansenii* and *Trichosporon cutaneum* are reported in vermicasts (Aira *et al*., 2006). Several studies have shown that vermicasts are rich in microbial population (Lee, 1985 and Parthasarathi and Ranganathan, 1999). Earthworm casts form a suitable base for free-living beneficial microbes, whose activity is essential for the release of nutrients to plants (Ross and Cairns, 1982) and they also act as carrier material for biofertilizers such as *Azotobacter chroococcum*, *Bacillus megaterium* and *Rhizobium leguminosarum* and *Rhizobium* and phosphate solubilizing bacteria (Manivannan and Daniel, 2007).

The microbial CFU of bacteria (225.33±2.52), fungi (155.00±4.00) and actinomycetes (234.67±2.08) in vermicast was significantly higher in T3 i.e. *C. auriculata* + *L. leucocephala* + cowdung + *E. eugeniae* on 45^{th} day compared to T1 bacteria (185.00±2.00), fungi (145.00±3.00) and actinomycets (195.00±2.00), T2 bacteria (180.33±0.58), fungi (140.00±1.00) and actinomycets (190.00±1.00) and T4 bacteria (220.00±1.73), fungi (150.33±5.51) and actinomycets (230.33±2.08), (Tables 1, 2and 3). Casts are rich in ammonia and partially digested organic matter, and thus provide a good substrate for the growth of microorganisms. The epigeic and anecic earthworms by their inherent nature of feeding on organic matter, provides available plant nutrients like nitrate, phosphate, sulphate, macro and micronutrients, enzymes and abundant microorganisms to the soil (Ranganathan, 2006).Studies carried out by Subler *et al* (1998) reported that vermicompost contains vermicasts which carry a mixture of hormone-like substances, known and unknown. One of the other factors, such as presence of beneficial microorganisms or biologically active plant growth influencing substances or phytohormones released by beneficial microorganisms in the vermicompost, might be involved in promoting plant growth.Several studies have shown the effect of earthworms on available mineral nutrients of the soil. It is evident from documents that soils with many earthworms generally have more exchangeable mineral nutrients than soils without earthworms. This is because the earthworms play an important role in litter decomposition and incorporation of plant residues into the soil during their burrowing, feeding and casting activities. Karmegam and Daniel (2009) and Krishnamoorthy and Vajranabhaiah (1986) had observed positive correlation between nutrients and earthworm population density in their studies.

4. Conclusion

The present study conducted on microbial CFU of bacteria, fungi and actinomycetes showed a higher load of microbes in vermicasts. Among the four treatments the microbial CFU in vermicast was significantly higher in T3 i.e. *C. auriculata* + *L. leucocephala* +

cowdung + *E. eugeniae*. The results of the statistical analysis also proved the same. Among the CFU, the actinomycetes load was higher followed by the bacteria and the fungi. The interaction between the microorganisms and earthworm is essential for decomposition and mineralization of organic wastes into useful manure. Casts are rich in ammonia and partially digested organic matter, and thus provide a good substrate for the growth of microorganisms. Earthworms by their inherent nature of feeding on organic matter, provides available plant nutrients like nitrate, phosphate, sulphate, macro and micronutrients, enzymes and abundant microorganisms to the soil. Vermicasts which carry biologically active plant growth influencing substances or phytohormones released by beneficial microorganisms in the vermicompost, might be involved in promoting plant growth.

5. References

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Annexure

Treatments	Total number of colony forming units of bacteria x 10^6 $CFUg^{-1}$				
	0d	15d	30d	45d	60d
T1	70.33±1.53	110.00±2.00	150.33±5.03	185.00±2.00	190.00±5.00
T2	70.33±1.53	105.00±1.00	145.00±4.00	180.33±0.58	185.00±1.00
T3	80.00±1.73	131.00±1.00	180.33±0.58	225.33±2.52	231.00±1.00
T4	80.00±1.73	125.33±2.52	175.00±1.00	220.00±1.73	225.67±0.58

Table 1: Total colony forming units of bacteria observed in vermicasts at different day intervals (0 to 60 d) in Treatments 1, 2, 3 and 4

- Values are mean ± Standard error (n=3)
- T1 - G.sepium + L.leucocephala + Cowdung(1:1:2) + E.eugeniae
- T2 - G.sepium + L.leucocephala + Cowdung(1:1:2) + E.fetida
- T3 - C.auriculata + L.leucocephala + Cowdung(1:1:2) + E.eugeniae
- T4 - C.auriculata + L.leucocephala + Cowdung (1:1:2) + E.fetida

Treatments	Total number of colony forming units of fungi x 10^4 CFU g^{-1}				
	0d	15d	30d	45d	60d
T1	61.00±1.00	91.00±2.00	121.00±1.00	145.00±3.00	151.00±1.00
T2	61.00±1.00	84.67±0.58	114.67±0.58	140.00±1.00	145.33±0.58
T3	71.00±1.00	100.67±1.15	131.00±1.00	155.00±4.00	160.67±0.58
T4	71.00±1.00	95.00±1.00	125.00±1.00	150.33±5.51	155.00±1.00

Table 2: Total colony forming units of fungi observed in vermicasts at different day intervals (0 to 60 d) in Treatments 1, 2, 3 and 4

- Values are mean ± Standard error (n=3)
- T1 - G.sepium + L.leucocephala + Cowdung(1:1:2) + E.eugeniae
- T2 - G.sepium + L.leucocephala + Cowdung(1:1:2) + E.fetida
- T3 - C.auriculata + L.leucocephala + Cowdung(1:1:2) + E.eugeniae
- T4 - C.auriculata + L.leucocephala + Cowdung(1:1:2) + E.fetida

Treatments	Total number of colony forming units of actinomycetes x10^4 CFU g^{-1}				
	0d	15d	30d	45d	60d
T1	80.67±2.52	120.67±2.52	160.67±2.52	195.00±2.00	200.33±5.03
T2	80.67±2.52	115.00±2.00	154.33±0.58	190.00±1.00	195.00±2.00
T3	91.00±2.00	140.00±1.00	190.67±1.53	234.67±2.08	240.67±2.52
T4	91.00±2.00	135.00±1.00	185.00±1.73	230.33±2.08	235.00±1.00

Table 3: Total colony forming units of actinomycetes observed in vermicasts at different dayintervals (0 to 60 d) in Treatments 1, 2, 3 and 4

- Values are mean ± Standard error (n=3)
- T1 -*G.sepium* + *L.leucocephala* + Cowdung(1:1:2) + *E.eugeniae*
- T2 -*G.sepium* + *L.leucocephala* + Cowdung(1:1:2) + *E.fetida*
- T3 -*C.auriculata* + *L.leucocephala* + Cowdung(1:1:2) + *E.eugeniae*
- T4 -*C.auriculata* + *L.leucocephala* + Cowdung(1:1:2) + *E.fetida*