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RESEARCH ARTICLE

A COMPARATIVE STUDY ON GUT MICROFLORA OF EARTHWORMS EUDRILUS EUGENIAE AND EISENIA FETIDA.

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Abstract

In the present study attempt was made to prepare vermicompost from the leaf materials such as *Gliricidia sepium* Jacq, *Leucaena leucocephala* (Lam.) De Wit and *Cassia auriculata* Linn using the earthworms, *Eudrilus eugeniae* (Kinberg) and *Eisenia fetida* (Savigny) along with cowdung in four different treatments. Fortnightly the analyses (0, 15, 30, 45 and 60 d) were done to evaluate the colony forming units (CFU) of bacteria, fungi and actinomycetes in earthworm guts (fore, mid and hind gut). The results showed that the higher load of CFU of bacteria (105 ± 1.15), fungi (100.33 ± 1.15) and actinomycetes (111.00 ± 1.00) were observed in the hind gut of *E.eugeniae* on 60 d in vermicompost prepared using *C.auriculata* + *L.leucocephala* + Cowdung mixture with *E.eugeniae* than in fore [bacteria (95.00 ± 2.00), fungi (91.00 ± 1.00) and actinomycetes (100.67 ± 0.58)] and mid guts [bacteria (100.00 ± 1.00), fungi (96.00 ± 1.00) and actinomycetes (105.67 ± 1.15)] and in other treatments.

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Introduction:-

Man knows earthworms ever since he started soiling his hand and called them as “intestines of earth”. The earthworms are natural resources of fertility and life, so they are popularly called as “friends of farmers”. Earthworms can serve as nature’s ploughman and they form nature’s gift to produce good humus by minimizing the time of humification of organic materials. Since the diversity of earthworm species varies with different soil types and different agro climatic conditions, the species suited to a particular region must be identified. The digestive tract of the earthworm is one of its unique features, being highly adapted according to its activities of burrowing and feeding. The earthworm gut is a natural bioreactor and the gizzard is a novel colloidal mill in which the feed is ground into particles smaller than one micron giving enhanced surface area for the microbial processing (Dkhar and Dkhar, 2004). The earthworm ingests soil, along with decomposing organic matter in it, which are mixed by powerful muscles and passed through its digestive tract. The digestive tract releases digestive fluids that contain enzymes which are mixed with the soil that has been ingested. The digestive fluids help in releasing sugars, amino acids and smaller sized organic molecules. These molecules are then absorbed via the membrane of the intestine, and used for energy and the synthesis of new cells. The earthworm gut provides a suitable place for the growth of bacterial colonies and this is evidence for the fact that earthworm castings contain significant number of bacteria that is present in the surrounding soil. The digested material is passed out through the anus in the form of castings. However, a better understanding of nutrient cycling and the factors governing their decomposition in soil is imperative for implementing sustainable management practices. Nutrient cycling in soil involves chemical, biochemical and physico-chemical reactions, with the biochemical reactions being catalyzed by soil enzymes associated with viable cells of microbial origin and plant roots. Therefore, any factor that affects soil microbial population will necessarily alter soil enzyme activity (Kiss et al., 1975). The whole world depends only on farmers for daily food. About 70 percent of the economy depends on agriculture only. Farmers regard earthworms as their friends and hold them in the high esteem as nature’s ploughman (Darwin, 1881). It is a well proved fact that

earthworms influence soil structure, decompose organic materials, recycle nutrients present in the soil through physical participation by feeding, fragmentation, aeration, turnover and dispersion; through chemical participation by digesting the organic substrates and by contributing nutrients to the soil through their metabolic by-products and dead tissues; and, through biological participation by providing shelter to the beneficial microbes in their gut and by activating soil microbial activity through the excretion of castings (Daniel and Karmegam, 2000 and Mahalingam and Daniel, 2008-a).

Methods:-

Vermicompost preparation:-

In 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 Wit and *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 26-28⁰ 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).

Microbial study:-

The total microbial counts in terms of colony forming units (CFU) of bacteria fungi and actinomycetes in the earthworm (fore, mid and hind) guts 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).

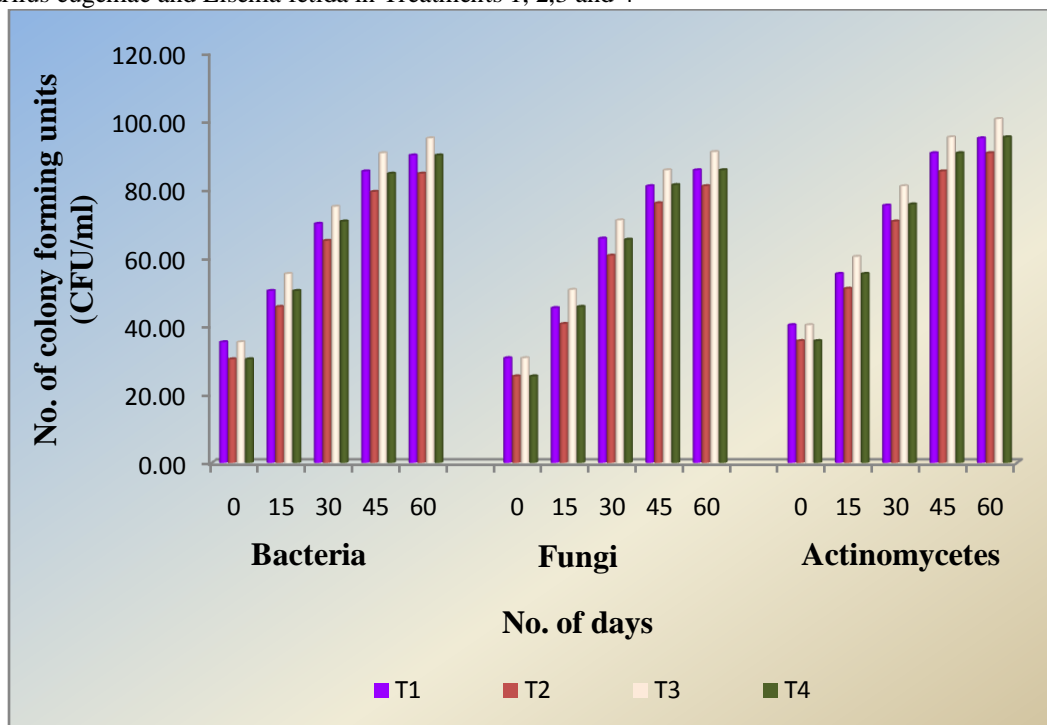
Statistical Analysis:-

The following statistical tools were used for the analyses and interpretation of the data. The experimental results are presented in the form of graphs using Microsoft Excel (Version 2003 and 2007). Mean and Standard Deviation were also calculated with the help of the same tool.

Results and Discussion:-

The results on the observation of total CFU of bacteria, fungi and actinomycetes in the fore gut of *E.eugeniae* and *E.fetida* in Treatments 1, 2, 3, and 4 are given in Fig 1. The results showed that the bacterial (95.00 ± 2.00), fungal (91.00 ± 1.00) and actinomycetes (100.67 ± 0.58) count was higher in fore gut of *E.eugeniae* i.e in T3 on 60th day compared to T1 bacteria (90.00 ± 3.00), fungi (85.67 ± 0.58) and actinomycetes (95.00 ± 1.73), T2 bacteria (84.67 ± 0.58), fungi (81.00 ± 1.00) and actinomycetes (90.67 ± 1.53), and T4 bacteria (90.00 ± 3.00), fungi (85.67 ± 0.58) and actinomycetes (95.33 ± 1.15).

Fig 1:- Total colony forming units of bacteria, fungi and actinomycetes observed in the fore gut of *Eudrilus eugeniae* and *Eisenia fetida* in Treatments 1, 2,3 and 4



T1 - *G.sepium* + *L.leucocephala* + Cowdung (1:1:2) + *E.eugeniae*

T2 - *G.sepium* + *L.leucocephala* + Cowdung (1:1:2) + *E.fetida*

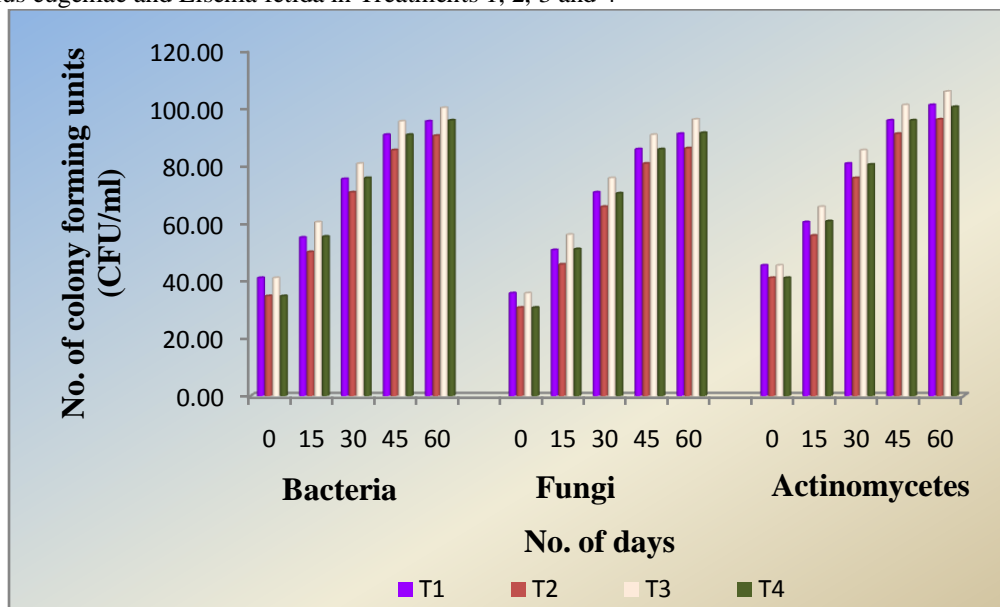
T3 - *C.auriculata* + *L.leucocephala* + Cowdung (1:1:2) + *E.eugeniae*

T4 - *C.auriculata* + *L.leucocephala* + Cowdung (1:1:2) + *E.fetida*

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. The joint action of earthworms and microorganisms characterizes the bio-oxidation of the organic material in the vermicomposting. The stimulation of the metabolic activity of the microorganisms, either living free and/or associated with the earthworm gut, and a concomitant exponential increase of the total biomass of earthworms characterize the mesophilic hydrolytic stage (Benitez et al., 1999). Therefore microorganisms are mainly responsible for the biochemical degradation of the organic matter during composting and vermicomposting processes and, in the latter, earthworms play a very important role in both microbial activity and in diversity. The vermicompost is an aerobically degraded organic matter, which has undergone chemical disintegration by the enzymic activity in the gut of earthworms and also of enzymes of the associated microbial population. (Anand et al., 1995 and Kumar et al., 2010).

The results on the observation of total CFU of bacteria, fungi and actinomycetes in the mid gut of *E.eugeniae* and *E.fetida* in Treatments 1, 2, 3, and 4 are given in Fig 2. The results showed that the bacterial (100.00 ± 1.00), fungal (96.00 ± 1.00) and actinomycetes (105.67 ± 1.15) count was higher in mid gut of *E.eugeniae* i.e in T3 on 60th day compared to T1 bacteria (95.33 ± 1.53), fungi (91.00 ± 1.00) and actinomycetes (101.00 ± 1.00), T2 bacteria (90.33 ± 0.58), fungi (86.00 ± 1.00) and actinomycetes (96.00 ± 0.00) and T4 bacteria (95.67 ± 1.53), fungi (91.33 ± 1.53) and actinomycetes (100.33 ± 1.15).

Fig 2:- Total colony forming units of bacteria, fungi and actinomycetes observed in the mid gut of *Eudrilus eugeniae* and *Eisenia fetida* in Treatments 1, 2, 3 and 4



T1 - *G.sepium* + *L.leucocephala* + Cowdung (1:1:2) + *E.eugeniae*.

T2 - *G.sepium* + *L.leucocephala* + Cowdung (1:1:2) + *E.fetida*.

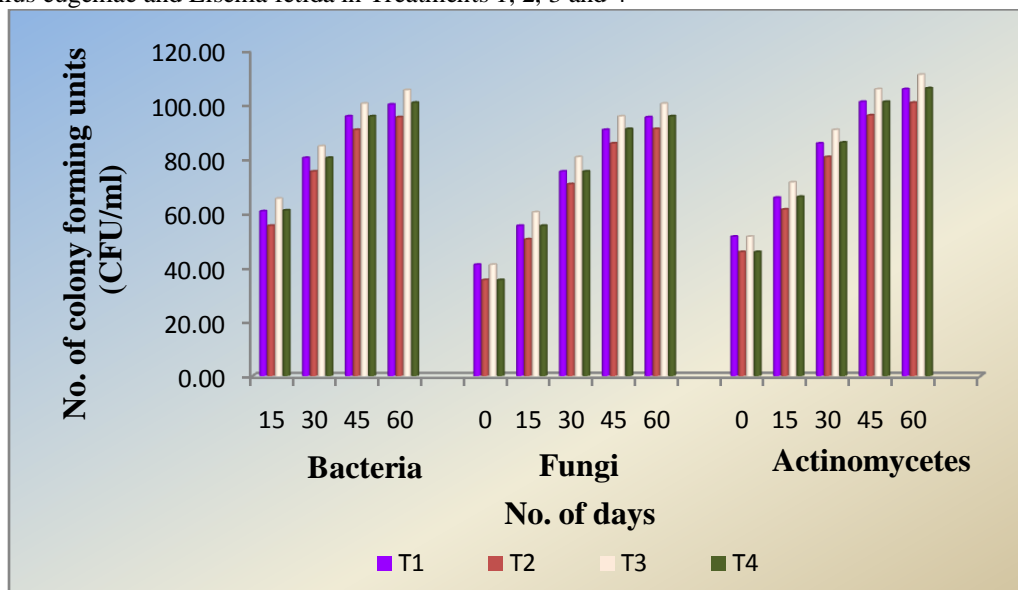
T3 - *C.auriculata* + *L.leucocephala* + Cowdung (1:1:2) + *E.eugeniae*.

T4 - *C.auriculata* + *L.leucocephala* + Cowdung (1:1:2) + *E.fetida*.

The gut microflora of the earthworms belong to bacterial colonies of *Acromobacter*, *Micrococci* and *Bacillus* and the fungal colonies of *Aspergillus fumigatus*, *Aspergillus flavus* and *Penicillium rubrum*. Edwards and Fletcher (1988) described the fungi and bacteria to be important nutrients for the earthworms. Gut transit time may be one of the most important factors determining microbial population growth in worm guts and is highly variable between different species and is dependent on factors like food quality (Hendriksen, 1991) and temperature (Barois and Lavelle, 1986). In earthworms with short gut transit times, there is probably not enough time for microbial multiplication. Whereas in earthworms with longer gut transit time, there may be enough time for microbial multiplication since these earthworms tend to feed on organic rich materials, where microbial colonization is already high (Kristufek et al., 1992).

The results on the observation of total CFU of bacteria, fungi and actinomycetes in the hind gut of *E.eugeniae* and *E.fetida* in Treatments 1, 2, 3, and 4 are given in Fig 3. The results showed that the bacteria (105.33 ± 1.15), fungal (100.33 ± 1.15) and actinomycetes (111.00 ± 1.00) count was higher in mid gut of *E.eugeniae* i.e in T3 on 60th day compared to T1 bacteria (100.00 ± 1.00), fungi (95.33 ± 1.15) and actinomycetes (105.67 ± 1.15), T2 bacteria (95.33 ± 1.15), fungi (91.00 ± 0.00) and actinomycetes (100.67 ± 1.15) and T4 bacteria (100.67 ± 0.58), fungi (95.67 ± 0.58) and actinomycetes (106.00 ± 1.00).

Fig 3:- Total colony forming units of bacteria, fungi and actinomycetes observed in the hind gut of *Eudrilus eugeniae* and *Eisenia fetida* in Treatments 1, 2, 3 and 4



- 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*

Highest load of bacteria, fungi and actinomycetes were observed in the hind gut of *E.eugeniae* on 60 d in vermicompost prepared using *C.auriculata* + *L.leucocephala* + Cowdung mixture with *E.eugeniae* was observed in T3 (Figs 3). In studies carried out by other authors (Karmegam and Daniel 2000-b and Singh and Sharma, 2002), they have reported that the microorganisms are stimulated during the passage through the gut of the earthworms where, water and soluble-C in the form of intestinal mucus produced by the earthworms awaken the dormant micro flora, thereby increase the decomposition of the stable forms of the organic matter and release the nutrients which in turn increase the soil fertility. Vermicompost and vermicast are very good sources for enriching the soil microflora. In the vermicasts the CFU of actinomycetes was significantly higher than that of bacteria and fungi. Edwards and Fletcher (1988) have observed an increased bacterial number from fore to hindgut and decreased amount of leaf material from fore-to hindgut in their studies. It has been speculated that the increase of bacterial numbers towards the end of the earthworm gut could be due to growth of bacterial cells or to an activation of dormant cells during gut passage (Kristufek et al., 1992). Filamentous fungi were detected in larger amounts only in the foregut of the earthworms and latter they disappeared because they were digested during passage through the crop and gizzard of the earthworms. However the spores of fungi which survive after gut passage will multiply in the mid and hind gut. Such results have been observed by Schonholzer et al (2002). However the bacterial population increased during the gut passage and they were found in higher number in fresh cast when compared to the fore gut. Nagarathinam et al (2000) and Karmegam and Daniel (2000-b) described that the alimentary canal of the earthworm carries great numbers of bacteria, fungi and actinomycetes. The major source of nutrients for earthworms is microorganisms and the earthworms promote microbial activity during the decomposition of organic matter. The process by which the microbes benefit from the mucus secretions of the earthworm and the process by which the earthworms benefit from the enhanced microbial decomposition of ingested organic matter make their relationship mutually symbiotic.

Conclusion:-

The studies carried out on microbial CFU of bacteria, fungi and actinomycetes in earthworms, *E.eugeniae* and *E.fetida* in the fore, mid and hind gut showed a higher load in the hind gut than in the fore gut and mid gut. In the present study increased bacterial, fungal and actinomycetes number from fore to hindgut were observed. It has been speculated that the increase of bacterial, fungal and actinomycetes numbers towards the end of the earthworm gut could be due to growth of bacterial, fungal and actinomycetes cells or to an activation of dormant cells during gut passage. The microorganisms are stimulated during the passage through the gut of the earthworms where, water and soluble-C in the form of intestinal mucus produced by the earthworms awaken the dormant micro flora, thereby

increase the decomposition of the stable forms of the organic matter and release the nutrients which in turn increase the soil fertility.

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