*ejpmr, 2015,2(5), 826-835*

**SJIF Impact Factor 2.026**

**EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH**

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

# A STUDY ON VERMICAST MICRO FLORA OF *EUDRILUS EUGENIAE* USINGDIFFERENT LEAF LITTERS

**Natarajan Manivannan[1], Gunalan Udhayakumar[2], Rajagopal Ravishankar[3], Nooruddin Thajuddin[4] and Thilagavathy Daniel[5], R.R.Thanighaiarassu[6], P. Sivamani[6*], D. Raghunathan[6]**

[1]Department of Industrial Biotechnology, Bharathidasan University Tiruchirappalli - 620 024, Tamil Nadu, India.

[2]Department of Biotechnology, Sathyabama University, Chennai – 600119.

[3]Department of Microbiology, National College (Autonomous), Tiruchirappalli - 620 001.

[4]Department of Microbiology, Bharathidasan University Tiruchirappalli - 620 024.

[5]Department of Biology, Gandhigram Rural University, Gandhigram – 624 302.

[6]Microlabs, Institute of Research and Technology, Arcot, Vellore Dt, Tamilnadu-632 503.

**Article Received on 24/07/2015** **Article Revised on 16/08/2015** **Article Accepted on 08/09/2015**

***Correspondence for Author**

**P. Sivamani**

Microlabs, Institute of Research and Technology, Arcot, Vellore Dt, Tamilnadu-632 503.

## ABSTRACT

Vermicomposting technology is simple biotechnological processes of composting with certain species of earthworms are used to enhance the process of waste conversion and produce a better end product. Earthworms are essential members of the soil macroflora altering the biological activity and physical structure of soils and stimulating organic matter degradation. Vermicast is the excreta of earthworms and it is an extremely helpful tool in neutralizing soil pH. They effectively harness the beneficial microflora and contain vitamins, enzymes, minerals and growth hormones. Vermicast contains significant number of bacteria, fungi and actinobacteria that is present in the surrounding soil. In present study *Eudrilus eugeniae* composting earthworm was used to prepare vermicompost from leaf litters of *Tephrosia purpurea* + Cow dung (1:1), *Cassia auriculata* + Cow dung (1:1), *Gliricidia sepium* + Cow dung (1:1), *Tephrosia purpurea* + *Cassia auriculata* + Cow dung (1:1:2), *Cassia auriculata* + *Gliricidia sepium* +Cow dung (1:1:2), *Tephrosia purpurea* + *Gliricidia sepium* + Cow dung (1:1:2) were subjected to decomposition for 90 d. The colony forming units (CFU) of bacteria, fungi and actinobacteria

of vermicast were analyzed on 90 d. The results showed more number of CFU of bacteria, fungi and actinobacteria on 90 d than in the initial and control.

**KEYWORDS:** *Eudrilus eugeniae*, *Tephrosia purpurea*, *Cassia auriculata*, *Gliricidia sepium*, Cow dung.

## INTRODUCTION

Earthworms are often referred to as farmer's friend and nature's ploughman. The major role of earthworms in the soil is decomposition of organic materials, developing soil structure and altering physico-chemical properties (Zhang and Schrader, 1993). The role of earthworm in organic matter decomposition, nutrient cycling and soil structure and plant productivity has been studied by several authors (Lavelle, 1988; Blair et al. 1995). The earthworms speed up the composting process and transform wastes into nutrient rich castings. Earthworm, *Eudrilus eugeniae* a tropical species commonly called African night crawler, is large in size, grows rapidly, breeds fast and is capable of decomposing large quantities of organic materials into usable vermicompost (Kale and Bano, 1991). Vermicompost is nothing but the excreta of earthworms, which is rich in humus and nutrients. Vermicomposting is a simple biotechnological process of composting, in which certain species of earthworms are used to enhance the process of waste conversion and produce a better end product. Vermicomposting differs from composting in several ways (Gandhi et al. 1997). It is a mesophilic process, utilizing microorganisms and earthworms that are active at 10 – 32°C. The process is faster than composting. The earthworm castings are rich source of beneficial microorganisms and all essential plant nutrients for growth (Tomati, et al. 1988; Edwards and Burrows, 1998). It provides excellent effect on overall plant growth, encourages the growth of new shoots and leaves and improves the quality and shelf life of the produce (Manivannan and Daniel, 2007).

An epigeic earthworm, *Eudrilus eugeniae*, has been identified as detritus feeders and can be used potentially to minimize the organic wastes from different sources (Suthar, 2007). Earthworm species belonging to the epigeic group live on or near the surface of the soil. Epigeic earthworms consume plant litter and litter inhabiting organisms and ingest little. So they are qualified as "litter transformers" (Lavelle et al. 1997). Therefore, composting biology and waste recycling efficiency of this species is well documented by various authors although some tropical and native earthworm species *E. eugeniae* has been used in the vermicomposting of organic wastes to produce biofertilizers (Suthar, 2007). Several studies have shown that epigeic activities induce an increase in microbial activities due to greater

surface area for decomposition, reduce immobilization by surface litter dwelling fungi and modify the composition of microorganism communities (Scheu et al. 1994; Parkinson et al. 1998). After reviewing the current literature, *E. eugeniae* was selected as the candidate species for vermicomposting of leaf litter.

Interactions with microbes were observed in earthworm burrow lining, cast, gut and drilosphere. Drilosphere is the soil area directly or indirectly influenced by earthworms' activity and constantly changing in space and time (Lavelle, 1988). Earthworms and microorganisms mineralize, humify organic matter and facilitate chelation of metal ions (Lavelle et al. 1995; Cai et al. 2002). Microorganisms were help earthworms in their growth (Pizl et al. 2003). *Eisenia fetida* Savigny hatched from microbiologically sterile cocoons could not reach sexual maturity in sterilized soil until mixed cultures of mobile protozoa were added in its food (Miles, 1963).

The objective of the present work was to evaluate the microorganisms between initial vermibed (INI), worm un-worked (WUW) and worm worked(W W ) leaf litters of *Tephrosia purpurea* + Cow dung (1:1), *Cassia auriculata* + Cow dung (1:1), *Gliricidia sepium* + Cow dung (1:1), *Tephrosia purpurea* + *Cassia auriculata* + Cow dung (1:1:2), *Cassia auriculata* + *Gliricidia sepium* +Cow dung (1:1:2), *Tephrosia purpurea* + *Gliricidia sepium* + Cow dung (1:1:2) were subjected to decomposition for 90 d. The colony forming units (CFU) of bacteria, fungi and actinobacteria of vermicast were analyzed on 90 d.

## MATERIALS AND METHODS

The total colony forming units (CFU) of bacteria, fungus and actinomycetes in vermibed substrates at the beginning of the experiment (Initial) and at the end the experiment (Worm-worked and Worm-unworked substrates) were enumerated using standard plate count method (Subbarao, 1995; and Kannan, 1996).

Ten gram of each sample was taken in a 250 ml sterile conical flask containing 100 ml of distilled water and shaken in a vortex mixture for 30 minutes. From this stock, various dilutions were prepared from 10 - 1 to 10 - 6 with sterile distilled water. The diluted samples were used for the analysis of total colony forming units. Media and procedure used in the present investigation is as described in table 1.

The numbers of colony forming units were counted after incubation i.e., 2 days for bacteria, 5 days for fungi and 12 days for actinobacteria as given in the following tables.

The statistical analyses were performed using SPSS Statistics 17.0 software (Statistical Program for Social Sciences 17.0). Mean and standard errors were calculated for the experimental analyses that were carried out in triplicates. Method of LSD and Duncan was employed to ascertain the values of the model parameters and ANOVA was used to establish their statistical significance at a confidence level of 99%.

## RESULTS

The results of the enumeration of the total colony forming units (CFU) of bacteria, fungi and actinobacteria in the Initial vermibed substrates (INI), Worm-unworked compost (WUW) after 90 d and Worm-worked compost (W W) after 90 days are given in Table 2, 3, and 4. The total CFU were higher in the 90 d old Worm-worked as well as 90 d old Worm-unworked composts than in the Initial (INI) raw material used for composting. The leaf litter of *T. purpurea* + *C. auriculata* + cow dung (1:1:2) used in Treatment 4 showed the most significant differences when compared to the other substrates. Two-way ANOVA for colony forming units (CFU) of bacteria, fungi, and actinobacteria were compared between treatments and INI, WUW and WW substrates are showed in Table 5.

The difference in the CFU of bacteria between the treatments is significant ($P<0.001$) and between the factors is also significant ($P<0.001$). Duncan post Hoc test result for colony forming units of the bacteria in the Initial and in the Worm-worked compost is shown in Fig. 1. The numbers of bacterial colonies were more in all the treatments than in the initial. Highest number of bacteria (376) was observed in Treatment 4, followed by Treatment 6 and then 5.

The highest number of fungi (249) was present in Treatment 4, followed by the Treatment 6 (231) (Fig. 2), and the highest number of actinobacteria (286) was observed in Treatment 4, followed by Treatment 6 (268) (Fig. 3).

The studies conducted on the microbial colony forming units in worm worked compost shows dominance over the worm unworked compost in the Table 2, 3 and 4. The worm worked compost showed increased load of microbial population over the other, providing that the symbiotic relationship with earthworms is helpful for the biodegradation of organic wastes. Combination of leaf litters showed enhancement in the number of microbes for efficient biodegradation of organic wastes (Fig. 1, 2 and 3). The colony forming units were remarkably high in the worm-worked compost and the statistical analysis also proved the

same in the Table 5. The interaction of earthworm and microbes together brought out effective biodegradation of wastes and converting them into useful compost. All the three microbes' *viz.*, bacteria, fungi and actinobacteria (Table 2, 3 and 4) showed remarkable increase in the number of colony forming units in the worm-worked compost over the worm-unworked compost.

**Table.1. Media and procedure followed for enumeration of total colony forming units (CFU) of bacteria, fungus and actinobacteria:**

| Organism studied | Media used | Dilution tested | Incubation days |
|---|---|---|---|
| Bacteria | Nutrient agar | $10^{-7}$ | 2 |
| Fungi | Rose bengal agar | $10^{-4}$ | 5 |
| Actinobacteria | Kenknight's agar | $10^{-4}$ | 12 |

**Table 2. Colony Forming Units (CFU) of bacteria in Initial vermibed substrate (INI) and Worm-unworked (WUW) and Worm-worked (WW) compost using *Eudrilus eugeniae* (90 days)**

| Treat -ment | Vermibed substrate used | Bacteria x $10^7$/ g | | |
|---|---|---|---|---|
| | | INI | WUW | W W |
| 1 | Leaf litter of *T. purpurea* + cow | 195±7.99 | 232±12.73 | 320.±21.88 |
| 2 | Leaf litter of *C. auriculata* + cow | 161±9.39 | 208±10.24 | 298±20.91 |
| 3 | Leaf litter of *G. sepium* +cow dung(1:1) | 180±9.22 | 222±13.76 | 302±21.06 |
| 4 | Leaf litter of (TP+CA)+CD(1:1:2) | 210±10.63 | 251±14.18 | 376±22.92 |
| 5 | Leaf litter of (CA+GS)+CD(1:1:2) | 170±11.58 | 214±15.36 | 321±21.60 |
| 6 | Leaf litter of (TP+GS)+CD(1:1:2) | 200±12.63 | 230±14.60 | 342.±20.12 |

LL- Leaf Litters; TP-*Tephrosia purpurea*; CA-*Cassia auriculata*; GS-*Gliricidia sepium*; CD-Cowdung

**Table 3. Colony Forming Units (CFU) of fungi in Initial vermibed substrate (INI) and Worm-unworked (WUW) and Worm-worked (W W) compost using *Eudrilus eugeniae* (90 days)**

| Treat-ment | Vermibed substrate used | Fungi x $10^4$ / g | | |
|---|---|---|---|---|
| | | INI | WUW | W W |
| 1 | Leaf litter of *T. purpurea* + cow dung(1:1) | 121±11.19 | 158±12.71 | 181.±14.18 |
| 2 | Leaf litter of *C. auriculata* + cow dung(1:1) | 100±10.36 | 134±11.27 | 166±15.65 |
| 3 | Leaf litter of *G. sepium* + cow dung(1:1) | 110±11.21 | 148±12.46 | 172±15.12 |
| 4 | Leaf litter of (TP+CA)+CD(1:1:2) | 159±14.68 | 214±13.11 | 249±18.08 |
| 5 | Leaf litter of (CA+GS)+CD(1:1:2) | 127±13.21 | 189±13.60 | 214±14.05 |
| 6 | Leaf litter of (TP+GS)+CD(1:1:2) | 141±12.09 | 199±14.94 | 231.±16.12 |

LL- Leaf Litters; TP-*Tephrosia purpurea*; CA-*Cassia auriculata*; GS-*Gliricidia sepium*; CD-Cowdung

**Table 4. Colony Forming Units (CFU) of actinobacteria in Initial vermibed substrate (INI) and Worm-unworked (WUW) and Worm-worked (W W) compost using *Eudrilus eugeniae* (90 days)**

| Treatment | Vermibed substrate used | Actinobacteria x $10^4$/ g | | |
|---|---|---|---|---|
| | | INI | WUW | W W |
| 1 | Leaf litter of *T. purpurea* + cow dung(1:1) | 79±6.19 | 108±8.33 | 211.±21.2 |
| 2 | Leaf litter of *C. auriculata* + cow dung(1:1) | 66±7.31 | 84±9.54 | 181±20.21 |
| 3 | Leaf litter of *G. sepium* + cow dung(1:1) | 70±9.18 | 98±8.56 | 197±19.26 |
| 4 | Leaf litter of (TP+CA)+CD(1:1:2) | 99±8.63 | 172±13.18 | 286±21.42 |
| 5 | Leaf litter of (CA+GS)+CD(1:1:2) | 76±9.55 | 143±18.36 | 245±20.10 |
| 6 | Leaf litter of (TP+GS)+CD(1:1:2) | 87±8.53 | 155±14.68 | 268.±19.1 |

LL- Leaf Litters; TP-*Tephrosia purpurea*; CA-*Cassia auriculata*; GS-*Gliricidia sepium*; CD-Cowdung

**Table 5. Two-way ANOVA for Colony Forming Units (CFU) of bacteria, fungi and actinobacteria in Initial vermibed substrate (INI) and Worm-unworked (WUW) and Worm-worked (WW) compost using *Eudrilus eugeniae* (90 days).**

**CFU of bacteria**

| Source | Sum of squares | df | Mean square | F | Sig |
|---|---|---|---|---|---|
| Treatment | 39933.852 | 5 | 7986.770 | 890.718 | P<0.001 |
| Factor (INI,WUW,WW) | 317468.685 | 2 | 158734.343 | 17702.71 | P<0.001 |
| Treatment * factor | 35666.759 | 10 | 3566.676 | 397.771 | P<0.001 |
| Error | 807.000 | 90 | 8.967 | | |
| Total | 393876.296 | 107 | | | |

**CFU of fungi**

| Source | Sum of squares | df | Mean square | F | Sig |
|---|---|---|---|---|---|
| Treatment | 23560.630 | 5 | 4712.126 | 624.276 | P<0.001 |
| Factor (INI,WUW,WW) | 174214.574 | 2 | 87107.287 | 11540.2 | P<0.001 |
| Treatment * factor | 4303.093 | 10 | 430.309 | 257.009 | P<0.001 |
| Error | 679.333 | 90 | 7.548 | | |
| Total | 205757.630 | 107 | | | |

**CFU of Actinobacteria**

| Source | Sum of squares | df | Mean square | F | Sig |
|---|---|---|---|---|---|
| Treatment | 14465.407 | 5 | 2893.081 | 368.458 | P<0.001 |
| Factor(INI,WUW,WW) | 251009.185 | 2 | 125504.593 | 15984.07 | P<0.001 |
| Treatment * factor | 402.148 | 10 | 40.215 | 5.122 | P<0.001 |
| Error | 706.667 | 90 | 7.852 | | |
| Total | 266583.407 | 107 | | | |

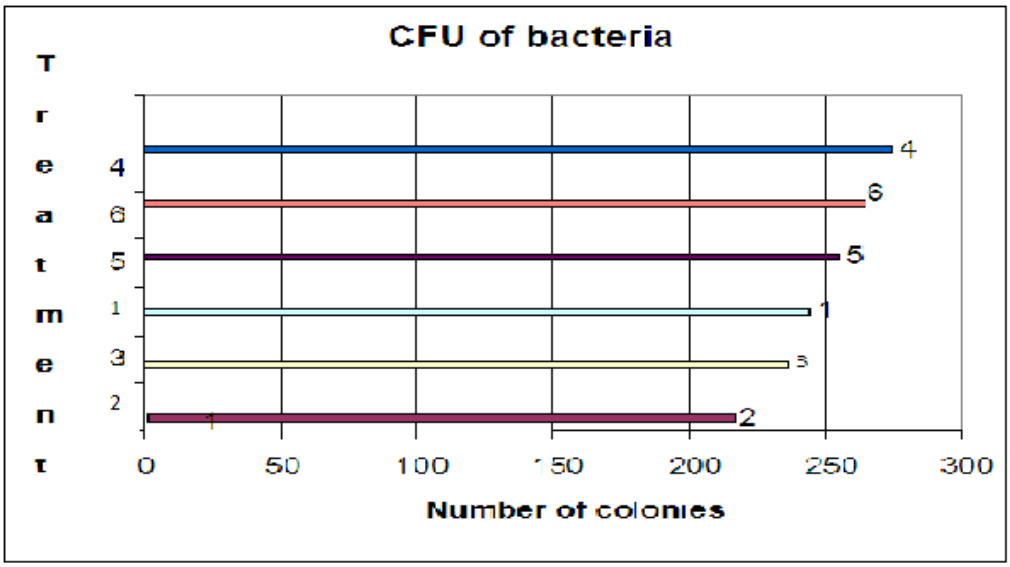


**Fig. 1. Duncan post Hoc test result for colony forming units of bacteria in the Worm-worked (WW) composts (90 d).**

Treatment 1. LL of TP + CD (1: 1),Treatment 2. LL of CA + CD (1: 1), Treatment 3. LL of GS + CD (1: 1), Treatment 4. LL of (TP + CA) + CD (1:1:2), Treatment 5. LL of (CA + GS) + CD (1:1:2), Treatment 6. LL of (TP + GS) + CD (1:1:2).

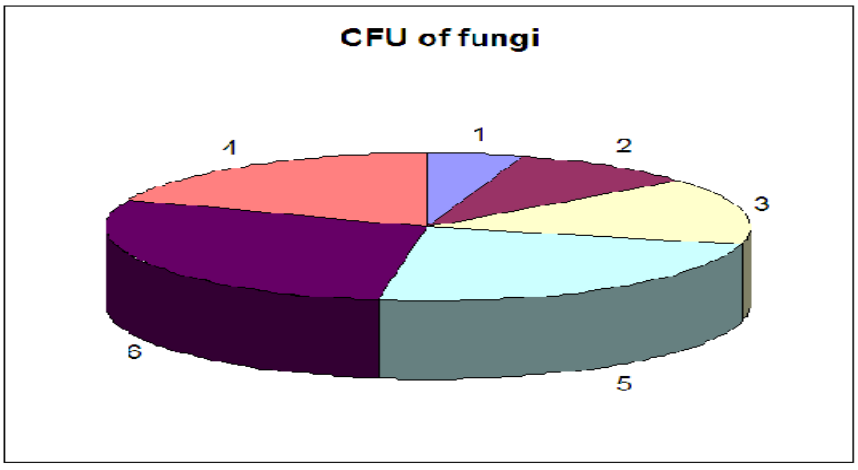


**Fig. 2. Duncan post Hoc test result for colony forming units of fungi present in the Worm-worked (WW) compost (90 d).**

Treatment 1. LL of TP + CD (1: 1), Treatment 2. LL of CA + CD (1: 1), Treatment 3. LL of GS + CD (1: 1), Treatment 4. LL of (TP + CA) + CD (1:1:2), Treatment 5. LL of (CA + GS) + CD (1:1:2), Treatment 6. LL of (TP + GS) + CD (1:1:2).

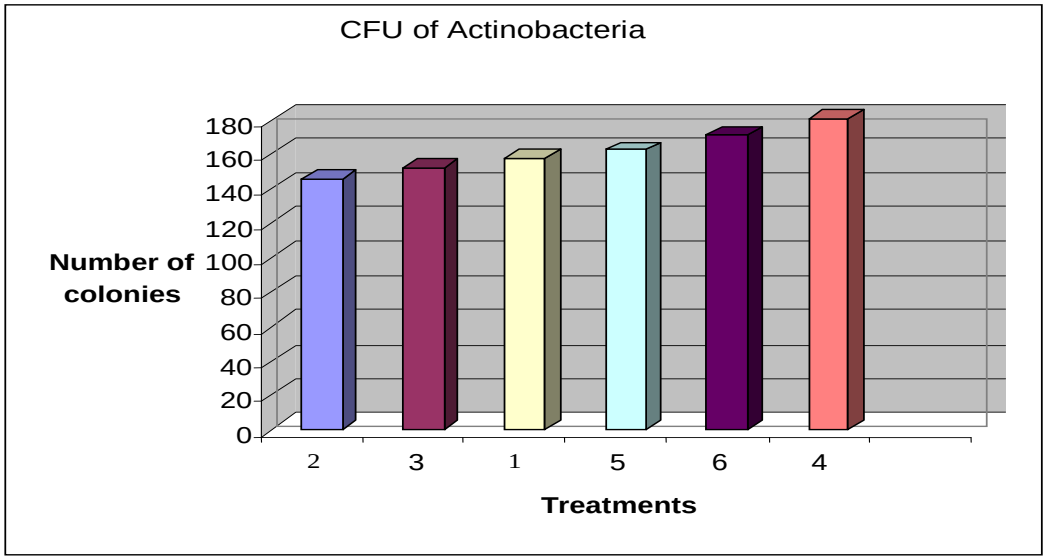


**Fig 3. Duncan post Hoc test result for colony forming units of Actinobacteria present in the Worm-worked (WW) composts (90 d).**

Treatment 1. LL of TP + CD (1: 1), Treatment 2. LL of CA + CD (1: 1), Treatment 3. LL of GS + CD (1: 1), Treatment 4. LL of (TP + CA) + CD (1:1:2), Treatment 5. LL of (CA + GS) + CD (1:1:2), Treatment 6. LL of (TP + GS) + CD (1:1:2).

## DISCUSSION

Studies on microbial colony forming units have established the supremacy of vermicompost over the worm-unworked compost by their presence in higher counts, indicating their symbiotic association with earthworms, which are essential for the biodegradation of organic wastes. The inter-relationship of microorganisms with macro organisms by their presence inside or outside the body of macro organisms and in the environment has been established already by Tiwari and Mishra (1993), Heijnen and Marinissen (1995), Lavelle et al. (1995) and Parthasarathi and Ranganathan (1999). Relations between earthworms and microorganisms are diverse and complex. Interactions between earthworms and microorganisms are also observed suggesting that these media increase microorganism activities. Moreover, food preferences are revealed for some earthworm species, indicating specific associations. Microorganisms are an unavoidable constituent of earthworms' natural diet (Edwards et al. 1996). Some microbes are preferentially ingested by earthworms while others are rejected. No study explains how earthworms can choose a particular

microorganism. The decaying of organic material is the most important action in the natural environment of vermicomposting worms. A natural microbial decomposition proceeds with a succession of microorganisms (Edwards 1985). Thus the present investigation brings out the actual microbial diversity among the worms from the vermicast of different leaf litters.

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