Estimating the Level of Phosphate Solubilising Bacteria and Azotobacter in the Vermicompost of Eudrilus Eugeniae and Perionyx Excavatus with Various Combinations of Cow- Dung and Saw-Dust

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Abstract*-** Vermicomposting using *Eudrilus eugeniae* and *Perionyx excavatus* was carried out from May to October 2012 by Tray method wherein the worms were fed with cow-dung and saw-dust. The phosphate solubilising bacteria and Azotobacter were estimated in both precompost and vermicomposts prepared in different ratios of the raw materials (1:1, 1:1.5, 1:2 and 1:3). The samples were serially diluted between $10^{\square\square}$ $\square\square 10^{\square\square}$ and inoculated on to the Pikovskaya's medium and Jensen's medium which were then incubated for 24 h. The total number of phosphate solubilising bacteria and Azotobacter colony was counted. Each colony was stained with methylene blue and identified by its morphological characters. Three different groups of Phosphate solubilising bacteria (*Bacillus, Streptomyces,and Pseudomonas)* and one nitrogen fixing bacteria *(Azotobacter)** were identified.

Index Terms- Vermicompost, Pre-compost, *Eudrilus eugeniae, Perionyx excavatus*, Phosphate solubiliser, Azotobacter.

I. INTRODUCTION

In sustainable agriculture, vermicompost plays a crucial role. In this technology, the organic wastes are converted into nutrient compounds with the help of earthworms as natural reactor [1]. The vermicompost contained desirable nutrients, plant growth hormones and high levels of soil enzymes [2,3]. Previously, the vermicompost was produced from various organic materials such as horticultural residues [4], mushroom wastes [5], horse wastes [6], pig wastes [7,8] brewery wastes[9], sericulture wastes[10], municipal sewage sludge [11,12] agricultural residues [13] weeds [14] cattle dung[15] industrial wastes [16,17] sludge from paper mills and dairy plants [18,19] domestic kitchen wastes[20], urban residues and animal wastes[21,22]. The vermicompost productions mainly rely on earthworm species, and the desirable composition of the feeding materials. The earthworm species life cycle, feeding habits and reproduction must be known in order to produce high quality vermicompost. The vermimicrobial populations play a crucial role to hold the nutrients over longer periods without adverse impacts on the environment; the microbial populations also help to solve the acid soil problems, because the pH of vermicompost materials is neutral to alkaline. The diverse population of the microorganisms favours the biochemical reactions between vermicompost and the plants. So it's very important to isolate and identify the microbes from vermicompost[23]**.** The microbial composition in vermicompost varies based on the type of earthworm and feeding materials. In this work, we used the following earthworm species *i.e*., *E. eugeniae* and *P. excavatus* to produce vermicompost. The *E. eugeniae* which is commonly called 'African Night crawler' has high rates of growth and reproduction and is capable of decomposing large quantities of organic wastes rapidly [24]. *P. excavatus*, a tropical earthworm is extremely prolific for use in vermiculture. It is a commercially produced earthworm. They are also known as "blues" or "Indian blues". This species is particularly good for vermicomposting in tropical and sub-tropical region [25]. Mayiladuthurai taluk (study area) in Nagapattinam district is regarded as a portion of "Granary of Tamil Nadu" supports diverse assemblage of earthworm species coupled with other agricultural wastes and hence the present study assumes importance.

II. MATERIALS AND METHODS

Collection of raw materials:

The raw materials such as cow-dung was collected from Mannampandal area, and the saw-dust collected from two different saw mills located at Mayiladuthurai in Nagai district.

Pre-composting and vermicomposting:

The pre-compost was prepared by mixing cow-dung and saw-dust in the ratio of 1:1, 1:1.5, 1:2 and 1:3 separately in plastic bowls and by sprinkling water for the first two days. After 20 days the pH of the pre-compost was adjusted to be neutral (pH 7). 6 kg of each ratio was taken in a set of five experimental and control plastic trays (46 cm length,26 cm width,13.5cm depth) for each species (*E. eugeniae* and *P. excavatus*). The earthworms were inoculated in the appropriate trays and the trays were covered with nylon mesh to protect them from the predators. Moisture was maintained in the composts for 40 days by sprinkling water daily. After the treatment the vermicomposts were collected, and then the phosphate solubilising bacteria and Azotobacter colonies were isolated and their populations were counted by using colony count method.

Isolation of phosphate solubilising bacteria:

2g of vermicompost as well as pre-compost and raw cow-dung were serially diluted with distilled water from $10^{\square\square}$ to $10^{\square\square}$ separately. 45g of Pikovskaya's medium (26) was dissolved in 1000 ml of distilled water and autoclaved for about 30 min.

Then the medium was poured in the petriplates and allowed to cool. After solidification the serially diluted samples were inoculated by pour plate method in the appropriate plates. The plates were incubated at 37°C for 48 h and the bacterial colonies with different phosphate solubilisers were counted and identified by their physical characteristic features such as size, form and shape, and by simple staining using methylene blue dye.

Isolation of Azotobacter in vermicompost:

2g of vermicopost as well as pre-compost and raw cow-dung were serially diluted with distilled water from $10^{\square\square}$ to $10^{\square\square}$separately. 47g of Jensen's medium (26) was dissolved in 1000 ml of distilled water and autoclaved for about 30 min. Then the medium was poured in the petriplates and allowed to cool. After solidification the serially diluted samples were inoculated by pour plate method in the appropriate plates. The plates were incubated at 37°C for 48 h and different types of Azotobacter were counted and identified by their different colour, physical characteristic features such as size, form and shape, and by simple staining using methylene blue dye. After forty eight hours they become brick red colour and they were confirmed as Azotobacter.

III. RESULTS AND DISCUSSION

Vermicomposts were prepared by using different composition of cow-dung and saw- dust by inoculating two different earthworm species *viz*., *E. eugeniae* and *P. excavatus* .The phosphate solubilising bacteria [27,28] and Azotobacter colonies [29] were isolated from each vermicompost as well as precompost. Totally, three different bacterial colonies (Bacillus, Streptomyces and Pseudomonas *sp*.) were isolated and identified based on their colony morphology as well as microscopical structure of the individual bacteria and it was shown in Fig 1. Bacillus, Streptomyces and Pseudomonas *sp*. and are all the most powerful phosphate solubilizers [30] and one nitrogen fixing bacteria[31] such as Azotobacter (Fig 2).

In the present study the Phosphate solubilisers and Azotobacter colonies length in compost were measured based on both before and after of *E. eugeniae* and *P. excavatus* and it was shown in Table 1. From the results, the *E. eugeniae* treated vermicompost showed a maximum phosphate solubilisers and azotobacter colonies length. The population of microorganism was significantly increased by 30 per cent in *E. eugeniae* treated samples and the results were shown in Table 2 and 3. The population of bacteria in microbially enriched manures (cow dung and saw dust) showed significantly increased by 50 per cent as compared to the manures treated with *E. eugeniae and P. excavatus*

The results further revealed that Phosphate solubilisers are the most powerful solubilisers as they contain insoluble mineral phosphates which get converted as the single phosphates sources [32]. Azotobacter in the presences of vermicompost is reported to be more effective than other bio-fertilizers. They mainly promote nitrogen fixing of the soil [33]. The phosphate solubilising bacteria and Azotobacter in vermicompost of an unusual waste (saw-dust) was checked and vermicompost of saw-dust with cow-dung at different ratios favoured the growth of certain phosphate solubilising bacteria such as Bacillus, Streptomyces, and Pseudomonas *sp*. and nitrogen fixing bacteria Azotobacter as well as the phosphate solubilising capacity of these bacteria has already been verified by many investigators [27,28]. Further, the results of present study support the role of earthworm in recycling the saw wastes in an effective manner with environmental protection. Hence, we suggest to utilising the saw-dusts in the preparation of vermicomposts using earthworms such as *E. eugeniae* and *P. excavatus* without polluting the environment. Further, the vermicomposts prepared by using saw-dusts as substrate would be more suitable natural fertilizer to improve the soil fertility since they contain significant quantity of Azotobacter and phosphate solubilising bacterial population. Further, vermicastings are effective bio–fertilizers containing beneficial soil micro-flora and earthworm cocoons. When covered with a layer of mulch in the soil, they produce a huge earthworm population of organic fertilizer and in this way it is an essential component of natural farming.

IV. CONCLUSIONS

In conclusion, the experimental data here demonstrate that the vermicompost produced by *E. eugeniae* and *P. excavatus* is capable of producing Azotobacter and phosphate solubilising bacterial populations. The results of the microbial population indicated that the Azotobacter and phosphate solubilising bacterial population is 50 % higher in *E. eugeniae* produced compost using cow-dung and saw-dust mixer. The valuable bacterial strains quantified in this study may be useful in the production high quality compost for sustainable agriculture.

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Figure captions:

Figure 1. Culture plate Results showing different Phosphate solubilizing bacteria in Pikovskaya's medium .

Figure 2. (a) Culture plate results showing Azotobacter in Jensen's medium. (b) Microscopic view results (40x) of Azotobacter collected from vermicompost, after staining with methylene blue dye

Table1. A comparison of mean length and width of Phosphate solubilizing bacterial (PSB) colonies and Azotobacter colonies in control and vermicomposts of two different species *(E. eugeniae* and *P. excavatus)*.

S. No.	Species		Mean Length of PSB and Azotobacter colonies (cm)	Mean Width of PSB and Azotobacter colonies (cm)
1	Control		0.84 ± 0.4094	0.48 ± 0.2513
2	Vermicompost	*E. eugeniae*	1.31 ± 0.3685	0.85 ± 0.3478
3		*P. excavatus*	0.85 ± 0.2989	0.49 ± 0.1526

S. No.	Different Ratios of cow-dung and saw-dust	Control		*E. eugeniae*		*P. excavatus*	
		Length (cm)	Width (cm)	Length (cm)	Width (cm)	Length (cm)	Width (cm)
1	Cow-dung	0.68 ± 0.22	0.34 ± 0.15	1.33 ± 0.56	0.84 ± 0.43	0.84 ± 0.18	0.47 ± 0.13

Table 2. A comparison of mean length and width of microorganisms treated with various ratios of cow-dung and saw-dust in vermicomposts of E. eugeniae and P. excavatus

2	1:1	0.59 ± 0.20	0.29 ± 0.09	1.07 ± 0.46	0.59 ± 0.32	0.89 ± 0.30	0.57 ± 0.23
3	1:1.5	0.72 ± 0.38	0.42 ± 0.17	0.87 ± 0.45	0.55 ± 0.33	0.94 ± 0.45	0.69 ± 0.36
4	1:2	0.70 ± 0.30	0.46 ± 0.23	1.24 ± 0.33	0.79 ± 0.38	0.78 ± 0.30	0.47 ± 0.19
5	1:3	1.42 ± 0.70	0.70 ± 0.23	0.90 ± 0.46	0.48 ± 0.23	0.83 ± 0.37	0.48 ± 0.18

Table 3. Analysis of Variance (ANOVA) to study the impact of length and width of phosphate solubilizing bacteria and Azotobacter in vermicomposts (E. eugeniae and P. excavatus)

S.no	Parameters	Sources	DF	Seq ss	Adj MS	F	P
1	Length	Species	2	2.89	1.47	9.48	0.00
2		Ratio	4	0.41	0.07	0.44	0.78
3		Dilution factor	4	1.74	0.43	2.81	0.03
4		Error	131	20.27	0.15		
5		Total	141	25.31			
6	Width	Species	2	1.43	0.71	10.20	0.00
7		Ratio	4	0.13	0.03	0.47	0.75
8		Dilution factor	4	0.65	0.16	2.35	0.06
9		Error	131	0.05	0.07		
10		Total	141	11.26			

Figure 1.

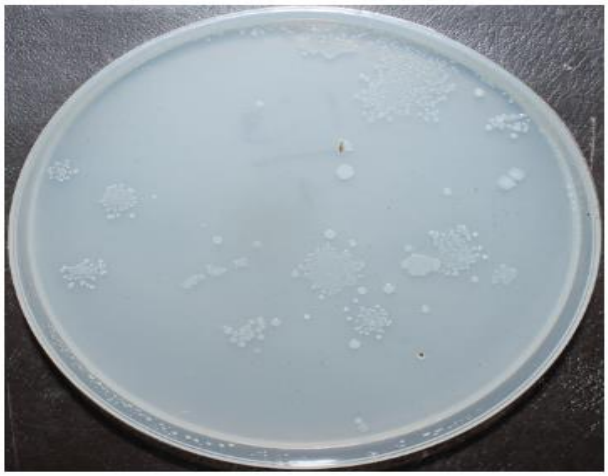

Figure2.