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Influence of *Jeevamrutha* (Biodynamic Formulation) on Agro-Industrial Waste Vermicomposting

Santhebennur Jayappa Veeresh[1] • Jogattappa Narayana[1*] • Jaime A. Teixeira da Silva[2]

[1] Department of P.G Studies and Research in Environmental Science, Bioscience Complex, Kuvempu University, Shankaraghatta-577451, Karnataka State, India
[2] Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Ikenobe 2393, Kagawa-ken, 761-0795, Japan

Corresponding author: * janaes@rediffmail.com

ABSTRACT

This study aimed to determine the influence of cow dung and a biodynamic microbial consortium (*jeevamrutha*) on the microbial population during the conversion of papermill and sugar factory sludge into beneficial vermicompost. The bacterial and actinomycete densities were highest in the *jeevamrutha*-treated group and fungal density was higher in the treatment group treated with cow dung. The microbial density in earthworms of treatment groups inoculated with or without microbial culture was significantly different to that of the control group. Furthermore, there was a significant relationship between microbial density and treatment groups. The inoculation of microbial consortia like *jeevamrutha* and cow dung together with organic substrates significantly enhances the microbial density throughout the process of decomposition.

Keywords: biodynamic agriculture, earthworms, microbial density, vermicompost
Abbreviations: AH, areca nut husk; **CD**, cow dung; **cfu**, colony-forming unit; **EM**, effective microorganism; **PD**, press dug; **PM**, press mud

INTRODUCTION

Vermicomposting is a decomposition process involving interactions between earthworms and microorganisms. Although the microorganisms are responsible for the biochemical degradation of organic matter, earthworms are the crucial drivers of the process by fragmenting and conditioning the substrate, increasing surface area for microbiological activity and altering its biological activity dramatically (Domínguez 2004). Earthworms can affect soil microflora and faunal population directly and indirectly by three main mechanisms: (1) comminution, burrowing and casting; (2) grazing; (3) dispersal. These activities change the substrate's physico-chemical and biological status and may cause a drastic shift in the density, diversity, structure and activity of microbial communities within the drilosphere (Brown 1995). Therefore, earthworm communities may influence the spatial variability of resources, altering their availability to microorganisms, thereby regulating nutrient cycling processes (Marinissen and De Ruiter 1993; Aira *et al.* 2006). Brown *et al.* (2000) stated that microorganisms are the main agents responsible for the decomposition process.

The general strategy of composting is to inoculate a microbial consortium in order to accelerate the rate of decomposition and introduce a higher population density of beneficial bacteria and fungi. The technique also stimulates the establishment of microbial diversity for enhanced growth and activity of mixed microbial populations that are indigenous to the organic substrates. The improved methods for composting are Indian Bangalore, Indorepit (FAO 1980) and Coimbatore methods (Manickam 1967), passive composting of manure piles (NRAES 1992), Berkley's rapid composting method – shredding and frequent turning (Raabe 2001), North Dakota State University hot composting – use of a mineral nitrogen activator (Smith 1995), EM-based quick composting (Hiraoka 2002), and IBS rapid composting technology (Cuevas 1997).

Apart from these methods, some progressive farmers have developed biodynamic composting technologies. Steiner prescribed 9 different preparations to aid the fertilization of soil which are the cornerstones of biodynamic agriculture, and described how these should be prepared; he believed that these preparations transferred supernatural terrestrial and cosmic "forces" into the soil (Kirchmann *et al*. 2008). These prepared substances are numbered 500 through 508, out of which formulation-500 (cow horn compost) and formulation-501 (horn-silica) are very popular and are being used by a large number of organic farmers in India. Formulations-502 to 507 are used as compost enrichers and promoters (Steiner 1974). Similarly, Subhash Palekar is one of the progressive farmers of Maharashtra, India; in his workshop on "Philosophy and Technology of Zero Budget Natural Farming he used a new biodynamic formulation termed *jeevamrutha* (Palekar 2006). Vanaja *et al*. (2009) stated that *jeevamrutha* is a plant growth-promoting substance containing beneficial microorganisms that provides the necessary nutritional requirement for growth and yield of a crop. The microorganisms that supply nitrogen like *Azotobacter*, *Acetobacter*, *Azospirillum* and phosphorus-solubilizing bacteria like *Pseudomonas* and potash-solubilizing bacteria like *Bacillus silicus* are present in dung that is used to prepare *jeevamrutha*. Microorganisms are well activated in soil following the addition of *jeevamrutha* which also maintains soil productivity. Manjunatha *et al.* (2009) reported that the use of *jeevamrutha* (indigenous species cow dung (CD) and cow urine, pulse flour, jaggery, rhizosphere soil solution)-treated organics, improves the physico-chemical and biological properties of soil, besides improving the efficiency of applied farmyard manure. They also confirmed that the potential of *jeevamrutha* is to supply materials and to act as food support for beneficial microbes. In view of the above, the current study focused on determining the influence of microbial inoculants such as conventional CD and a biodynamic formulation i.e., *jeevamrutha*, on bacterial, fungal and actinomycete densities during vermicomposting. Another objective was to determine the effects of duration of composting and inocu-

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Table 1 Treatment for rapid vermicomposting process.

Treatment*	Ingredients	Weight (kg)	Total weight (kg)
Control	AH + PD + PM	0.5 + 2 + 2	4.5
T1	AH + PD + PM	0.5 + 2 + 2	4.5
T2	AH + PD + PM + CD	0.5 + 2 + 2 + 0.5	5
T3	AH + PD + PM + J	0.5 + 2 + 2 + 45 ml	4.5

AH = areca nut husk ; PD = press dug ; PM = press mud + *jeevamrutha*; CD = cow dung.
* For T1, T2 and T3, beddings were removed on the 21st day of vermicomposting.

lation of the microbial consortium on the microbial population during industrial sludge composting.

MATERIALS AND METHODS

Collection of substrates

The earthworms for composting, *Eudrilus eugeniae* (Kinberg), were obtained from the Banashankari earthworm-rearing centre, Dummahalli, Shivamogga, India. There are two main agro-based industries located in Bhadravathi Town; Mysore Paper Mill (MPM) and Sugar Factory (SF), both having a single effluent treatment plant. Press dung (PD) was collected from the primary treatment unit and liquid biomass, termed bio-sludge or press mud (PM), was collected from the secondary treatment unit. PD consists of a large lignocellulosic part that is filtered in the primary treatment plant, while PM consists of both soil and lignocellulosic parts that is removed from the secondary treatment unit. To maintain favorable conditions, areca nut husk (AH), procured from the orchards of Bhadravathi Taluk, was mixed with the composting material to facilitate aeration during vermicomposting. Fresh CD was collected from the local farmer cow yards of Shankarghatta village and *jeevamrutha* was prepared in the laboratory.

Preparation of *jeevamrutha*

A standard procedure was used (Shankaran 2009): 125 g of CD, 125 ml of cow urine, 25 g of dicotyledonous seed (mung bean, *Phaseolus mungo*) powder, 250 g of old jaggery (obtained from the jaggery house, Bhadravati town), 1 handful of fertile soil and 2.5 L of tap water were used to prepare 2.5 L of *jeevamrutha.* All the ingredients were mixed in a plastic pot; the mouth of the pot was covered with wet gunny cloth and the pot was kept in the dark for 72 hrs. During this period the content was mixed thoroughly with a wooden stick every 24 hrs. After the 72-hr incubation period, *jeevamrutha* was stored in a polythene bottle at 4°C until further use.

Experimental treatments

The collected organic substrates were maintained at 60-80% relative humidity at 25-28°C by spraying the surface of each type of residue with tap water (500 ml/day). After 2 days of moistening, the organic substrates were subjected to different treatments by mixing different proportions of ingredients (**Table 1**). Composting was carried out in labeled rectangular plastic polystyrene tubs measuring 0.45 m × 0.30 m × 0.15 m (length × width × depth) in triplicate.

Partial decomposition

Partial decomposition was carried out by sprinkling water on the vermibeds to 60-80% relative humidity and all treatments were mixed weekly. All vermibeds were maintained in similar conditions for up to 15 days for thermal stabilization, initiation of microbial degradation and softening of waste. T3 was treated by 45 ml of *jeevamrutha* with 7-day intervals up to the 21st day of the vermicomposting process.

Vermicomposting

After initial decomposition of organic substrates, 3-4 week old clitellated and non-clitellated earthworms (*Eudrilus eugeniae*) were inoculated (optimum earthworm density = 10 g worms per 1 kg organic waste) (Domínguez 2001) into labeled plastic polystyrene tubs. Vermicomposting was terminated at the end of the 21st day after which the worms were separated from the vermibeds. The fungal, bacterial and actinomycete population from each treatment was determined by the dilution plate technique (Walksman 1917) every 7 days. In each treatment, composite 10 g of decomposing organic substrate samples were taken and 1 g from each sample was suspended in 1 ml sterile saline (1 g NaCl in 100 ml distilled H_2O) in a sterile test tube and thoroughly vortexed. The tubes from different treatments were used as inocula for enumerating fungi, bacteria and actinomycetes. From this stock various dilutions were prepared i.e., 10^{-1} to 10^{-6} with sterile distilled water. One ml of the 10^{-6} dilution was transferred to a Petri dish containing soil extract agar (James 1958) medium, 1 ml of the 10^{-4} dilution to a Petri dish containing streptomycin rose Bengal agar (Martin 1957) medium and 1 ml of the 10^{-5} dilution to a Petri dish containing starch casein agar (Kuster and Williams 1964) medium and inoculated by the spread plate technique to study the make-up of bacteria, fungi and actinomycetes, respectively. The bacterial count was determined after incubating for 2 days at 28°C; actinomycetes and fungi were counted after 10-15 and 7 days' incubation at 28 and 25°C, respectively. Results are presented as the number of colony-forming units (cfu) expressed per 1 g of decomposing organic substrate dried at 105°C (Arun 2006).

Statistical analysis

Data was evaluated with SPSS v. 12. Differences in the means of microbial density in different treatment and control groups were assessed by the paired sample *t*-test. A Pearson correlation coefficient was made to know the relationship existing between composting duration, microbial consortiums inoculation and microbial densities.

RESULTS AND DISCUSSION

Bacterial density

Bacterial density in the different treatment groups is presented in **Table 2**. It ranged from 1159.33 ± 31.67 to 563.33 ± 28.67 cfu $g^{-1} \times 10^{-6}$ on the first day of degradation of organic substrates. This stage had the highest bacterial density compared to latter stages of the vermicomposting process. Gaur *et al.* (1980) also found that the mesophillic bacterial type was dominant in the early stages of composting. The bacterial population decreased on the 7th and 14th days of decomposition of organic substrates with a gradual increase observed at the end of the experiment. The maximum level of bacterial colonies was recorded in T3 i.e., *jeevamrutha*-inoculated treatments compared to other treatment groups on the 21st day of composting. Mader *et al*. (1995) also reported that biodynamic FYM increased soil microbial biomass and biological activity compared to fertilisation without biodynamic preparations during there study on effects of low and high external input agriculture on soil microbial biomass and activities for sustainable agriculture. The bacterial population showed strong positive correlation with treatment groups and actinomycetes density (**Table 5**). The paired sample *t*-test showed the significant difference between control treatments and earthworm inoculated treatments but the *jeevamrutha*-inoculated treatment group showed highly significant difference to the control group in all the stages of vermicomposting process (**Table 2**). The variation in composition of composting beds affects the density of bacteria and also the actinomycetes' density regulated by bacterial density. Apart from the bedding material,

Table 2 Bacterial density (cfu $g^{-1} \times 10^{-6}$) during rapid vermicomposting (mean ± SD) and the variation between the control group and the treatment groups.

Treatments	0	7	15	21
Control	563.33 ± 28.67	586.33 ± 36.67	590.33 ± 29.67	610.67 ± 30.33
T1	565.00 ± 33.00	446.67 ± 38.33**	512.67 ± 29.33**	633.67 ± 33.33*
T2	825.00 ± 33.00**	612.00 ± 32.00**	591.00 ± 39.00	786.00 ± 28.00**
T3	1159.33 ± 31.67**	1068.33 ± 30.67**	930.33 ± 42.67**	956.67 ± 31.33**

Paired sample *t*-test; * ($P < 0.01$); ** ($P < 0.001$), df = 2.

Table 3 Actinomycete density (cfu $g^{-1} \times 10^{-5}$) during compost-vermicomposting (mean ± SD) and the variation between the control group and the treatment groups.

Treatments	0	7	15	21
Control	136.91 ± 5.09	137.82 ± 10.18	139.46 ± 8.54	144.72 ± 9.28
T1	136.67 ± 11.33	124.67 ± 6.33	126.00 ± 8.00**	158.67 ± 9.33**
T2	156.33 ± 11.67	137.67 ± 9.33	129.33 ± 8.67**	184.00 ± 11.00**
T3	274.00 ± 9.00**	239.00 ± 12.00**	236.00 ± 10.00**	252.00 ± 9.00**

Paired sample *t*-test; * ($P < 0.01$); ** ($P < 0.001$), df = 2.

Table 4 Fungal density (cfu $g^{-1} \times 10^{-4}$) during compost-vermicomposting (mean ± SD) and the variation between the control group and the treatment groups.

Treatments	0	7	15	21
Control	112.78 ± 9.22	114.44 ± 10.56	115.11 ± 10.89	116.65 ± 10.35
T1	110.00 ± 8.00	126.67 ± 6.33	152.00 ± 9.00**	143.90 ± 12.10*
T2	196.00 ± 15.00*	204.00 ± 11.00**	256.00 ± 8.00**	212.00 ± 13.00**
T3	131.00 ± 11.00*	136.00 ± 9.00*	169.00 ± 9.00**	157.00 ± 12.00**

Paired sample *t*-test; * ($P < 0.01$); ** ($P < 0.001$), df = 2.

earthworms also play a crucial role in alteration of microbial density. Aira *et al*. (2002) stated that earthworms are important drivers of the process, conditioning the substrate and altering its biological activity. The burrowing and casting activities of earthworms contribute to the activity of soil microorganisms (Edwards and Bohle 1996) and nutrient-enriched earthworm casts are good media supporting microbial growth (Lee 1985). The final products from the experiment revealed that highest bacterial density was found in the treatment group of earthworms + *jeevamrutha*-inoculated soil when compared to all other control and treatment groups.

Actinomyceties density

The variation of actinomycete density among the different treatment groups is presented in **Table 3**. The population of actinomycetes ranged between 136.67 ± 11.33 and 274.00 ± 9.00 cfu $g^{-1} \times 10^{-5}$ in all the decomposing organic substrates at the initial stage of the vermicomposting process. The actinomycete population also showed the same trend as bacterial density i.e., the density decreased on the 7th and 14th days of decomposition of organic substrates and a gradual increase was observed at the end of the experiment. The highest actinomycete population (252 ± 9 cfu $g^{-1} \times 10^{-5}$) was recorded in the treatments treated with *jeevamrutha* at the last stage of the vermicomposting process. A correlation analysis of the actinomycete population showed a significant positive relationship with the treatment groups and with bacterial density (**Table 5**). These results revealed that the variation in treatment composition and density of bacteria affects actinomycete density. Insam *et al.* (2002) noted that actinomycetes compete with others organisms for nutrients and can inhibit microbial growth due to the production of antibiotics, lytic enzymes or even by parasitism; the interaction between various functional groups of microorganisms depends on nutrient resources and the biochemical mechanisms of organic and inorganic matter transformation changes. The paired sample *t*-test showed significant differences in actinomycete density in earthworm-inoculated treatment groups when compared to the control group (**Table 3**). The *jeevamrutha*-inoculated treatment group showed highly significant values during all stages of the vermicomposting process (**Table 3**).

Table 5 Relationship between treatment groups, duration of composting and microbial density.

	Bacteria	Actinomycetes	Fungi
Treatment	0.807**	0.779**	0.482**
Duration of composting	-0.039	0.064	0.084
Bacteria		0.952**	0.105
Actinomycetes			0.023

** Correlation is significant at $P = 0.01$ (2-tailed).

Fungal density

The variation in fungal density in the different treatment groups is presented in **Table 4**. The fungal population ranged from 110 ± 8 to 196 ± 15 cfu $g^{-1} \times 10^{-4}$ in decomposing organic substrates on the first day of the vermicomposting process. The population increased dramatically up to the 14th day; thereafter, a decrease was observed until the end of the organic substrate decomposition period, except for the control group, in which the density of fungi progressively increased but was comparatively less than other treatment groups. The highest (212 ± 13 cfu $g^{-1} \times 10^{-4}$) fungal populations were found in T2 i.e., treatment treated with CD at end of the vermicomposting process. In the *jeevamrutha*-treated treatment group, fewer fungi were noticed compared to bacteria. Carpenter *et al.* (2000) noted the same thing during their study on the effects of biodynamic preparations on compost development; they found that a larger proportion of bacteria and smaller proportion of fungi were observed in biodynamic (BD) composts than in the control. The fungal indicators (content of fatty acids) 18:1 w9c was significantly lower ($p < 0.05$), and 18:2 w6c also tended to be lower in completed BD composts. The phospholipid fatty acid indicators of bacteria 17:0 anteiso, 17:0 iso, and 17:1 w6c were greater while 16:1 w7c was lower in BD composts vs. control composts. However, the fungal population in our study showed a strong positive correlation with the treatment groups (**Table 5**). This finding corroborates earlier studies by Oyun *et al*. (2006) and Couteaux *et al.* (1991) which suggested that fungi are more strongly influenced by substrate quality whereas the bacterial population is largely regulated by predation by the fungal and actinomycetes population. The paired sample *t*-test showed a significant difference between control treatments and earthworm-inoculated treatments, but the CD-inoculated treatment group showed a highly significant difference with

the control group during all stages of the decomposition process (**Table 4**).

The above results showed that the inoculation of a microbial consortium during organic substrate decomposition enhances the microbial density and concomitant increase in the rate of decomposition. Singh and Sharma (2002) also made the same observation regarding the role of bioinoculants i.e., *Pleurotus sajor-caju*, *Trichoderma harzianum*, *Aspergillus niger* and *Azotobacter chroococcum*, in predecomposition of mixed solid waste and horticulture waste (70: 30) on subsequent vermicomposting; they observed that this system not only improved the quality of the product but also reduced the stabilization period. Manjunatha *et al*. (2009), in a study on the effect of farmyard manure treated with *jeevamrutha* on yield attributes, yield and economics of sunflower *(Helianthus annuus* L.) reported that the application of *jeevamrutha* to soil increased the activity of microbes by enhancing the solubilisation and uptake of nutrients.

CONCLUDING REMARKS

The process of conversion of agro-industrial waste to manure through rapid vermicomposting revealed that the inoculation of a biodynamic consortium (*jeevamrutha*) to organic substrates enhanced the bacterial and actinomycete densities than treatments including CD and control groups. However, mixing CD together with decomposing materials exhibited the highest fungal density compared to other treatment groups. When *jeevamrutha* was combined with earthworms, there was a significant improvement of microbial and bacterial density, which was significantly correlated with the actinomycete population. Finally, this study concludes that *jeevamrutha* and CD are better microbial consortia to enhance microbial density and vermicomposting than if none were to be used.

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