

Pre-composting of *Ficus racemosa* using microbial decomposers

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ABSTRACT

The rate of decomposition can be enhanced by treating the litters initially with certain efficient microflora. Inoculants such as *Bacillus subtilis*, *Trichoderma viridae*, *Trichoderma harzianum* are known to degrade cellulose and lignin. Inoculation with phosphate solubilising *Pseudomonas fluorescence* may help to solubilise phosphorus and increase its availability to plants. Inoculation of *Lactobacillus* spp (EM) is known to accelerate the process with specific antagonistic properties with other microbes. Therefore these microbes were used as inoculants during pre-decomposition of the litters of *Ficus racemosa* mixed with different proportions of cow dung to reduce the time of composting

Keywords; *Bacillus subtilis*, *Ficus racemosa*, *Lactobacillus* spp, *Pseudomonas fluorescence* *Trichoderma harzianum* and *Trichoderma viridae*.

INTRODUCTION

Composting is a biological process through which microorganisms convert the waste organic materials into useful end products [1], which is useful to soil conditions and plants as an organic fertilizer [2]. This process uses various microorganisms such as bacteria, fungi and actinomycetes to breakdown the organic compounds into simpler substances..

Composting process can be divided into two main phases the active phase (consisting of sequential mesophilic, thermophilic and cooling steps) and the curing phase (alternatively termed the maturation phase). During active phase bio-degradable materials are broken down, transformed and partially mineralized in a series of steps, organic matter becomes stabilized as a consequence of the intense microbial activity. During composting substrates that are rich in cellulose and lignin the fungi serves as important degraders throughout the composting process. Usually, in the curing phase of composting the ratio of fungi to bacteria increases due

to the competitive advantages of fungi under poor substrate availability, meaning the predominance of difficult to degrade compounds such as lignin and humus.

Microorganisms play a predominant role in the mineralization of organic compounds, which is particularly evident during leaf litter decomposition. Microorganisms require carbon for growth and nitrogen for protein synthesis. Successful composting depends on several factors such as C/N ratio, particle size, aeration and humidity [3]. Both bacteria and fungi are present and are active in a typical composting process. Bacteria dominate the microbial community during the degradation phase [4]. During this phase large quantities of dissolved organic carbon are usually available in the substrate depending on the substrate (eg. Green waste and household waste) nitrogen may also be available in significant quantity. Fungal activity is mainly important in the maturation phase of composting [5]. Fungi decompose the complex carbon compounds advance the accumulation of organic material, and hold nutrients in the fungal biomass, dropping escape of nutrients out of the root zone. Fungus contains certain cellulolytic enzymes that decomposes organic substances [6] and can be used as a vector in composts, since it breaks down the carbon source and have been reported to have positive effect on plant growth and development.

In the present study, a commercial additive is added under the trade name AMIRTHA-D which contains an effective decomposer of the *Bacillus* sp, *Lactobacillus* sp, *Trichoderma* sp and *Pseudomonas* sp to the substrate containing mixture of leaf litters *Ficus racemosa* and cow dung in order to study the beneficial role of these microbes in decomposing leaf litters to produce compost to enhance nutritive value.

MATERIALS AND METHODS

The raw materials used in the present study for composting are:

Leaf litters of *Ficus racemosa*, Cow dung and Microbial fungal strains *Trichoderma viridae*, *Trichoderma harzianum* and the bacterial strains- *Pseudomonas fluorescens*, *Bacillus subtilis* and *Lactobacillus* sp (the additive AMIRTHA- D)

Collection of leaves and Cow dung

Leaves of *Ficus racemosa* were collected from in and around the College campus at Courtallam. Cow dung was procured from the livestock farm at Tenkasi and the collected leaves and cow dung was shade dried before using in the experiment.

Microbial source

A commercial additive under the trade name AMIRTHA-D an effective decomposer composed of the fungal strains *Trichoderma viridae*, *Trichoderma harzianum* and the bacterial strains- *Pseudomonas fluorescens*, *Bacillus subtilis* and *Lactobacillus* sp (Effective Microorganism) were procured from a non government Organization unit Kalpaviruzham at Tenkasi. These strains were obtained individually and also in combination with good compatibility.

Experimental Setup

Leaf litters of *Ficus racemosa* (FR) mixed with cow dung (CD) was used as substrate for composting. The experiments were conducted in plastic troughs of 10 L capacity with 5kg of the substrate. The moisture content of the substrate in each trough was maintained between 50 % and 60 % during the study period by periodic sprinkling of water which is considered as optimum for composting [7]. After 6 days of prior composting the microbial cultures were inoculated individually and also in different combinations in composters A -I. The composters are arranged as follows A - *Pseudomonas fluorescens*, B - *Bacillus subtilis*, C - *Trichoderma viridae* , D - *Trichoderma harzianum*, E - *Lactobacillus* spp (EM), F - Control (without inoculants), G - *Pseudomonas fluorescens* + *Bacillus subtilis* , H - *Trichoderma viridae* + *Pseudomonas fluorescens* , I - *Pseudomonas fluorescens* + *Bacillus subtilis* + *Trichoderma viridae* + *Trichoderma harzianum* + *Lactobacillus* spp. To each of the treatment the microbial cultures individually were inoculated using 50ml broth culture per kg substrate and in combinations 20 ml broth culture per kg substrate was added after diluting with distilled water. For mesophilic aerobic digestion, turning was done manually every 4 days and the temperature was not allowed to exceed 26°C by maintaining the moisture by adding water when necessary. The substrate with different treatments maintained in the reactors A - I was composted in triplicates for a period of 30 days. Samples from each reactor (A - I) were collected before and after inoculating microbes, for physico-chemical analysis of the composted product. Composting of *F. racemosa* was carried out in separate plastic troughs (A-I) .After the microbial inoculation into the composters, they were kept separately and composting was carried out for a period of 30days. The experiment was run in triplicate. The results (average of the triplicates) of the analyzed parameters are presented.

Compost analysis:

The pH and electrical conductivity (EC) were determined in 1: 10 compost - water suspension, according to the method of [8]. Total organic carbon (TOC) was determined by chromic acid wet digestion method [9] and total nitrogen (TN) by Kjeldahl microanalysis [10]. Total phosphorus (TP) was determined spectrophotometrically and potassium content (TK) was detected by the flame emission technique [11]. The C/N ratio was calculated from the obtained value of total organic carbon and the total nitrogen present in the compost. All the samples were analyzed in triplicate and the results were averaged for statistical analysis.

Statistical analysis

All the samples were analyzed in triplicate and the results were averaged and tested for their statistical significance using one way analysis of variance (Anova) to determine the differences among the parameters analyzed in different treatments during the composting process. Tukey's test was performed for various parameters. The probability levels used for statistical significance of tests were $p < 0.05$.

RESULTS AND DISCUSSION

Composting is the most suitable technique for transforming organic waste into usable agricultural amendments. Although the waste composition is very diverse, lignocellulose is the most abundant component which is responsible for limiting degradation [8]. The physico-chemical characteristics of the microbially composted leaf litters were analyzed and the results are tabulated in Table 1. During composting period microorganisms altered the properties like pH, EC, TN, TP, TK, TOC contents and C/N ratio towards desired levels over natural decomposition process. They stabilize the pH, EC and effectively mineralize N, P, K and carbon than the natural process.

Composting the leaf litter of *Ficus racemosa*

Table 1 presents the results of composting *F. racemosa* during the initial and final period, in all the composters FR-A to FR-I. After composting *F. racemosa* mixed with appropriate quantity of cow dung the results of the parameters like pH, EC, TOC, TN, TP, TK and C/N ratio of the compost inoculated with and without microbes were analyzed and the obtained results are tabulated in Table 2.

The pH in all the composter initially was more or less neutral ranging between (6.7 ± 0.065) to (7.8 ± 0.050) . But at the end of composting the pH has become alkaline in all the composters with highest reaching in FR - H (8.2 ± 0.016) and FR - I (8.5 ± 0.024) . Electrical conductivity (EC) in all FR composters has relatively increased with FR - I (1.84 ± 0.41) , FR - H (0.92 ± 0.25) and FR - G (0.89 ± 0.14) as highest on analysis of the final compost. Thus pH and EC have shown considerable variation in all FR composters (A-I) in comparison with that of control and with each other. The C/N ratio in FR - I compost was (47.89 ± 0.04) initially and finally it was significantly reduced to $(28.84 \pm 0.01) \%$. This reduction balancing the ratio of C to N has been noted in all FR composter when compared to control. The reduction in the ratio between C and N is indicative of compost maturity, which is the result of induced decomposition and nitrogen immobilization. The variation observed in physico-chemical characteristics of the end products of composted *Ficus racemosa* is dependent on different microbial inoculants used. The reduction in TOC and C/N ratio are presented through a diagram in Fig 1. There is an increase in electrical conductivity from the initial to final in all the FR composters A to I, Similar observations are reported by [12]. Increase in pH of the *F. racemosa* compost was observed. It was highest at 8.5 ± 0.024 pH in FR - I composter. According to [13] this variation in pH is influenced by several factors like temperature, moisture and aeration.

Fig. 1: Variations in Physico –chemical characteristics of nine types of composted

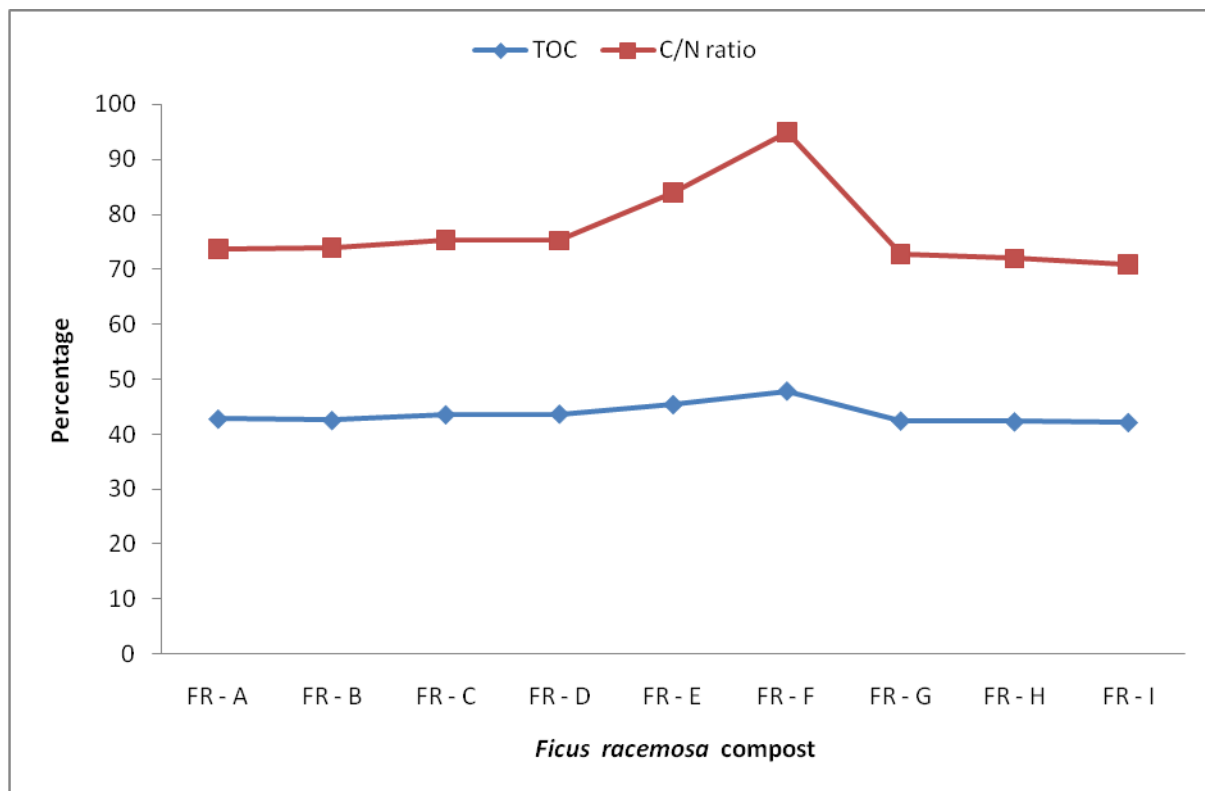
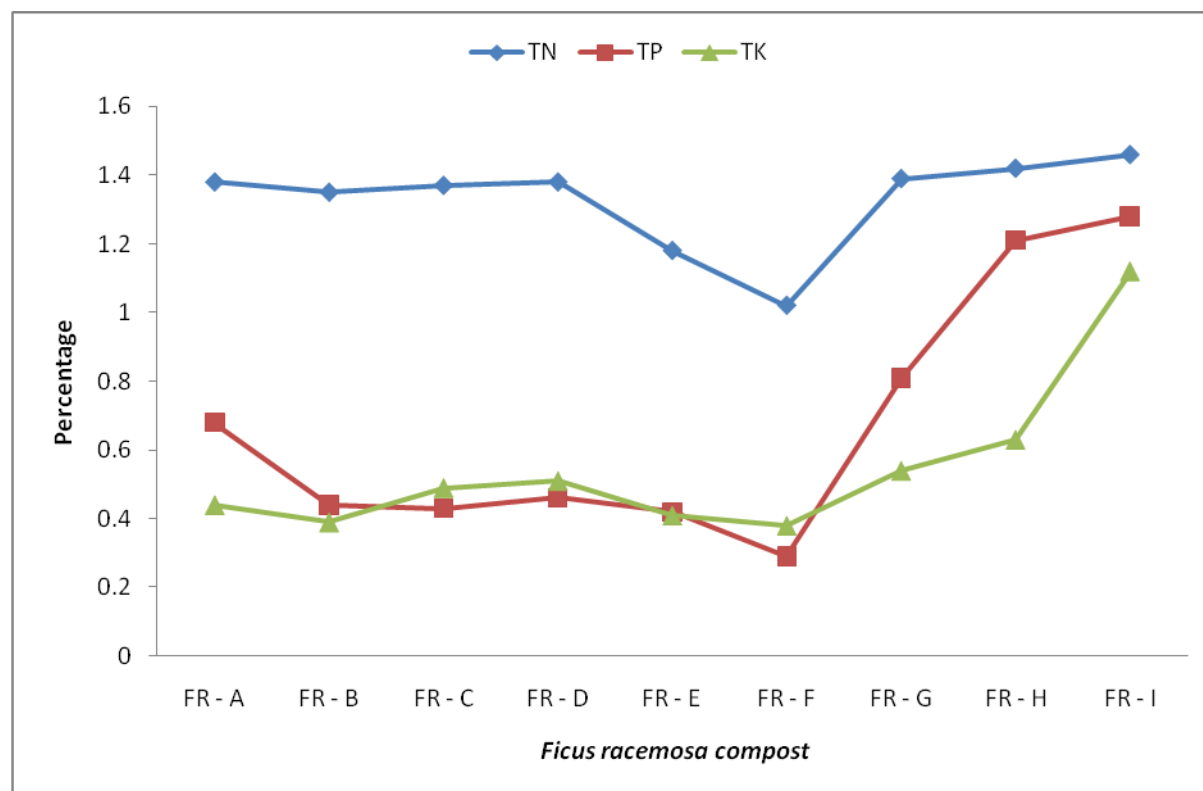
Ficus racemosa

Fig. 2: A diagrammatic representation of the available NPK content in nine types of composted *Ficus racemosa*



Total nitrogen (TN) is relatively higher in all the composters when compared with that of control composter, TN range was from 1.02 ± 0.012 in FR - F to 1.46 ± 0.024 in FR - I. However in the present study the change was noticeable from initial to final, when the C/N ratio is less than 20, the compost is mature and can be used without any restrictions. C/N ratio is a good indicator for compost stability and maturity and reduction of C/N ratio in mature compost is indicative of the oxidation of carbon [14]. Fig 3.2 depict the available TN, TP and TK content of the composted *F. racemosa* of all the nine composters (FR-A to FR - I). Statistical analysis of the data reveals that the availability of nitrogen among the composters was insignificant at ($p < 0.05$) but the available nitrogen content in all the composters was relatively higher compared to control. It is highest (1.46 ± 0.024) in FR - I composter. Similarly Total phosphorus (TP) and Total potassium (TK) contents are also higher in compost of FR - I (1.28 ± 0.03) (TP) and FR -I (1.12 ± 0.03) (TK) respectively. Stabilized compost showing nearly a pH of 7.1 is compatible with agronomic uses, along with a C/N ratio below 20. In fresh composting of substrates the nitrogen is decreased due to ammonia volatilization. In compost, the autotrophic nitrifying bacteria convert ammonia into nitrates [15]. During successful composting complete destruction of *Salmonella* sp and reduction in harmful microorganisms such as faecal *Coliforms* and *Streptococci* occurs as reported by [16]. The phytotoxic materials and microbial intermediate metabolite gets stabilized during the process of composting [17] through humification.

From this study it is clear that the leaf litters must be treated with microbial consortium to enhance the productivity of the earthworm and to enhance the nutritive value of the compost.

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Table : 1 Variations in physico-chemical characteristics of composted (FR) *Ficus racemosa* (Mean \pm SD,n=3)

Physico-chemical Parameter	Sampling Days	COMPOSTERS									F-value	P-value
		FR - A	FR - B	FR - C	FR - D	FR - E	FR - F	FR - G	FR - H	FR - I		
pH	Initial	6.8 \pm 0.033	6.9 \pm 0.016	6.8 \pm 0.045	6.8 \pm 0.047	6.9 \pm 0.049	7.8 \pm 0.050	6.9 \pm 0.032	6.7 \pm 0.065	6.9 \pm 0.063	0.269	0.96
	Final	8.1 \pm 0.067	7.9 \pm 0.150	8.5 \pm 0.042	7.8 \pm 0.051	7.6 \pm 0.069	8.9 \pm 0.026	8.1 \pm 0.028	8.2 \pm 0.016	8.5 \pm 0.024		
EC	Initial	0.39 \pm 0.02	0.36 \pm 0.04	0.38 \pm 0.12	0.42 \pm 0.01	0.47 \pm 0.06	0.31 \pm 0.03	0.53 \pm 0.07	0.61 \pm 0.01	0.67 \pm 0.05	1.256	0.36
	Final	0.58 \pm 0.04	0.83 \pm 0.01	0.71 \pm 0.11	0.60 \pm 0.17	0.52 \pm 0.65	0.38 \pm 0.02	0.89 \pm 0.14	0.92 \pm 0.25	1.84 \pm 0.41		
TOC %	Initial	50.47 \pm 0.021	50.01 \pm 0.024	51.21 \pm 0.029	50.02 \pm 0.033	51.82 \pm 0.028	48.57 \pm 0.020	50.04 \pm 0.037	49.47 \pm 0.024	49.81 \pm 0.016	0.169	0.98
	Final	42.75 \pm 0.020	42.50 \pm 0.024	43.55 \pm 0.028	43.67 \pm 0.012	45.41 \pm 0.029	47.91 \pm 0.016	42.41 \pm 0.029	42.26 \pm 0.026	42.12 \pm 0.024		
TN %	Initial	1.05 \pm 0.024	1.06 \pm 0.021	1.08 \pm 0.041	1.04 \pm 0.023	1.03 \pm 0.028	0.93 \pm 0.063	1.02 \pm 0.012	1.03 \pm 0.021	1.04 \pm 0.022	0.352	0.92
	Final	1.38 \pm 0.020	1.35 \pm 0.016	1.37 \pm 0.035	1.38 \pm 0.020	1.18 \pm 0.016	1.02 \pm 0.012	1.39 \pm 0.014	1.42 \pm 0.016	1.46 \pm 0.024		
TP %	Initial	0.28 \pm 0.02	0.26 \pm 0.01	0.21 \pm 0.02	0.29 \pm 0.17	0.24 \pm 0.21	0.12 \pm 0.34	0.48 \pm 0.42	0.54 \pm 0.21	0.62 \pm 0.45	1.871	0.18
	Final	0.68 \pm 0.06	0.44 \pm 0.04	0.43 \pm 0.12	0.46 \pm 0.01	0.42 \pm 0.11	0.29 \pm 0.06	0.81 \pm 0.08	1.21 \pm 0.05	1.28 \pm 0.03		
TK %	Initial	0.38 \pm 0.03	0.37 \pm 0.01	0.38 \pm 0.09	0.36 \pm 0.14	0.31 \pm 0.08	0.36 \pm 0.05	0.49 \pm 0.04	0.52 \pm 0.02	0.64 \pm 0.03	3.298	0.04
	Final	0.44 \pm 0.12	0.39 \pm 0.11	0.49 \pm 0.12	0.51 \pm 0.02	0.41 \pm 0.05	0.38 \pm 0.07	0.54 \pm 0.15	0.63 \pm 0.07	1.12 \pm 0.03		
C/N ratio	Initial	48.06 \pm 0.19	47.17 \pm 0.18	47.41 \pm 0.16	48.09 \pm 0.12	50.31 \pm 0.16	52.22 \pm 0.11	49.05 \pm 0.09	48.03 \pm 0.08	47.89 \pm 0.04	0.297	0.94
	Final	30.97 \pm 0.08	31.48 \pm 0.09	31.78 \pm 0.07	31.64 \pm 0.11	38.48 \pm 0.06	46.97 \pm 0.02	30.51 \pm 0.03	29.76 \pm 0.02	28.84 \pm 0.01		

Mean values are statistically significantly (P< 0.05)

A - *Pseudomonas fluorescens***B** - *Bacillus subtilis***C** - *Trichoderma viridae***D** - *Trichoderma harzianum***E** - *Lactobacillus spp(EM)***F** - Control (without inoculums)**G** - *Pseudomonas fluorescens* + *Bacillus subtilis* **H** - *Trichoderma viridae* + *Pseudomonas fluorescens***I** - *Pseudomonas fluorescens* + *Bacillus subtilis* + *Trichoderma viridae* + *Trichoderma harzianum* + *Lactobacillus spp(EM)***FR** – *Ficus racemosa*