



PRE-COMPOSTING OF FICUS BENGHALENSIS USING MICROBIAL CONSORTIUM

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Abstract:

The organic waste leaves *Ficus benghalensis* were allowed for microbial composting. In which the result showed while leaf litters of *Ficus benghalensis* with cow dung and selective useful microbial bacterial strains *Pseudomonas fluorescence*, *Bacillus subtilis* and *Lactobacillus* spp (the additive AMIRTHA-D) and fungal strains *Trichoderma viridae* and *Trichoderma harzianum* are allowed for composting, the composting period altered the physic-chemical properties like p^H , EC, N, P, K, TOC contents and C/N ratio drastically changed in the end product compost during decomposition process.

Key Words: *Ficus Benghalensis*, *Pseudomonas Fluorescence*, *Bacillus Subtilis*, *Lactobacillus Spp*, *Trichoderma Viridae* & *Trichoderma Harzianum*

1. Introduction:

Composting is a biological process through which microorganisms convert the waste organic materials into useful end products (Che Jusoh *et al.*, 2013), which is useful to soil conditions and plants as an organic fertilizer (Hanapi *et al.*, 2013). This process uses various microorganisms such as bacteria, fungi and actinomycetes to breakdown the organic compounds into simpler substances. In natural environments, leaves and other branches that fell down to the ground form a rich, moist layer of mulch that protects the roots of plants and provides a home for nature. . Composting is a feasible process of treating solid waste for beneficial use and destroying pathogens, disease and undesirable weed seed with the help of mesophilous and thermophilous microbes. 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. The curing phase is characterized by the conversion of a part of the stabilized organic matter into humus like matrix of nutrients and organic matter referred to as mature compost. 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.

Odour emission is a major problem for composting plants in the early stage of composting process, is generated by the degradation of substrates such as proteins and lipids. Hundreds of odorous substrates have been identified in compost gases, and their quality, quantity depends on the feedstock and condition of the composting (Gallego *et al.*, 2012). Some microbial groups such as *Lactobacillus* spp are reported to be responsible for the emissions. Such a composting process often struggles with an extended period of low microbial activity as well as high odour emission in the initial composting stage (Kumar *et al.*, 2010). To overcome this high odour emission period the early stages of the composting process should be steered towards conditions that favour beneficial composting microbes (eg. *Bacillus* sp and *Actinobacteria*) while discouraging microbes that cause odour problem mainly (*Clostridia* sp and *Lactobacillus* sp) (Kurola *et al.*, 2011). 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 of *Ficus benghalensis* and cow dung in order to study the beneficial role of these microbes in decomposing leaf litters of two different avenue trees and to produce compost to enhance nutritive value.

2. Materials and Methods:

The raw materials used in the present study for composting are: Leaf litters of *Ficus benghalensis*, 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 benghalensis* 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 benghalensis* (FB) 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 (Gajalakshmi and Abbasi, 2008). 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. benghalensis* 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 Gupta (2000). Total organic carbon (TOC) was determined by chromic acid wet digestion method (Walkley and Black, 1934) and total nitrogen (TN) by Kjeldahl microanalysis (Piper, 1996). Total phosphorus (TP) was determined spectrophotometrically and potassium content (TK) was detected by the flame emission technique (Tandon, 1993). 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 $p < 0.05$.

3. 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 (Dixon and Langer, 2006). The physico- chemical characteristics of the microbially composted leaf litters were analyzed and the results are tabulated in Table 3.1. 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.

3.1 Composting of *Ficus Benghalensis*: Table 3.1 affords the results of composting *F. benghalensis* along with cow dung for a period of 30 days. The table presents in detail the initial and final nutrient level of composted litter. The physico – chemical parameters like pH, EC, TOC, TN, TP, TK and C/N ratio of the composted leaf litter *F. benghalensis* were analyzed and the obtained results are described. Estimating the pH periodically, reveal that there was an increase in the pH value during composting from 7.7 ± 0.012 to 8.7 ± 0.020 respectively. The pH was 7.9 ± 0.044 in FB – B, 7.8 ± 0.081 in FB – A, 8.2 ± 0.021 in FB – G and 8.4 ± 0.145 in FB – I composters and in others it ranges between 7.7 ± 0.012 to 8.7 ± 0.020 . This increase in pH may be attributed in the formation of ammonium ions (NH_4^+) during decomposing the organic matter (Pramanik *et al.*, 2007). The pH value of composted *F. benghalensis* (FB) was found to be almost neutral to alkaline and stabilized during curing phase, which may be due to the buffering nature of humic substances. The pH value obtained in the present study are lined with the report of Bernal *et al.*, (2009) who considers the pH range of about 6.7 – 9.0 to be the optimum values for composting which supports good microbial activity during the process of composting.

Table 3.1: Variations in physico-chemical characteristics of composted (FB) *F. benghalensis* (Mean $\pm$ SD, n=3)

Physico-Chemical Parameters	Sampling Days	Composters									F-value	P-value
		FB - A	FB - B	FB - C	FB - D	FB - E	FB - F	FB - G	FB - H	FB - I		
pH	Initial	6.2 $\pm$ 0.034	6.00 $\pm$ 0.134	6.3 $\pm$ 0.111	6.2 $\pm$ 0.034	6.3 $\pm$ 0.067	8.4 $\pm$ 0.423	6.5 $\pm$ 0.084	6.4 $\pm$ 0.113	6.6 $\pm$ 0.155	0.382	0.90
	Final	7.8 $\pm$ 0.0	7.9 $\pm$ 0.0	7.7 $\pm$ 0.0	7.9 $\pm$ 0.0	7.8 $\pm$ 0.0	8.7 $\pm$ 0.0	8.2 $\pm$ 0.0	8.3 $\pm$ 0.0	8.4 $\pm$ 0.0		

		41	.044	012	135	042	020	021	011	015		
EC	Initial	0.42±0.024	0.38±0.015	0.39±0.037	0.40±0.025	0.36±0.056	0.54±0.042	0.61±0.051	0.63±0.048	0.65±0.032	0.663	0.71
	Final	0.89±0.042	0.94±0.047	0.79±0.034	0.81±0.045	0.77±0.032	0.76±0.041	0.96±0.038	1.02±0.036	1.12±0.027		
TOC %	Initial	48.12±0.024	47.20±0.018	46.14±0.032	45.12±0.028	43.18±0.038	51.06±0.041	47.12±0.064	47.21±0.062	48.28±0.048	0.380	0.91
	Final	36.97±0.012	36.83±0.02	36.88±0.025	37.11±0.02	36.98±0.011	48.99±0.027	36.72±0.01	36.61±0.017	36.54±0.016		
TN %	Initial	1.27±0.024	1.28±0.026	1.29±0.030	1.21±0.023	0.96±0.026	0.91±0.022	1.34±0.032	1.36±0.046	1.37±0.024	2.177	0.13
	Final	1.40±0.042	1.41±0.121	1.38±0.011	1.39±0.041	1.09±0.065	0.98±0.036	1.76±0.023	1.84±0.037	1.98±0.015		
TP %	Initial	0.29±0.006	0.31±0.002	0.33±0.005	0.31±0.001	0.30±0.007	0.28±0.001	0.46±0.004	0.49±0.003	0.51±0.002	1.474	0.28
	Final	0.32±0.011	0.34±0.008	0.41±0.006	0.38±0.014	0.31±0.010	0.29±0.009	0.92±0.012	1.42±0.018	1.58±0.007		
TK %	Initial	0.21±0.04	0.30±0.017	0.28±0.11	0.32±0.15	0.24±0.50	0.18±0.02	0.41±0.14	0.46±0.25	0.47±0.41	1.982	0.16
	Final	0.38±0.26	0.42±0.16	0.39±0.19	0.48±0.09	0.28±0.04	0.27±0.03	0.62±0.06	0.74±0.02	1.08±0.01		
C/N ratio	Initial	37.88±0.28	36.87±0.31	35.76±0.29	37.28±0.26	44.97±0.22	56.11±0.34	35.16±0.21	34.71±0.26	35.24±0.18	1.724	0.21
	Final	26.40±0.06	26.12±0.03	26.72±0.05	26.69±0.02	33.92±0.06	49.98±0.03	20.86±0.02	19.89±0.04	18.45±0.01		
Mean Value are Statistically Significant (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)

FB - *Ficus Benghalensis*

Figure 3.1: Variations in Physico –chemical characteristics of nine types of composted *Ficus benghalensis*

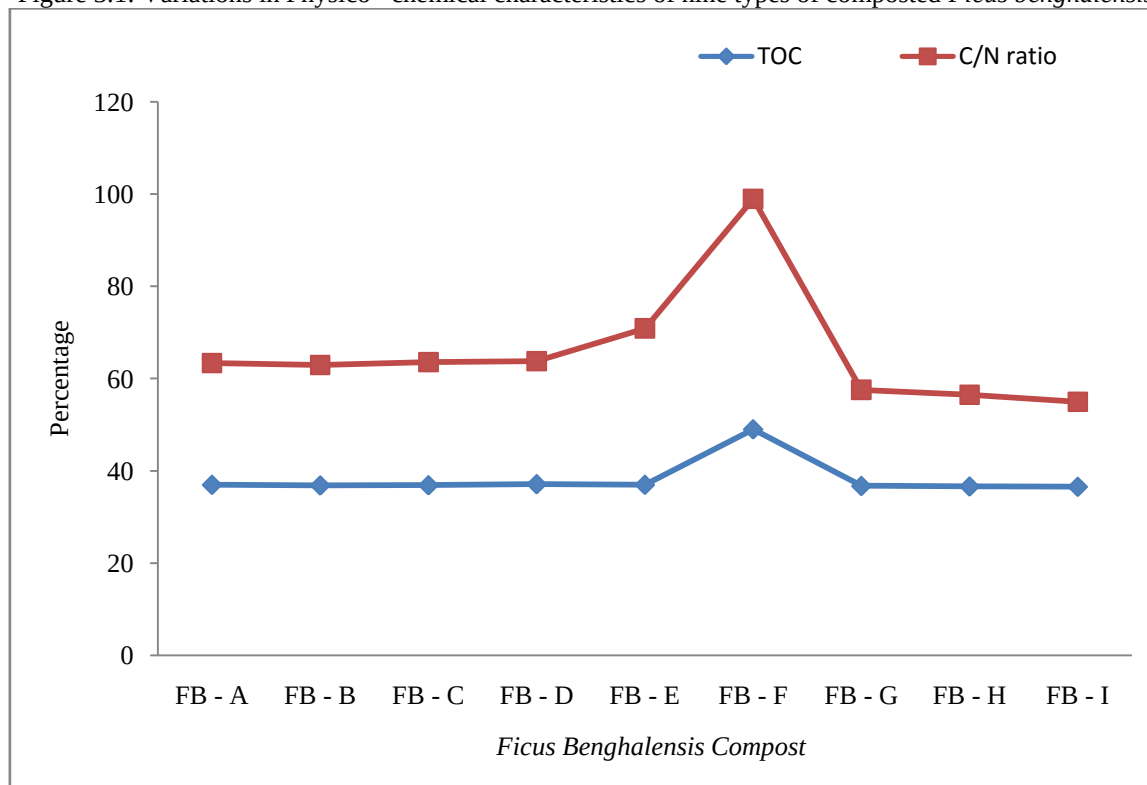
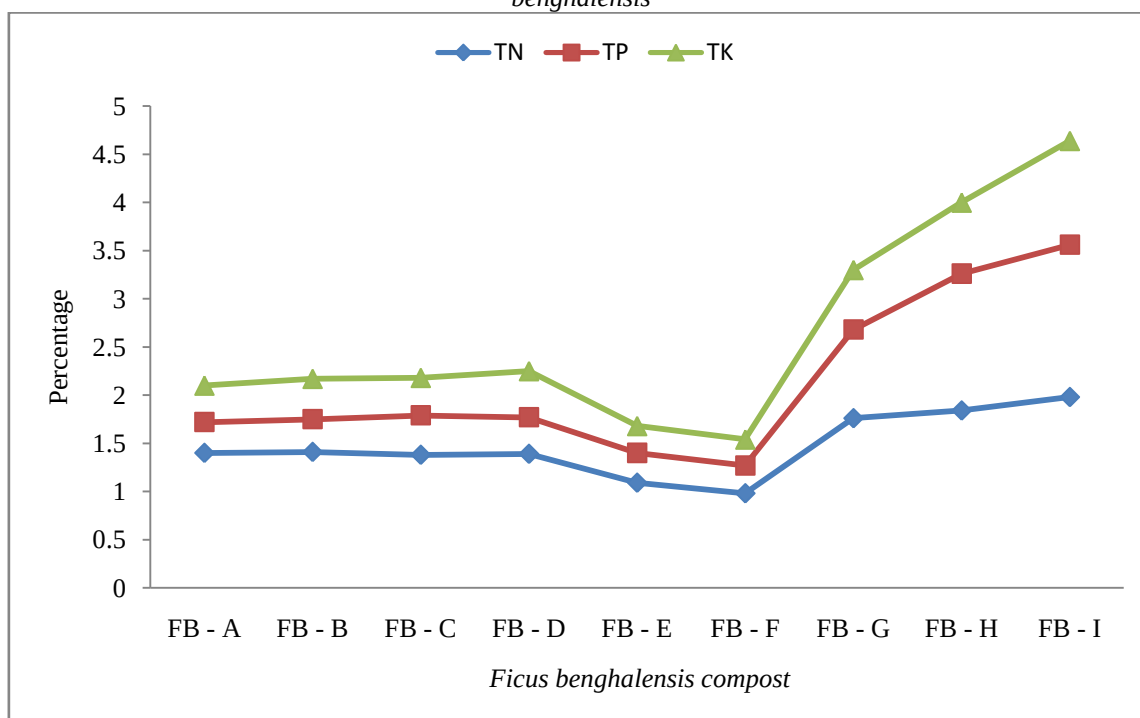


Figure 3.2: A diagrammatic representation of the available NPK content in nine types of composted *Ficus benghalensis*



The electrical conductivity (EC) of the substrate varied with the progression of composting. A gradual increase in EC was observed at the end of composting for a period of 30 days. The EC of the composted product observed in composter FB – I value 2.12 ± 0.027 is higher. In comparison with the control 0.76 ± 0.041 , FB – F there is considerable variation in all composters of *F. benghalensis*. These high values may be due to the effect of the concentration of salts as a consequence of biodegradation of organic matter (Campell *et al.*, 1997).

Information in the Fig 3.1 reveals a decrease in total organic carbon (TOC) at the end of composting in all the composters during decomposition with microbial inoculants. The reduction in TOC among the treatments was found to be insignificant ($p < 0.05$) among the composters. The reduction in TOC was found to be maximum especially in FB – I composter with all the five microbes in combination. Similarly gradual increase in total organic nitrogen is seen, resulting in a much balanced C/N ratio particularly in FB – H and FB – I composters. The TOC content decreased 48.99 ± 0.027 to 36.54 ± 0.016 during composting with inoculants. The observed results is in support to the findings of Singh and Sharma (2002), who have reported a similar loss of 30.10% to 26.48% of TOC content during composting. This reduction in TOC appears to be the effect of microbial inoculants added in the present study which increased the initial degradation process during composting with decreased stabilization time. During the active phase of the composting process the organic carbon (OC) decreases in the material due to decomposition of organic matter by the micro organisms. This loss of organic matter decreases the C/N ratio of the substrate. The degradation rate of the organic matter decreases gradually as composting progresses because of the reduction in available carbon sources, and synthetic reactions of new complex and polymerized organic compounds (humification) prevail over mineralization during the maturation phase. This process leads to stabilized end-products which acts as slow- release fertilizers for agricultural purposes. However, the major concern of manure composting is to control Organic carbon (OC) losses since they reduce the agronomic value of compost and contribute to greenhouse gas emissions (Hao *et al.*, 2004). The losses of organic carbon were significantly affected by composting. It was found that, the percentage of organic carbon decreased, which shows the decomposition of waste by microbial population (Mondini *et al.*, 2003). Part of the carbon in the decomposition residue is evolved as CO_2 and a part was assimilated by the microbial biomass (Cabrera *et al.*, 2005).

Total nitrogen (TN) includes all forms of nitrogen, organic nitrogen, ammonium nitrogen ($\text{NH}_4\text{-N}$) and nitrate nitrogen ($\text{NO}_3\text{-N}$). The measure of TN content includes both organic and inorganic forms of nitrogen in compost. In mature composts most of the nitrogen is in organic form. The TN content of all compost reactors varied from 0.98 ± 0.036 to 1.98 ± 0.015 . The TN content was higher in FB – I 1.98 ± 0.015 followed by FB – H 1.84 ± 0.037 when compared to the control FB – F 0.98 ± 0.036 respectively. Similar results was obtained by Porkodi and Amruththa (2014) who have observed a maximum increase in nitrogen content from 0.62 % to 0.89 % during composting market waste. This increase in TN content of the compost is not only due to increment of nutrient but also due to mineralization of organic matter by microorganisms. Earlier studies have shown that the *B. subtilis* and *P. fluorescens* are able to fix atmospheric nitrogen. In our study also, it is

observed that whenever the inoculation of substrate was done by *B. subtilis* and *P. fluorescens* microbes alone or in combination, the nitrogen percentage invariably increased in all the composters.

Total Phosphorus (TP) and Total Potassium (TK) content were recorded to be highest in all the composters. The highest phosphorus content was recorded in composter FB – I wherein the content increased from 0.51 ± 0.002 to 1.58 ± 0.007 followed by FB – H 1.42 ± 0.018 during composting compared to the control. Acid production by the activity of microorganisms during decomposition of organic waste is the major mechanism involved in the solubilization of insoluble phosphorus (Pramanik *et al.*, 2007). The increase in the concentration of potassium is observed in all the composters with inoculants compared to control. The highest value was recorded in composter FB – I and FB – H compared to control FB – F. The present observation is in accordance with the findings of Wania *et al.*, (2013) who also observed an increase in total potassium content from 0.60 % to 1.01 % in kitchen waste composting. The insoluble potassium salts can be solubilized by the decomposition of the wastes. In the present study the potassium concentration varies from 0.29 ± 0.03 to 1.08 ± 0.01 . Potassium increase during the period of composting and effective use of fibrous materials could prevent the loss of potassium from the compost formed (Gallaerdo *et al.*, 1987).

The data on the C/N ratio, is one of the most widely used indices for the maturity of organic waste. The results obtained from the present investigation revealed that the C/N ratio decreased from the initial to the final in all composters. The initial C/N ratio is in the range of 56.11 ± 0.34 to 34.71 ± 0.26 % (Table 3.1). The application of microbial inoculants however increased the nitrogen content accompanied by a decrease in TOC in the resulting compost with a maximum C/N ratio reduction of 18.45 ± 0.01 % in the composter FB –I while it was 49.98 ± 0.03 in the control without microbes.

The results of the present study indicate gradual increase in conductivity with progress of composting *F. benghalensis*. The EC in FB – I composter was significantly higher 2.12 than that in other composters. The increase in EC is an indicator of successful composting process. Similarly pH was also obtained to be within the optimum range in the FB – I and FB-H composters. When the pH is slightly alkaline or acidic it is suitable for applying to soil. The total organic nitrogen (TN) was found to be higher in FB – G, H and FB – I. This is because the free nitrogen is fixed by the non-symbiotic nitrogen fixing bacteria.

Besides the combinations studied, the activity of the fungus and the bacterial combinations were able to degrade the substrate well. The reduction in the content of TOC was significant in all the compost (A-I), and it is 6.74 fold more in FB – I when compared to control (FB – F) where it is only 2.07 %. This has been reflected in a better balancing of the C/N ratio in FB – I compost. In comparison to control (FB – F) composter C/N ratio in all the other composters is well balanced. Decrease in C/N ratio is an indicative for degradation of organic waste by the microbial action (Bonazzi *et al.*, 1990). Compost is applied to soil to prevent nitrogen immobilization and to prevent and control soil nitrogen deficiency (Illmer and Schinner, 1997).

The Data in Fig 3.2 states that the available nitrogen, phosphorus and potassium content is higher in the composted *F. benghalensis* of the FB- I composter followed by FB- H. In other treatments also N, P and K content was significantly higher when compared to compost in control (FB – F). The N, P and K concentrations indicate the immobilization of nutrient content. The nutrient content of the composted bio-waste was found to be higher than the non-composted waste as reported earlier (Joseph, 1995). Compost when added to the soil increases the nitrogen, phosphorus and potassium content in the soil. Similarly, when compost is added to soil that increases water retention capacity and also increases the temperature of the soil which improves the soil quality and enables seedling development. Compost was reported to suppress the Dollar spot (*Sclerotinia homoeocarpa*) of Turf grass and other plant diseases through a combination of physico-chemical and biological mechanisms (Boulter *et al.*, 2002). Compost when used as manure provides nitrogen which in turn increases the yield. The pH gets maintained due to the buffering capacity of compost (Buchanan and Gliessman, 1991). Compost is an environmentally and economically valuable material. Composted waste is used as a container media for plants (Inbar *et al.*, 1988). 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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