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Comparative Performance of Microbial Cultural and Earthworm in Composting of Tender Coconut Waste into High Quality Organic Manure

Kalpana Motha[1*], A.V.D. Dorajerao[2] and S. Vishala[1]

[1]*Horticultural Research Station, Dr YSRHU, Vijayarai, India*
[2]*College of Horticulture, Dr YSRHU, Venkataramannagudam, India*

**Corresponding author*

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A B S T R A C T

Consumption of tender coconut is increasing day by day. Due to heavy consumption of these tender nuts in urbans, the disposal also indirectly affects public health. Biodegradation of tender coconut took more than two years under natural condition. Hence, a study was taken on "Conversion of tender coconut waste into high quality organic manure using microorganism and earthworms". The tender coconut waste was pretreated with lime, rock phosphate, cowdung and glyricidia and treatment was imposed viz., T_1-TCW+earthworm (*Eudrilus eugeniae*), T_2-TCW + *Trichoderma viridae* + *Pleurotus sajorcaju*, T_3-TCWonly (without earthworms/microorganism), T_4-RTCW (Raw tender coconut waste). The results of the experiment revealed that vermicomposting of tender coconut waste with *Eudrilus eugeniae* significantly reduces the time of composting (60 days) with highest compost recovery (680kg/one tonne) compared to microbial compost (90 days) with compost recovery of 490kgs/one tonne of the substrate. Vermicomposting of tender coconut waste resulted in increase of pH, decrease of electrical conductivity (dms^{-1}), cellulose (%), lignin (%) and significantly narrow down the carbon nitrogen ratio. The raw tender coconut contains nitrogen (0.53%), phosphorus (0.10%) and potash (2.23%), on composting, there was increase of 1.55% (N), 0.23% (P) and 2.24% (K) in vermicompost whereas in microbial compost 0.90% (N), 1.11% (P) and decrease in K by 0.95%. The vermicompost was also rich in micronutrients *viz.*, manganese (164ppm), Iron (8381 ppm), Zinc (134.60 ppm), copper (38.33 ppm). A total microbial load (cfu's) was observed high in vermicompost (486.61 cfu/g) compared to microbial compost (391.14 cfu/g).

Introduction

Andhra Pradesh is one of the major coconut growing states in the country and the state commands an area of nearly 1.02 lakh hectares with annual production of 1092.7 million nuts. Nearly 30-40% of the production is utilized for tender nut water consumption in the state. The tender coconut is able to provide many benefits for health and welfare to human beings who consume it. The consumption of tender nut during peak summer season is approximately 3,71,350 nuts/day and the disposal of empty nuts is creating lot of sanitary problem in town and cities. Due to heavy consumption of these tender nuts in urbans, the disposal also indirectly affects public health. When broken in half, leaving the fruit exposed the two concavities are ideal for the accumulation of rainwater, which could turn this into a dangerous outbreak of the mosquito vector of dengue, *Aedes aegypti* (Joan *et al.*, 2001). Besides, the tender coconut waste contains 11.86 to 13.91 % lignin and 20.2 – 24.30% cellulose materials. These lignocellulosic biomass are highly recalcitrant and strongly resists biodegradation due to high lignin content, high crystallinity, high degree of polymerization and presence of acetyl groups on hemicellulose. The presence of lignin in the biomass leads to the protective barrier that prevents plant cell destruction by microbial enzymes. Hence, under natural condition, the degradation of tender coconut waste took more than two years for complete decomposition. Reports have shown that a good amount of potash can be squeezed out of empty shells and can be used as manure, but its labourious process. In order to avoid this problem, a study was conducted during 2007-2009 at Horticultural Research Station, Dr. Y.S.R. Horticultutal University, Ambajipeta on "Conversion of High quality organic manure from tender coconut waste using microorganisms and earth worms.

Materials and Methods

Collection, multiplication of microbial culture and earthworms

Isolation and mass multiplication of *Trichodermaviridae*

The bioagent *Trichoderma viridae* is isolated by serial dilution and plate count method. About 100gof soil sample was collected from coconut rhizosphere in random and about 10g of soil was diluted in 90 ml of distilled water (10^{-1}). For isolation of *Trichoderma viridae* 10^{-4} dilution was taken and isolation on Trichoderma specific media by spread plate method. After incubation of 2-3 days, Trichoderma colonies were identified and pure cultured on potato dextrose agar media. The culture was talc formulated using talc powder and carboxy methyl cellulose.

Isolation and mass multiplication of *Pleurotus sajorcaju*

Pleurotus sajorcaju is the edible oyster mushroom having the lignolytic ability. The mushroom spawn was collected from Regional Mushroom Laboratory, Visakapatnam and Regional Coir Board, Dowleswaram. The spawn was cultured on potato dextrose agar media and it was multiplied on different substrate like jowar grains and rice husks.

Isolation and mass multiplication of earth worm *Eudrilus eugenia*

The tender nut waste was chopped, mashed and mixed with cowdung slurry in 1:2 ratio in cement tub. The substrate was allowed for partial decomposition for one month and adult earthworms were released into tub @15-20 worms per one kg of substrate. Then the substrate was mulched with wet gunny bag in order to maintain moisture in the tub. During the vermiprocessing, the earthworms get acquitted with the tender nut waste and multiplied rapidly.

Pretreatment of tender coconut waste

The tender nut waste was chopped into 5 cm bits. Pretreatment with urea, lime rock phosphate, additives such as glyricidia, cowdung was done. For one tonne of raw material, urea @ 5kg, lime @ 5 kg, rock phosphate @ 20 kgs, cowdung @ 200 kgs, glyricidiae @ 200 kgs was added and incubated the material for about 30 days. The heap is periodically watered to maintain moisture level and two three tunnings were made at regular intervals. The lignocellulose degrading microorganisms such as *Pleurotus* *sajorcaju*, *Trichoderma viridiae* @ 2kgs/tonne of material were spread uniformly and tested their efficacy to degrade the tender coconut waste (Plate-1).

For vermicomposting the tender coconut waste, the raw was chopped into 5cm bits, a fresh cow dung slurry @200kgs/ tonne of tender nut waste is spread uniformly over the tender nuts in layers and incubated for one month for partial decomposition of the material.

The substrate was maintained at 28–32 ^{0}C and 60–70% relative humidity. Sufficient moisture with 60-70% is maintained by regular sprinkling of water. One or two turnings were made at intervals. After one month, the African night crawler, *Eudrilus Eugenia* were released into the tub@2000 no's/tonne of raw material and incubated by regular watering to maintained the moisture (Plate-2).

Analysis of the raw tender coconut waste, microbial compost and vermicompost for nutrient content

Generally tender coconuts of 5th, 6th 7th month old nuts are sold in the market. So the tender nuts of different age groups viz., 5th, 6th and 7th month @ three nuts per age group were collected from the palms and chopped into small bits, dried in the oven at temperature of 50-60^{0}c temperature for about 48 hours.

Then the dried bits were made into fine powder with blender. The powdered material was sieved and packed according to the age of the nuts and analysied for physico-chemical properties of tender coconut waste at Central Tobacco Research Institute, Rajahmundry. Further microbial compost and vermi compost samples of about 100g were weighed, shade dried and send to Central Tobacco Research Institute, Rajahmundry for physico- chemical properties.

Results and Discussion

The analysed data on physico-chemical properties of raw tender coconut waste was presented in the Table 1, revealed that the pH was slightly acidic ranged from 6.0 to 6.5 with an average of 6.2. The electrical conductivity ranged from 2.25 – 2.75 dsm^{-1} and organic carbon percentage ranged from 35.60 – 42.20 respectively in different age group of tender coconuts. The tender coconut waste was rich in major and micro nutrients and the nitrogen percentage ranged from 0.45 – 0.62, phosphorous ranged from 0.09 – 0.12 percent while potassium ranged from 2.00 to 2.41 percent. Besides major nutrients, the tender nut waste is rich in iron, manganese, zinc and copper (ppm) etc. Iron ranged from 889 – 1261 ppm and copper from 5.77 – 6.76 ppm. The tender nut waste also contain high amount of cellulose and lignin percentage and the cellulose ranged from 20.23 – 24.30, while lignin percentage ranged from 11.86 to 13.91 percent.

These lignocellulosic biomasses are highly recalcitrant and strongly resist biodegradation as it leads to the protective barrier that prevents plant cell destruction by microbial enzymes. Hence, under natural condition, the degradation of tender coconut waste took more than two years for complete decomposition. So lignocellulosic biomass of tender coconut waste has to be treated so that the cellulose fibers are exposed to microbial actions. Hence, the tender coconut waste was pretreated with urea, lime, rock phosphate, cow dung and green manure and treatments were imposed as T_1- TCW + Earthworms, T_2- TCW + the microbial culture, T_3 – TCW only (control). The treatments were replicated thrice after pretreatment. The results of the experiment (Fig. 1) revealed that the biodegradation and decomposition of tender nut waste with earthworms *Eudrilus eugainiae* (T_1) significantly reduces the time of composting when compared to microbial composting (T_2) and control. (T_3). Vermicompost from tender coconut waste was obtained within 68.91 days followed by microbial composting (90.65 days) as against control (180) with compost recovery of 680.60kgs, 490.35kgs of the one tonne of substrate respectively compared to the control (180.75 kgs).

The physico chemical properties of final vermicompost (T_1) and microbial compost (T_2) were compared with untreated tender nut waste (T_3) and raw tender nut waste (T_4). It is observed from the Table 2, that pH of the vermicompost (T_1) and microbial compost (T_2) are neutral as against slightly acid pH in untreated control (T_3) and raw tender nut waste (T_4). It was evident that electrical conductivity, Organic carbon (%), cellulose (%), lignin (%), Carbon Nitrogen ratio decreased significantly in vermicompost (T_1) and microbial compost (T_2) as against untreated tender coconut waste (T_3) and raw tender coconut waste (T_4).

There was a considerable increase in pH, major nutrients *viz.*, nitrogen, phosphorous and potash content and drastic increase in the micronutrient such as Manganese, Iron, Copper, Zincin both vermi compost and microbial compost when compared with the values of untreated tender nut waste (T_3) and raw tender nut waste (T_4). The initial pH of raw tender coconut waste was ranged from 6.00- 6.50 with an average of 6.27and there was an increase of pH in vermi-compost (7.1) and in microbial compost (7.2) as against 6.50 in untreated tender nut waste (T_3) and 6.27 of raw tender nut waste (T4). The electrical conductivity of raw tender coconut waste was ranged from 2.25–2.75 dsm^{-1} with an average of 2.50dsm^{-1}. There was a decrease of electrical conductivity in vermi-compost (0.91dsm^{-1}) and in microbial compost (0.9dsm^{-1}) as against 1.94 dsm^{-1}in untreated tender nut waste (T_3) and initial electrical conductivity (2.50 dsm^{-1}) of tender nut waste.

It was noticed from the Table 2, that in raw tender coconut waste the cellulose and lignin percentage ranged from (20.23–24.30%) and (11.86–13.91%) with an average of cellulose (21.99%) and lignin (12.63%). With the comparison of T_3-untreated tender nut waste (21.56% and 12.63%) and T_4- raw tender nut waste (21.99% and 17.51%), there was a decrease of cellulose and lignin percentage in T_{1-} vermi-compost (10.70 and 4.28%) and T_2-microbial compost (15.64 % and 5.86%) respectively. The CN ratio was narrow down from 55.44 in untreated tender nut waste (T_3) and 64.26 in raw tender coconut waste (T_4) to 15.35 in vermi-compost (T_1) and 13.96 in microbial compost (T_2).

The nutrient status in tender nut waste as affected by the treatments was presented in the Table 3. It was observed from the table that nitrogen, phosphorus and potassium percentage in raw tender coconut waste was ranged from 0.45-0.62% (N), 0.09- 0.12% (P) and 2.00-2.41% (K) with an average of 0.53 % (N), 0.10% (P) and 2.23 % (K). On composting, there was an increase of nitrogen percentage of 1.55% and 0.90 % in vermi-compost (T_1) and microbial compost (T_2) respectively as against 0.64% untreated control (T_3) and 0.53% in raw tender nut waste (T_4). With regard to phosphorus percentage, there was a decrease of phosphorus % in vermicompost (T_1) (0.23%) and an increase in microbial compost (T_2) (1.11%) when compared to 0.45% un treated control (T_3) and 0.10 % raw tender nut waste (T_4).while potassium content showed increased in vermicompost (T_1) (2.48%) and in microbial compost (T_2) (0.95%) as against (0.51 %) in untreated control (T_3) whereas decrease in potash percentage was observed in microbial compost (T_3) (0.95%) compared to raw tender coconut waste (T_4) (2.23%).

Table.1 Initial physico - chemical properties in tender coconut waste

S. No.	Physio-chemical properties	Tender nut waste				
		5[th] month old nut	6[th] month old nut	7[th] month old nut	Range	Mean
1.	pH	6.50	6.00	6.30	6.0 – 6.5	6.27
2.	Electrical Conductivity (dsm-1)	2.75	2.25	2.50	2.25 – 2.75	2.50
3.	Organic Carbon	42.20	37.89	35.60	35.60 – 42.20	38.56
4.	Cellulose (%)	20.23	21.43	24.30	20.23 – 24.30	21.99
5.	Lignin (%)	12.11	11.86	13.91	11.86 – 13.91	12.63
6.	Nitrogen (%)	0.62	0.45	0.53	0.45 – 0.62	0.53
7.	Phosphorus (%)	0.12	0.09	0.10	0.09 – 0.12	0.10
8.	Potassium (%)	2.00	2.28	2.41	2.00 – 2.41	2.23
9.	Mn (ppm)	12.15	10.16	18.33	10.16 -18.33	13.55
10.	Fe (ppm)	908.33	889.00	1261.66	889.00- 1261.66	1019.66
11.	Zn (ppm)	10.23	11.48	12.91	10.23 – 12.90	11.54
12.	Cu (ppm)	5.77	6.16	12.91	5.77 – 12.91	8.28

Table.2 Physico-chemical properties of Tender Coconut Waste as affected by the treatments

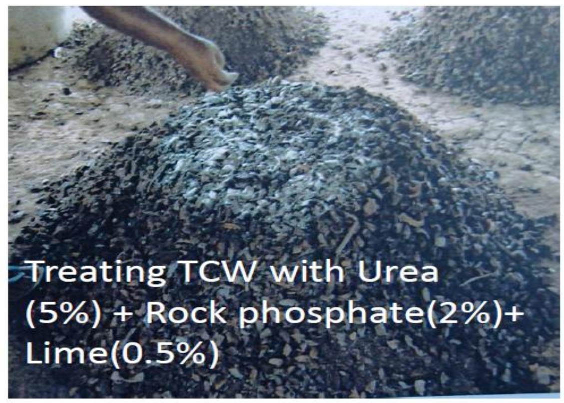
S. No.	Physio-chemical properties	Vermi-composting (T_1)	Microbial composting (T_2)	Untreated Control (no microbes/ Earthworm) (T_3)	Raw tender coconut waste (T_4)
1.	pH	7.10	7.20	6.50	6.27
2.	Electrical Conductivity (dsm-1)	0.91	0.93	1.94	2.50
3.	Cellulose (%)	10.70	15.64	21.56	21.99
4.	Lignin (%)	4.28	5.86	17.51	12.63
5.	CN ratio	15.35	13.96	55.44	64.26

Table.3 Mean nutrient status of tender nut waste as affected by the treatment

S. No.	Nutrient status	Vermi-composting (T_1)	Microbial composting (T_2)	Untreated Control (no microbes/ Earthworm) (T_3)	Raw tender coconut waste (T_4)
1.	Nitrogen (%)	1.55	0.90	0.64	0.53
2.	Phosphorus (%)	0.23	1.11	0.45	0.10
3.	Potassium (%)	2.48	0.95	0.51	2.23
4.	Mn (ppm)	164.00	99.00	32.00	13.55
5.	Fe (ppm)	8381.00	13193	4548	1019.66
6.	Zn (ppm)	134.60	66.00	18.00	11.54
7.	Cu (ppm)	48.33	41.00	16.23	8.28

Table.4 Mean microbial load in composted material with different agents

S. No.	Particulars	Vermi-composting (T_1)	Microbial composting (T_2)	Untreated Control (no microbes/ Earthworm) (T_3)
1.	Bacteria x 10^6	409.66	372.00	270.00
2.	Fungi x 10^4	41.33	37.00	22.00
3.	Actinomycetes x 10^5	35.66	31.00	18.00
Total		486.65	438.32	310.00



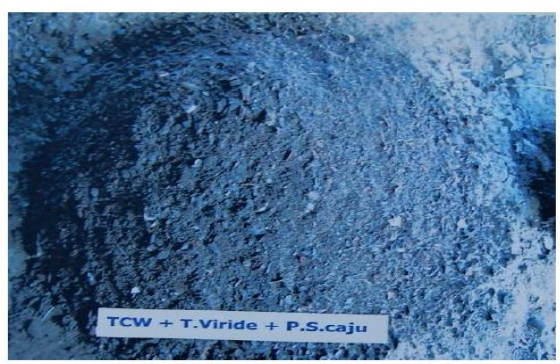


Plate-1:Protocol for microbial composting of Tender Coconut Waste

Raw Tender Coconut Waste

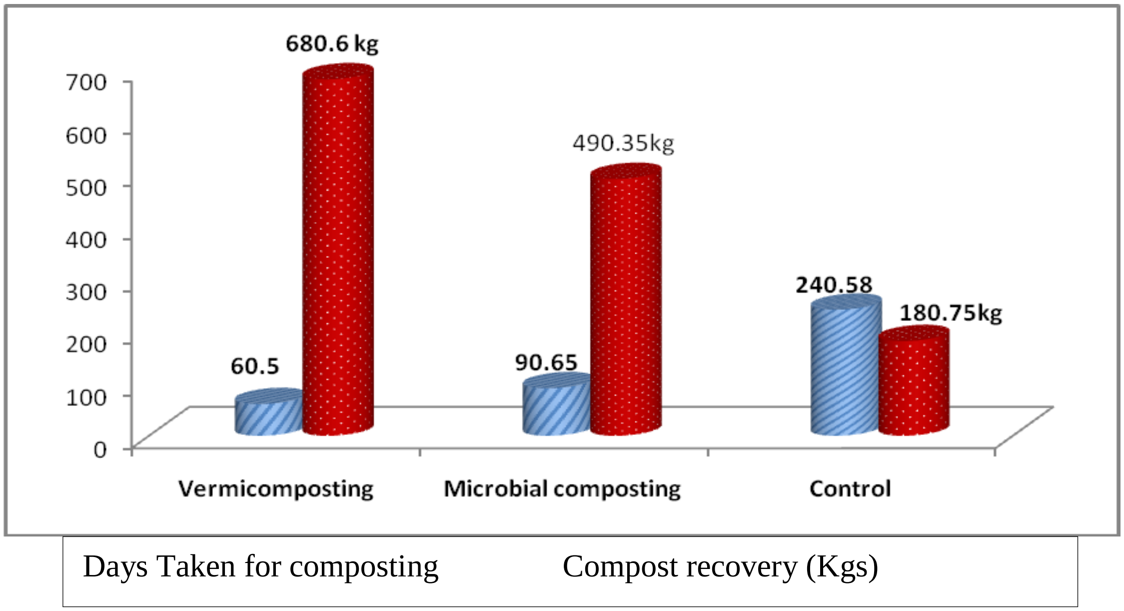
Cowdung slurry @200kg/ton of TCW

Release of earthworms @2000no's /t

Vermicompost of TCW

Plate-2:Protocol for Vermicomposting of Tender Coconut Waste

Fig.1 Days taken for composting and compost recovery as affected by the treatments



The micro nutrients Viz., Manganese (Mn), Iron (Fe), Zinc (Zn) and Copper (Cu) were increase in vermi-compost (T_1) and microbial compost (T_2) as against the untreated control (T_3) and raw tender nut waste (T_4). The increased percentage of Manganese in vermicompost (T_1) and microbial compost (T_2) are (164 ppm and 99 ppm) as against 32ppm and 13.55ppm in untreated control (T_3) and raw tender nut waste (T_4). An increase of iron, zinc and copper was observed in vermi compost are (8381 ppm,134.60ppm and 48.33 ppm) and in microbial compost (13,193 ppm, 66 ppm, 41 ppm) compared to only 4548 ppm,18ppm and 16.23ppm in untreated control (T_3) and 1019.55ppm,11.54ppm, 8.28ppm of iron, zinc and copper in raw tender nut waste (T_4) respectively.

A total microbial load (CFU's) was observed high in vermicompost (T_1) (486.65 CFU/g.) and microbial compost (T_2) (438.32 CFU/g.) compared to untreated control (T_3) (310.00 CFU/g). Among all the microbes bacteria recorded highest in vermicompost of tender coconut waste (409.66 x 10^6 CFU/g.) compared to fungi (41.33 x 10^4 CFU/g.) and actinomycetes (35.66 x 10^5 CFU/g.) (Table 4). This could be attributed that the casts are usually rich in ammonia and partially digested organic matter and thus provide a good substrate for growth microorganisms.

Tender coconut waste was collected from different places of the villages and nearby towns. The tender coconut waste contains 11.86 to 13.91 % lignin and 20.23 – 24.30% cellulose materials. These lignocellulosic biomasses are highly recalcitrant and strongly resist biodegradation due to high lignin content, high crystallinity, high degree of polymerization and presence of acetyl groups on hemicellulose. The presence of lignin in the biomass leads to the protective barrier that prevents plant cell destruction by microbial enzymes. Hence, under natural condition, the degradation of tender coconut waste took more than two years for complete decomposition. So lignocellulosic biomass of tender coconut waste was treated so that the cellulose fibers are exposed to microbial actions. This pretreatment can be done either by physical, chemical or biological methods. The physical and chemical treatments are more preferred than biological methods. The physical treatment comprises of grinding, size reduction, fine pulverization etc. whereas chemical treatments generally use of acid, alkali, alkaline peroxide, oxidation, ammonia etc. at specific temperature and pressure. Hence, the tender coconut waste was pretreated with urea, lime, rock phosphate, cow dung and green manure. Liming can enhance humification process, urea provides nitrogen that acts as a source for reproduction and multiplication of microorganisms and also small quantities of nitrogen enhances the lignin degradation whereas rock phosphate had acid pH that acts on lignin material.

Vermicomposting involves the bio-oxidation and stabilization of organic material by the joint action of earthworms and microorganisms whereas Composting involves the accelerated degradation of organic matter by microorganisms under controlled conditions, in which the organic material undergoes a characteristic thermophilic stage (Lung *et al.,* 2001). In vermiprocessing, the tender coconut bits were pretreated with cow dung slurry and incubated for one month, then the earthworms *Eudrilus euginiae* was released @ 2000 no.s/tonne, that took 68 days for conversion of raw material into vermicompost with compost recovery of 68% of the substrate. Whereas in microbial composting, the tender coconut bits were pretreated with urea, rock phosphate, lime, cow dung and green manure and incubated for about two months, then released microbial culture @ 2kgs/tonne took 90 days for conversion of the raw material with compost recovery of 56% of the substrate. Thus, the degradation of tender coconut waste with earthworms significantly reduced the time of composting. In other words, the vermiprocessing took 3 months for conversion of tender coconut waste, whereas microbial composting took 5 months for conversion of raw substrate. The results are on par with the findings of Prakash *et al.,* 2008 who opined vermicomposting process accelerates the mineralization activity there by attributed to the increase of nutrients in vermicasts. Earthworms are very effective in initiating the decomposition processes and paving the way for subsequent microbial action (Pearson, *et al.,* 1963).

There was increase pH in the final compost (vermicompost/ microbial compost) compared to the untreated control and raw tender nut waste. Other authors have found similar results in vermicomposting experiments, and have suggested that the mineralization of N and P compounds, the release of CO_2 and organic acids from microbial metabolism, and the production of humic and fulvic acids, as possible causes of the decrease in pH during vermicomposting (Ndegwa and Thompson, 2001; Kaushik and Garg, 2004). Muthukumaravel *et al.,* 2008 opioned that the increased pH values at the final stage of composting can be attributed to the decomposition of nitrogenous substrates, resulting in the production of ammonia which formed a large proportion of the nitrogenous matter excreted by the earthworms. Electrical conductivity (dsm^{-1}) and carbon nitrogen ratio are important parameters that determine the compost maturity. Electrical conductivity (dsm^{-1}) and carbon nitrogen ratio gradually reduced with the ageing of composting. An ideal compost should have EC < 1dsm ^{1}. The final vermi-compost and microbial compost had electricial conductivity 0.91 and 0.93 dsm^{-1} respectively. During the process of vermicomposting and microbial composting, the minor production of soluble metabolites such as ammonium (NH_4), as well as precipitation of the dissolved salts may lead to lower EC values (Mitchell, 1997). The C to N ratio indicates the degree of decomposition of a waste, as carbon is lost as CO_2 during bio oxidation, whereas N is lost at a lower rate, and therefore the more decomposed a waste, the lower the C to N ratio. The carbon nitrogen ratio in vermi compost and microbial is narrow down to 15.35 and 13.96 respectively. Similar results were reported by Cristina Lazcano *et al.*, (2008), the C to N ratio was significantly lower in the treatments involving vermicomposting, which indicates that they underwent more intense decomposition.

There was decrease of lignin and cellulose material in vermi compost than in microbial compost. The reason attributed was that earthworms secrete enzymes *viz.,* cellulose, proteases, lipases, analyses, acid phosphotases and alkaline phosphates that brings rapid biochemical conversion of lignocellulytic material in the waste (Rajiv K. Sinha *et al.*, 2002). Also, the gut of earthworms is inhabited by millions of decomposer microorganisms that accelerate the decomposting process.

A significant increase in the major nutrients and micronutrients was observed in vermicompost except phosphorus and iron compared to microbial compost. The increased levels of macro and micro nutrients in the vermicompost and microbial compost agree with the results of the earlier studies (Alidadi *et al.,* 2005; Parthasarathi, 2007; Thanga Mariappan and Vijayalakshmi, 2010; Nahrul and Astimar, 2011). Blanchart *et al.*, (1997) opined that the increased organic carbon and nitrogen content of the worm gut might also stimulate the microbial activity. Microbes play an important part as a diet of earthworms which even prefer organic matter with high concentration of microbial life.

Furthermore, there was an increase in microbial population in vermicompost because, the casts are usually rich in ammonia and partially digested organic matter that provides a good substrate for growth of microorganisms and also some of the intestinal mucus secreted during passage through the earthworm gut is ingusted with the casts where it continues to stimulate microbial activity and growth Prakash *et al.*, (2008). Sivasankari and Anandharaj (2016) reported that the highest load of bacteria fungi and actinomycetes were observed in the hind gut of *Eudrilus eugeniae* on 60 day in vermicompost prepared using *C. auriculata* + *L. leucocephala* + Cowdung mixture with *E. eugeniae.*

In the light of present investigation carried out, vermicomposting of tender coconut waste with *Eudrilus eugeniae* significantly reduces the time of composting with highest compost recovery, reduced CN ratio, lignin cellulose content, highest major, micronutrients and microbial population.

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