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Effective Coir Pith Composting using Microbial Consortium for Better Hydrolytic Enzymes Production in the Composting Process

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A B S T R A C T

Coir pith is a byproduct of coir industry generated during extraction of coir fiber from coconut husk. Approximately two tons of coir pith is released during the processing of a ton of coir. In the present study, Coir pith collected from Sri Kamachi Amman coir industries, Cumbum, Theni disttrict, Tamil Nadu is utilized. A Coir pith composting is an aerobic process. Single, dual, and consortium of microbial inoculants were added @ two litres of broth culture ton^{-1} of coir pith heap. For the nitrogen source the Urea was added @ 0.5 per cent level. The coir pith was heaped above the soil and spread to the length of 4 feet × 3 feet breadth. The experimental design adopted for coir pith waste composting is as follows: The treatment T_1, T_2 and T_3 respectively used as single inoculant culture of *Cellulomonas fimi, Pleurotus sajor caju* and *Phanerochaete chrysosporium* respectively. Treatment T_4, T_5 and T_6 used the dual inoculation of *Cellulomonas fimi* + *Phanerochaete chrysosporium* (T_4), *Cellulomonas fimi* + *Pleurotus sajor caju* (T_5) and *Pleurotus sajor caju* + *P. chrysosporium* (T_6) respectively. The treatment T_7 included all the three inoculants (consortium) *Cellulomonas fimi* + *Phanerochaete chrysosporium* + *Pleurotus sajor caju* respectively. All the treatments were sampled from 0th day until 90th day at 15 days interval for determining xylanase, dehydrogenase, laccase and Mn peroxidase activities. The triple inoculants treatment found to be the best microbial consortium combination for consistent production of hydrolytic enzymes during the period of coir pith composting.

***Key words*:** Microbial inoculants, Coir pith waste, *Cellulomonas fimi, Pleurotus sajor caju*, *Phanerochaete chrysosporium*

The coconut palm is a long-lived plant; it has a single trunk, 20-30 meter tall, its bark is smooth and gray, marked by ringed scars left by fallen leaf bases. The tree can live as long as 100 years producing an annual yield of 50 to 100 coconuts. Coconut palms are found throughout the tropics, and can also be successfully grown in areas that receive only mild frosts. 90% of the world's coconut production for exports, sources from the Asia-Pacific region, though coconut products are an increasing source of revenues for many other developing areas. The economic importance of this tree crop is evident from the fact that it is grown in more than 90 countries across the world in an area of 14.2 million hectares producing about 57.5 billion nuts or 10.5 million tonnes of copra. However, Philippines, Indonesia, India and Srilanka account for 78 per cent of the area and production [1-2].

Coir, also known as coir dust, coir meal, coir pith, and coir fibers, may provide an alternative to peat since it is a biodegradable and renewable by-product. Social and ecological questions concerning child labor, inadequate wastewater management, and transportation should be additionally considered. From the perspective of substrate properties, coir pith has high water capacity and easily available water. It contains more lignin and less cellulose than peat, thus being more resistant to microbial breakdown. It is also easily renewable, which improves the water absorption of substrate mixtures and water distribution in the growing medium [3]. The agro-residues were densified using ram and die type briquetting machine without adding any binder material. It was revealed that the briquettes are well suited for the energy generation [4-6].

In the organic waste decomposition, the microorganism reproduces themselves and release carbon dioxide, water, other organic products and the final product of the composting process consists of most resistant residues of the organic matter breakdown product, the biomass of dead microorganism and other microorganisms together

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with product from chemical reaction between these matters [7-8]. If the moisture is below 40 per cent, decomposition will be slow; if the moisture content is more than 60 per cent anaerobic conditions occur [9-11]. In composting plant materials, the hydrolysis of polysaccharide constituents by the secreted enzymes could be expected to produce a mixture of sugars. Important enzymes involved in composting process include cellulase, hemicellulase and phosphatase. High levels of cellulase activities have been detected throughout the active phase of composting [12-14]. High temperatures support degradation of recalcitrant organics such as lignocellulose and elimination of pathogenic microorganisms [15]. The true maturity of compost can be assessed by measuring the maturity indices such as C:N ratio, lignin, cellulose, humus composition, phenol contents and quality of major nutrients, secondary nutrients and micronutrients during the period of composting [16-18]. The application of coir pith compost showed better performance by increasing soil fertility, soil microorganisms and gave the highest yield [19-21]. In the current study microbial combination were studied is better combinations for the production of hydrolytic enzymes for effective coir pith composting.

MATERIALS AND METHODS

Method of compost preparation

Collection of raw materials:

The raw material Coir pith is collected from Sri Kamachi Amman coir industries, Cumbum, Theni district, Tamil Nadu state.

Isolation and screening of microorganisms from coir pith waste

Bacteria and fungi used in the study were designated the culture used in the study are *Pleurotus sajor caju*, *Cellulomonas fimi* and *Phanerochaete chrysosporium* compost *culture* maintained at the culture collection unite and Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University.

Estimation of xylanase activity

One ml of one per cent Xylan solution in acetate buffer and one ml of appropriately diluted enzyme sample were pipetted out in a test tube and incubated at, 27°C for 15 min. Further, the reaction was stopped by the addition of two ml of dinitrosalicylic acid reagent (One g dinitrosalicylic acid, 200 mg phenol and 50 mg sodium sulphite in 100 ml, one per cent NaOH). The mixture was heated in a boiling water bath for 5 min. When the tubes were warm, one ml of 40 per cent potassium sodium tartarate solution was added and cooled down in running tap water. The volume was made to 10 ml by the addition of six ml of water. Then, the absorbance was measured at 560 nm in a calorimeter. Blank solution was prepared in the above-described method without the enzyme. Standard graph was prepared with 0-100 mg of xylose. One unit of xylanase activity (U) is defined as that releasing one µmol of xylose equivalents min^{-1} mg^{-1} of protein.

$$1U = \mu mol\ min^{-1}\ mg^{-1} \text{ of protein}$$

The reducing sugars produced by the action of endo and exo cleaving enzymes react with dinitro salicylic acid and reduce it to a brown-coloured product, nitro aminosalicylic acid [22].

Estimation of dehydrogenase activity (Cassida et al., 1964)

The dehydrogenase activity was determined based on the biological reduction of Triphenyl Tetrazolium Chloride (TTC) by the enzyme sample prepared from compost materials. Six ml of enzyme samples from compost preparations were taken in 50 ml serum vials and 0.2 g $CaCO_3$ was added to each vial and thoroughly mixed. The contents of vial were fully saturated with one ml of three per cent aqueous solution of TTC, one ml of one per cent sucrose solution and 2.5 ml distilled water. Then, the vials were sealed and incubated at 37°C for 24 h. The concentration of Formazon for each sample was determined by referring to a standard curve of the Triphenyl Formazole (TPF) in methanol and expressed as µg h^{-1} g^{-1} of the sample.

Estimation of manganese peroxidase activity

Manganese peroxidase activity was determined by monitoring the oxidation of guaiacol (2-methoxyphenol) as the substrate at 465 nm with extinction coefficient, ε_{465} = 12100 M-1cm-1, [23]. The reaction mixture contained 0.5 M sodium succinate buffer (pH 4.5), 4 mM guaiacol, 1 mM $MnSO_4$, 600µl of microbial culture filtrate, and 1 mM H_2O_2.

Estimation of laccase activity

The laccase activity was determined via the oxidation of ABTS as the substrate [24]. The reaction mixture contained 0.5 mM ABTS, 0.1 M sodium acetate buffer (pH 5.0) and 10 -100 µl culture supernatant. Oxidation of A B T S was monitored spectrophotometrically by determining the increase in absorbance at 420 nm, (A420nm) with a molar extinction coefficient, ε_{420} = 36000 M-1cm-1. One unit (U) of enzyme activity was defined as the amount of enzyme oxidizing 1mole of substrate per minute under assay conditions.

Preparation of microbial inoculants for coir pith compost

Single, dual, and consortium of microbial inoculants were added 1x $l0^9$ cfu ml^{-1} @ two litres of *Pleurotus sajor caju*, *Cellulomonas fimi* and *Phanerochaete chrysosporium* broth culture ton^{-1} of coir pith heap. For the nitrogen source the Urea was added @ 0.5 per cent level. The following treatment are scheduled the study of the hydrolytic enzymes activity and composting.

Composting technology for coir pith waste

Coir pith composting is an aerobic process. It should be heaped above the soil coir pith is spread to the length of 4 feet and breadth of 3 feet. Initially coir pith should be put up for 3 inch height and thoroughly moistened. The fiber is removed from the source itself. Coir pith alone transported to experimental site. An experiment was conducted to study the effect of individual, dual, and consortium of microbial inoculants were added @ two ml of broth culture (two litres per ton) of coir pith with 1x $l0^9$ cfu ml^{-1}. For the nitrogen source the Urea was added @ 0.5 per cent level. Over coir pith one portion of coir pith is added and the same input mentioned above is incorporated. Repeat the process to make a heap up to 4 feet height.

Moisture maintenance and turning

Moisture level of 55 to 60 per cent was maintained by uniform sprinkling of water at regular intervals. The optimum moisture is maintained for uniform composting. The compost heap was turned once every 15 days Turnings and trimming were given at fortnight intervals.

Qualitative analysis

The samples were collected periodically at an interval of 15 days to 90 days. Sampling of compost was done by following the method described by Faure and Deschamps (1990). Samples were air dried, powdered and used for analyzing the physio-chemical properties. The microbial population was also assessed by following the standard methods.

Statistical analysis: The experimental data were processed statistically by applying the technique of analysis of variance in randomized block design [25].

RESULTS AND DISCUSSION

In the composting process, the effective coir pith compost inoculants such as *Pleurotus sajor caju, Cellulomonas fimi* and *Phanerocheate chrysosporium* were used as the treatment scheduled for studying the hydrolytic enzymes (xylanase, dehydrogenase, Laccase and Mn peroxidase activity over the period of composting. Xylanase are enzymes that are capable of degrading xylan units yielding large quantities of monomeric xylose units. The xylanases activities during composting periods are presented in (Table 1). The peak xylanase activity in all the treatments was found to be on the 30th day and thereafter a sharp decline was noticed. Among the treatments, T_7 recorded peak xylanase activity on the 30th day of composting (31.13 U ml^{-1}). The T_6 was comparable to T_7 and recorded 29.79 (U ml^{-1}) Individual inoculant treatment recorded less than 22.00 (U ml^{-1}) on the 30th day. After the 30th day, all treatments showed insignificant xylanase activity.

Table 1 Effect of individual, dual and consortium on the xylanase activity during the coir pith composting period

Treatments	Xylanase (U ml^{-1})						
	Composting period (In days)						
	0	15	30	45	60	75	90
T_1: *Cellulomonas fimi* (CPB3)	0.08	7.20	19.35	9.47	4.23	4.15	2.95
T_2: *Phanerocheate chrysosporium* (CPF7)	0.08	7.22	19.15	9.51	4.26	3.96	3.01
T_3: *Pleurotus sajor caju* (CPF13)	0.08	7.23	21.68	9.60	3.25	3.08	2.96
T_4: *Cellulomonas fimi* (CPB3) + *P.chrysosporium* (CPF7)	0.08	7.25	27.28	10.15	6.23	5.13	4.92
T_5: *Cellulomonas fimi* (CPB3) + *Pleurotus sajor caju* (CPF13)	0.08	7.27	28.17	10.37	5.15	4.75	3.95
T_6: *Pleurotus sajor caju*(CPF13) + *P.chrysosporium* (CPF7)	0.08	7.28	29.79	10.65	4.43	4.23	3.98
T_7: *Cellulomonas fimi* (CPB3) + *P. Chrysosporium* (CPF13) + *Pleurotus sajor caju* (CPF13)	0.08	9.35	31.13	12.14	6.95	6.25	5.92
T_8: Control	0.08	3.33	11.25	6.12	2.13	2.01	2.01
SEd	-	0.07	0.12	0.08	0.05	0.04	0.02
CD(P=0.05)	NS	0.15	0.25	0.19	0.11	0.09	0.05

Xylanases are the enzymes capable of removing the constituents of the xylan backbone. [26] emphasized the role of xylanases of thermophilic fungi. The isolates obtained in the present study were screened for xylanolytic activity. The isolates CPB-3, CPF-4 and CPF-13 did not only produce higher quantities of xylanase (34.53, 86.00 and 85.16 U ml^{-1}) but also found to withstand increased temperature and higher pH. [27] showed multiple forms of xylanases, which differ in stability catalytic efficiency and absorption and activity on substrate. A possible role for the production of xylanase isozymes of different molecular size might be to allow their diffusion in plant substrate. For majority of xylanases, optimum pH ranged from 4.5 to 6.5 was found to be stable at high temperature.

Table 2 Effect of individual, dual and consortium of inoculants on the dehydrogenase activity during the coir pith composting period

Treatments	Dehydrogenase activity (mg formazole formed h^{-1}g^{-1})						
	Composting period (In days)						
	0	15	30	45	60	75	90
T_1: *Cellulomonas fimi* (CPB3)	1.20	1.72	1.92	2.86	2.13	2.01	1.72
T_2: *Phanerocheate chrysosporium* (CPF7)	1.20	1.86	1.99	2.71	2.33	2.04	1.74
T_3: *Pleurotus sajor caju* (CPF13)	1.20	1.73	1.97	2.95	2.45	2.01	1.75
T_4: *Cellulomonas fimi* (CPB3) + *P.chrysosporium* (CPF7)	1.20	1.90	2.86	3.95	3.15	2.89	2.40
T_5: *Cellulomonas fimi* (CPB3) + *Pleurotus sajor caju* (CPF13)	1.20	1.85	2.72	3.45	3.23	2.42	2.45
T_6: *Pleurotus sajor caju*(CPF13) + *P.chrysosporium* (CPF7)	1.20	1.92	2.06	3.28	3.15	2.95	2.96
T_7: *Cellulomonas fimi* (CPB3) + *P. Chrysosporium* (CPF13) + *Pleurotus sajor caju* (CPF13)	1.20	2.13	3.18	4.18	4.14	3.20	3.13
T_8: Control	1.20	1.25	1.23	1.26	1.43	1.22	1.20
SEd	0.02	0.11	0.12	0.13	0.09	0.08	0.11
CD(P=0.05)	NS	0.23	0.25	0.28	0.20	0.18	0.22

The survival and microbial activity during the entire composting process was studied by estimating dehydrogenase activity at 0th, 15th, 30th, 45th, 60th, 75th and 90th day of composting and the results are presented in (Table 2). All the treatments showed dehydrogenase activity throughout the composting periods but the degree of activity varied during the composting periods. Individual inoculated treatment T_1, T_2 and T_3 were more or less on par in dehydrogenese activity. The dual inoculant treatment T_4 performed better than T_5 and T_6. The triple inoculation treatment T_7 recorded maximum dehydrogenase activity on the 45th day (4.18 mg of formazole formed h^{-1} g^{-1}) and on

60th day (4.14 mg of formazon formed h^{-1} g^{-1}). All the treatments showed increased enzyme activity from 30th day onwards upto 75 days. There was least dehydrogenase activity in the uninoculated control (T_8).

Table 3 Effect of individual, dual and consortium on the laccase activity during the coir pith composting period

Treatments	Laccase (U ml^{-1})						
	Composting period (In days)						
	0	15	30	45	60	75	90
T_1: *Cellulomonas fimi* (CPB3)	0.12	0.23	0.41	0.52	0.59	0.15	0.15
T_2: *Phanerocheate chrysosporium* (CPF7)	0.12	0.23	0.42	0.56	0.55	0.14	0.14
T_3: *Pleurotus sajor caju* (CPF13)	0.12	0.22	0.41	0.54	0.55	0.15	0.14
T_4: *Cellulomonas fimi* (CPB3) + *P.chrysosporium* (CPF7)	0.12	0.25	0.45	0.56	0.55	0.15	0.14
T_5: *Cellulomonas fimi* (CPB3) + *Pleurotus sajor caju* (CPF13)	0.12	0.23	0.47	0.57	0.56	0.16	0.14
T_6: *Pleurotus sajor caju*(CPF13) + *P.chrysosporium* (CPF7)	0.12	0.23	0.46	0.57	0.56	0.15	0.15
T_7: *Cellulomonas fimi* (CPB3) + *P. Chrysosporium* (CPF13) + *Pleurotus sajor caju* (CPF13)	0.12	0.26	0.47	0.58	0.58	0.21	0.21
T_8: Control	0.02	0.13	0.14	0.14	0.24	0.28	0.29
SEd	-	0.01	0.02	0.03	0.04	0.01	0.01
CD(P=0.05)	NS	0.02	0.05	0.07	0.08	0.03	0.02

Laccase activity plays an important role in the breakdown of lignin in large quantities. The laccase activities recorded during composting periods (Table 3). The individual inoculants treatments reveal that T_2 recorded maximum laccase activity on the 45th day (0.56U ml^{-1}) followed by T_3 (0.54 U ml^{-1}) also on the 45th day and 0.41U ml^{-1} by T_1 on the 30th day. Among the dual inoculants treatments, T_5 performed better compared to T_4 and T_6. The triple microbial treatment T_7 recorded highest enzyme activity on the 45th day (0.58 Uml^{-1}). Interestingly, T_7 starts its maximum enzyme activity from 30th day onwards and the activity lasted upto 75th day. The treatment T_7 recorded an activity of (0.21U ml^{-1}) on the 75th day, whereas others recorded comparatively low enzyme activity on 75th day.

Table 4 Effect of individual, dual and consortium on the Mn peroxidase activity during the coir pith composting period

Treatments	Mn peroxidase (U ml^{-1})						
	Composting period (In days)						
	0	15	30	45	60	75	90
T_1: *Cellulomonas fimi* (CPB3)	0.12	0.13	0.14	0.14	0.09	0.05	0.05
T_2: *Phanerocheate chrysosporium* (CPF7)	0.12	0.13	0.15	0.18	0.15	0.14	0.14
T_3: *Pleurotus sajor caju* (CPF13)	0.12	0.12	0.13	0.15	0.15	0.15	0.14
T_4: *Cellulomonas fimi* (CPB3) + *P.chrysosporium* (CPF7)	0.12	0.15	0.16	0.16	0.15	0.15	0.14
T_5: *Cellulomonas fimi* (CPB3) + *Pleurotus sajor caju* (CPF13)	0.12	0.13	0.17	0.17	0.16	0.16	0.14
T_6: *Pleurotus sajor caju*(CPF13) + *P.chrysosporium* (CPF7)	0.12	0.13	0.16	0.17	0.16	0.15	0.15
T_7: *Cellulomonas fimi* (CPB3) + *P. Chrysosporium* (CPF13) + *Pleurotus sajor caju* (CPF13)	0.12	0.14	0.17	0.18	0.18	0.17	0.17
T_8: Control	0.02	0.03	0.04	0.04	0.05	0.05	0.05
SEd	-	0.01	0.01	0.01	0.01	0.01	0.01
CD(P=0.05)	NS	0.02	0.03	0.02	0.03	0.02	0.02

The Mn peroxidase activities recorded during composting periods are presented in (Table 4). The individual inoculants treatments reveal that T_2 recorded maximum Mn peroxidase activity on the 45th day (0.18 U ml^{-1}) followed by T_3 (0.15 U ml^{-1}) also on the 45th day and (0.14 U ml^{-1}) by T_1 on the 30th day. Among the dual inoculants treatments, T_5 performed better compared to T_4 and T_6. The triple microbial treatment T_7 recorded highest enzyme activity on the 45th day (0.18 Uml^{-1}). Interestingly, T_7 starts its maximum enzyme activity from 30th day onwards and the activity lasted upto 60th day. The importance of developing industrial organism having multiple enzyme system complexes. Keeping in view of this concept, the present study attempted to isolate and screen organisms capable of producing Dehydrogenase, xylanases, laccase and Mn peroxidase at higher levels of temperature and varied pH. Based on their potential abilities' isolates evolved as a consortium [28].

The xylanase of the screened isolates were potential sources for fast degradation of complex biomolecules, the study resulted in three most efficient isolates viz., C*ellulomonas fimi* (CPB-3), *Pleurotus sajor caju* (CPF-4) and *Phanerochaete chrysosporium* (CPF-13). For biodecomposition of coir pith, an attempt was made to develop composting of coir pith using the best isolates viz., CPB-3, CPF-4 and CPF-13 as single inoculants (T_1, T_2, T_3), as dual inoculants (T_4, T_5, T_6) and as triple inoculants or consortium (T_7) and yielded significant results. The composting samples were analyzed over a period of 0, 15, 30, 45, 60, 75 and 90 days for the activity of Dehydrogenas, xylanases, laccase and Mn Peroxidase. The study revealed that triple inoculants treated sample showed significant enzyme activity even on the 30th day of sampling.

CONCLUSION

The present study was undertaken to develop suitable composting technologies involving effective decomposing microbes for proper management of coir pith waste and convert them into enriched compost. The results of the various experiments conducted in the study revealed that, the triple inoculants consortium combining *Pleurotus*

sajorcaju (CPF-4), *Cellulomonas fimi* (CPB-3), and *Phanerocheate chrysosporium* (CPF-13) are found to be suitable inoculants mix for the effective composting of coir pith.

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