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Substitution of Urea with Fungi and Nitrogen Fixing Bacteria for Composting Coir Pith

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Coir pith can be converted into rich organic manure for horti, flori-and agriculture by the action of certain strains of white rot fungi. *Pleurotus spp.* are efficient lignin degrading agents, however apart from the studies on *Pleurotus sajor caju* in coir pith biodegradation, degradation of coir pith by other species of fungi is not well understood. The present study was mainly to identify which of the fungal mushroom species is more potent in degrading the coir pith and converting it into efficient organic manure, and whether the incorporation of some bacterial species could improve the composting process. The study revealed that, there is an effective degradation of lignin and a notable increase in the NPK content when nitrogen fixing bacteria were used as the main source of nitrogen during composting of coir pith instead of urea. The replacement of inorganic urea with biological agents such as nitrogen fixing bacteria will not only be economical but also enrich the fertility of soil in a sustainable manner. The coir pith composting can hence be made wholly organic and eco-friendly.

Key words: Lignin, *Bacteria, Fungi,* compost

India is one of the leading producers of coconut. It is an important oil seed and cash crop grown in south Indian States especially Kerala, Tamil Nadu and Karnataka. Coconut husk is the basic raw material of the coir industry. Coir fibre extracted from husk is used in production of mats, matting, rubberized coir mattresses, yarn, ropes etc. After extraction of coir fibre from husk, the, coir pith is unutilized. Coir pith is known as coir dust and is the major byproduct of the coir fibre extraction industries.

Coir pith constitutes as much as 70 per cent of the coconut husk. It is a fluffy, light, spongy material with increased water-holding capacity and extremely compressive and has a sizable percentage of combustible matter along with low ash content. Essentially it is a lignocellulosic material that decomposes very slowly in soil because its pentosan/lignin ratio is 1:0.30; the minimum required for moderately fast decomposition in the soil is 1:0.50.(*Ghosh et al.,2007*). Coir pith is resistant to biodegradation as it contains lignin (33-35%), which is an aromatic polymer composed of phenyl propane subunits including coumaryl, guaicyl and syringyl moieties that are covalently linked together by a variety of bonds, mainly â-aryl ether bonds. It is also present in the fibre and is responsible for the stiffness of coir. It is thought that the lignin has its origin in carbohydrates. Oyster mushroom belonging to *Pleurotus* species have the ability to degrade lignin slowly under favorable conditions.This included the selection of a suitable species of Basidiomycetes fungus called *Pleurotus sajor caju*, which has the ability to slowly degrade lignin and is capable of detoxifying phenolic compounds and also producing bio-polymersing enzymes. The cellulosic compounds present in the coir waste support the initial growth of this fungus and acts as co-substrate for lignin degradation.

The characteristic feature of *Pleurotus sajor caju* is that they contain higher protein content (28.03%), compared to common vegetables. It contains all the amino acids essential for human nutrition found in mushrooms and especially cystine, lysine, threonine and tryptophan which are present in appreciable amount. It degrades the phenolic group, as observed by the decrease

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in methoxy content; they also cause an oxidative shortening of side chain. Cleavage of the ring proceeds while still attached to the polymer. Enzymes such as laccase, phenol oxidases are also involved in the process of lignin degradation. It is essential to study the degradation of lignin in the biodegradation process.

Urea acts as an important nitrogen supplier to the coir pith to enhance their NPK value and make it efficient organic manure. The present study is targeted to replace the inorganic urea with the Nitrogen fixing bacteria like *Rhizobium, Azotobacter and Azospirillum*. A consortium using these microflora with appropriate fungal species for composting helps to make coir pith a perfect soil conditioner and a bio-organic manure.

Materials and Methods

Coir pith collected from the accumulated areas of Kalavoor, Alappuzha district was used in the study. The microbial species were procured from the Microbiology Division of the Central Coir Research Institute (CCRI), Alappuzha. The experimental protocol and biochemical analysis of coir pith was carried out at Rajiv Gandhi Chair in Contemporary studies, Cochin University of Science and Technology (CUSAT) during September 2007 to November 2007.

Five samples of 1 kg coir pith each along with duplicates were laid on a terraced floor as separate heaps.12 g of *Pleurotus sajor caju* spawn was thoroughly mixed with the first sample of coir pith. 12 g of *P.sajor caju* supplemented with 5 g of urea was given to the second sample. The third, fourth and fifth samples of coir pith were treated with 12 g of *P.sajor caju* and 5 g of Nitrogen fixing bacteria.(*Rhizobium, Azotobacter* and *Azospirillum*) respectively. The nitrogen fixing bacteria used were immobilized in lignite. The moisture content was maintained at 200% and the samples were monitored regularly for 30 days.

The composted coir pith was subjected to analysis for lignin content, nitrogen, potassium and phosphorus after the study period. Lignin was estimated by the Modified Klason lignin assay method. Nitrogen was estimated by Kjel dahl method. The estimation of Phosphorous was done with Spectrophotometry and Potassium by Flame photometry.

Results and Discussion

The lignin content, pH and organic carbon of coir pith before and after decomposition with different mushroom species was estimated and the results obtained are presented in Table 1.

The lignin content of raw coir pith invariably contains 32 % lignin. Where as the coir pith when treated with mushroom species, the lignin content showed varying levels. The decomposition ranged from 18 to 30.1%. The minimum value was obtained when the coir pith was treated with *Calocybe indica*, while the maximum value was evident when the pith was treated with *Pleurotus florida*. From the results it is clear that *C .indica* was found to be effective mushroom species in coir pith lignin degradation.

The pH of the raw coir pith on 15th day of composting and 30th day of composting did not show any variation and it remained at 6.3 in both the treatments. The pH was always towards acidic and at no point of time it was either neutral or towards alkaline. When treated with different mushroom species, the pH ranged between 6.4 and 6.8, the minimum in *P. florida* and maximum in *C. indica.* When the values of pH were examined on the 30th day, it was evident that the values showed a marginal increase in all the treatments when compared to 15th day of treatment; however the increase noticed was not significant.

The organic carbon content of coir pith on 15th and 30th day did not show any variation and it was 26.28%. Where as the values under treatment at time intervals showed variation. The organic carbon content of coir pith after 15 days of treatment with different species of mushroom showed that the values ranged between 26.17% in *P.sajor caju* to 27.38% in *C. indica*. In the case of treatments with 30 days of composting, the organic carbon content decreased in all the treatments when compared to 15 days of exposure. The organic carbon content ranged between 26.16 and 27.26 %.

Nitrogen, phosphorus and potassium (NPK) content of raw and treated coir pith with mushroom species on 15th and 30th day of composting are presented in Table 2. The results of the study revealed some interesting information on different variables on account of decomposition.

The nitrogen content of raw coir pith kept as control under 15 and 30 days was found to be not changed, the value being 0.73 % in both the cases. But under 15 days of treatment with different species of mushroom, the nitrogen content registered values between 0.81 and 0.93 %, *P. eous* exhibited the minimum value and *C. indica* the maximum value. On 30th day, in general all the values showed a marginal increase than the values registered for 15 days of treatment. The values however, ranged between 0.84 and 0.96 %. From the values obtained it is confirmed that the *C. indica* was found to be more effective in enhancing nitrogen content as a function of decomposition. *P. eous* was less efficient when compared to other species tested.

The phosphorus content of the control pith both in 15 days treatment and 30 days treatment was the same, the value being 0.24 %. The values for 15 days treatment with different species of mushroom indicated that the phosphorus content was maximum in coir pith treated with *P. sajor caju* (0 .29 %) while it was minimum in *P. eous* (0.25 %). In 30 days of exposure the values generally increased in all the treatments which ranged from 0.29 % to 0.48 %. *P. sajor caju* was found to be more effective in enhancing the phosphorus content when compared to other species of mushrooms.

Table 1. Lignin content, pH and Organic carbon content of raw and treated coir pith mushroom species

Treatment	Lignin Content (%)		pH		Organic Carbon (%)	
	0 Day	30th Day	15th Day	30th Day	15th Day	30th Day
Raw Coir pith	32	32	6.3	6.3	6.28	6.28
Pleurotus sajor caju	32	20	6.7	6.8	6.17	6.16
Pleurotus florida	32	30.1	6.4	6.7	7.14	6.21
Pleurotus eous	32	26	6.6	6.7	7.25	6.31
Calocybe indica	32	18	6.8	6.9	7.38	7.26

The potassium content of coir pith did not show any change in controls. However, the values under treatments with mushroom species showed variations. In 15 days treatment, the potassium content ranged between 0.30 and 0.40%, but when treated for 30 days, the potassium values increased and the enhancement ranged between 0.32 and 0.41 %. It is thus clear that there is a direct relationship exists between potassium content and the duration of treatment. In both the treatments, *P.sajor caju* was found to be effective in enhancing the potassium content followed *by P. florida* and *C. indica*.

Table 3 presents the results of the treatments on decomposition of lignin in coir pith. When coir pith was treated with *P.sajor caju* and *P. florida*, it was found that *P.sajor caju* decomposes lignin more effectively (28 %) than *P.florida* (30 %). When the chemical fertilizer, urea was added as an additional component along with *P.sajor caju* and *P. florida*, the results revealed that the decomposition process was better in coir pith treated with *P.sajor caju*. As the values observed in coir pith treated with *P.florida*, the difference noticed was just marginal i.e. from 30 to 30.1 %, indicating that it was not so effective in degrading the lignin. As an alternate to chemical fertilizer,

Table 2. Nitrogen, phosphorus and potassium content of raw and treated coir pith with mushroom species

Treatment	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
	15th Day	30th Day	15th Day	30th Day	15th Day	30th Day
Raw Coir pith	0.73	0.73	0.24	0.24	0.28	0.28
Pleurotus sajor caju	0.84	0.86	0.29	0.48	0.42	0.41
Pleurotus florida	0.86	0.89	0.28	0.36	0.39	0.38
Pleurotus eous	0.81	0.84	0.25	0.29	0.48	0.32
Calocybe indica	0.93	0.96	0.28	0.41	0.37	0.38

when the bacterial strains were added, the results showed an encouraging trend. When *Rhizobium* + *P.sajor caju* was added with coir pith, the bacterial strain degrades the lignin more effectively than the chemical fertilizer and the value obtained was 18%. In the case of *Rhizobium* +*P. florida*, the value obtained was 24 % and this value was also better than the value obtained with Urea. As Azotobacter was added with *P.sajor caju*, the degradation of lignin was 20 % and it was 28% when *P. florida* and *Azotobacter* combination was tried. The *Azospirillum* + *P.sajor caju* + coir pith combination revealed that the lignin degradation was 19.2 % and that of *Azospirillum* + *P. florida* + coir pith combination the results obtained was 29.2 %. It is thus clear that the bacterial strains are more effective than the chemical fertilizer, the urea tested. Among the bacterial strains tested *Rhizobium* combination performed well when compared to that of the other two strains tested.

Table 3. Lignin degradation using different concentration of mushroom species and bacterial

Treatment	Lignin content in raw coir pith (%)	Lignin content (after composting) (*P.sajor caju*) (%)	Lignin content (after composting) (*P.florida*) (%)
Pith+spawn	32	28%	30
Pith+spawn+urea	32	20%	30.1
Pith+spawn+*Rhizobium*	32	18%	24
Pith+spawn+*Azotobacter*	32	20%	28
Pith+spawn+*Azospirillum*	32	19%	29.2

The production of organic carbon, enhancement of nitrogen, phosphorus and potassium as a function of different treatments (coir pith, mushroom species, urea and bacterial strains) is presented in Table 3 and 4. Table 3 reveals that the organic carbon content showed a decreasing trend, however the rate of decrease was more in bacterial strains than that of urea. Among the bacterial strains, *Azospirillum* was more effective in reducing the organic carbon content.

In all the combinations, the values of nitrogen, phosphorus and potassium showed an increasing trend when compared to the chemical fertilizer, the urea. The nitrogen content was maximum (0.572 %) when treated with *Azospirillum* and the content was minimum (0.524 %) when treated with urea. The phosphorus and potassium content was maximum (0.14 %& 0.73 %) in *Azotobacter* while it was minimum (0.09 %& 0.69 %) in urea.

Organic carbon and NPK contents obtained under the combination of the spawn of *P sajor caju*, urea and bacterial strains presents a picture that the organic carbon values showed a declining trend when treated with urea and bacterial strains. But when compared the values obtained with urea and bacterial strains, the bacterial strains degrade the organic content mush faster than that of urea. In the case of bacterial strains, *Azospirillum* was found to be more effective in enhancing nitrogen and phosphorus while *Rhizobium* was more potent in enhancing the potassium content. As for nitrogen and phosphorus contents are concerned, it was more in *Azospirillum* (0.699 & 0.18 %) and minimum in urea (0.599 & 0.13 %). Maximum value of potassium (0.89 %) was discernible in *Rhizobium* while it was minimum in urea (0.85 %).

It is reported that coir pith contains 35% lignin and that it has to be degraded to make the fibre smooth and soft. Lignin in coir pith has to be degraded so that it can be used as good base material for the growth of several crops and can be used as good manure. Several trials have been worked out to degrade coir pith lignin using mushroom and bacterial species and obtained encouraging results. The white rot fungi belonging to the basidiomycetes have been widely used to degrade lignin and the results reported by authors are encouraging (Akin *et al.,* 1995). Ruggeri and Sassi (2003) and Bosco *et al*. (1999) have reported that *P. chrysosporium* is an effective agent to degrade lignin and produce lignocellulytic enzymes and has direct application in lignocellulose bioconversion processes. The present study reports that when mushroom species have been used to decompose coir pith, considerable quantities of lignin is degraded. The present study reports a reduction of about 18 to 30.1 % of lignin. Among the four mushroom species tested for the process of degradation, the species, *Calocybe indica* was found to be more effective than the other species. From the literature it is seen that *P.sajor caju* produces enzymes such as lignin peroxidase, manganese peroxidase and phenol oxidases in meager quantities. The enzyme production by other species of mushroom is not well known. The reason for the increased activity of *C. indica* in the process of lignin degradation might be due the nature of its enzyme profile and the quantity of production.

Table 4. Organic carbon (oc) & NPK values of compost under treatment with *Pleurotus florida* and bacteria

Treatment	OC (%)	N (%)	P (%)	K (%)
Pith+spawn	24.88	0.457	0.08	0.74
Pith+spawn+urea	29.56	0.524	0.09	0.69
Pith+spawn+*Rhizobium*	28.98	0.439	0.12	0.71
Pith+spawn+*Azotobacter*	28.68	0.556	0.14	0.73
Pith+spawn+*Azospirillum*	26.34	0.372	0.11	0.72

From the available studies it is observed that mushroom species with its enzymatic action degrade only the lignin content and leaving the other component intact (Arora *et al.,* 2002). But the present study confirms that when the coir pith was treated with four species of mushrooms, the nutrient content of the coir pith showed variation.

The enzymes from white rot fungi that catalyze the initial depolymerization of lignin are extra cellular and unusually non specific (Cullen and Kersten, 2004). Lignin degradation by white rot fungi has been extensively studied, and results revealed that the three kinds of extra cellular phenol oxidases, namely lignin peroxidase (LiP). Manganese peroxidase (MnP) and laccase (Lac), are responsible for initiating the depolymerization of lignin (Moriya Ohkuma *et al., 2001*) The present work is in line with these findings that, the depolymerization of the lignin causes the reduction in their percentage from

32% to 18% (with *C. indica*) in the composted coir pith. It is assumed that these enzymes are also produced by the Nitrogen fixing bacteria and are involved in the lignin depolymerization along with the enzymes secreted by the fungus.

This work is in harmony with the findings of Somasundaram Rajarathnam *et al.* (1998) that they indicate, one of the four strains of *Pleurotus* tested for the degradation showed maximum activities of laccase and polysaccharide degrading enzymes that could be correlated with high weight loss, reduction in the yield of lignin. Out of the four test organisms used in the present study, all the organisms showed a definite capability to degrade the lignin and thereby exhibited a drastic reduction in lignin percentage.

Venkitaswamy (2003) reported maximum yields of coconuts when coir pith compost was added with trace amounts of chemical fertilizers (NPK). But the present study of composting with Nitrogen fixing bacteria, causes the enhancement of NPK content within the coir pith compost itself by their action, and can cause the improved activity as organic manure.

Janshekar and Fiechter (2004) observed poor biodegradation of lignin when bacterial cultures were used to decompose the coir pith and concluded that the poor degradation may not be influenced by the culture medium composition or culture conditions but it may be due to the inability of the bacterial species tested to degrade lignin. But the present observation revealed that the bacterial strains when added with mushroom species, the lignin degradation increased, revealing the fact that combination of bacterial and fungal strains have the capability to degrade lignin to a considerable extent.

Thus the present study confirms that the individual mushroom species or mushroom species in combination with Nitrogen fixing bacteria not only influences lignin degradation but also enhances the NPK production and the combination of both have the capacity to degrade lignin to a considerable extent.

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