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Pleurotus florida and nitrogen fixing organism (*Azotobacter vinelandii*) for the biodegradation of coir pith

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Abstract
This study reports the biodegradation of coir pith by using *Pleurotus florida* along with nitrogen fixing organism and also reports the findings on the growth trials carried out using biodegraded coir pith on some plant. It is concluded that the coir pith can be converted to effective organic manure with biodegradation with *Pleurotus florida* and Nitrogen fixing bacteria *Azotobacter vinelandii*. The combined action of these organisms with the white rot fungi, there was a marked reduction in the lignocelluloses content and particle size, enhancement of NPK content and the resultant product can be used as effective manure for plants.

Keywords: *Pleurotus florida*, *Azotobacter vinelandii*, biodegradation

1. Introduction
Coir pith or coir dust is a major by product of coir fiber extraction industries [1]. It decomposes very slowly in soil as its pentosan-lignin ratio is below 0.5, and because of chemical and structural complexity of lignin-cellulose complex [2]. Normally coir pith is dumped as agricultural waste and accumulates as a waste product as heaps of course and fine dust [3]. High content of lignin (28.25%) in coir pith causes very slow decomposition following which it is used as raw organic manure for crops [4]. Some mushrooms belonging to *Pleurotus* species degrade lignin slowly under favorable conditions. Microorganisms produce extracellular enzymes (Ligninase and Cellulases) to degrade lignin in lignocelluloses of plant biomass [5]. Degradation of coir pith can be effectively carried out with suitable species of basideomycetes fungus and in combination with nitrogen fixing bacteria. Lignin (3×1011 metric tons on planet with an annual biosynthetic rate of 2×1010 tons) constitutes second most abundant group of biopolymers in biosphere [6]. Cellulosic compounds present in coir waste support initial growth of fungus and acts as co-substrate for lignin degradation. Coir wastes after biodegradation can be effectively used as manure for increasing yield of crops [7]. The present study reports the biodegradation of coir pith by using *Pleurotus florida* along with nitrogen fixing organism and also reports the findings on the growth trials carried out using biodegraded coir pith on some plants.

2. Materials and Methods
2.1 Sample collection
Coir pith was collected from the Coir Board, Thanjavur. Bacteria (*Azotobacter vinelandii*, MTCC No. 124) was procured from Microbial Type Culture Collection (MTCC), Chandigarh.

2.2 Estimation of chemical properties
Lignin: The lignin in coir pith was determined by Klason lignin method [8].
Nitrogen: Nitrogen in the coir pith samples were estimate by Alkaline permanganate method using Kjeldahl distillation Unit [9].
Phosphorus: Phosphorous was estimated by Vanado Molybdo phosphoric yellow colour method.
Potassium: Potassium was estimated by Flame photometry.
Ammonia: Estimation of ammonia in coir pith sample is done by nesslerization method.

2.3 Estimation of enzyme activity
Lignin peroxidase: Lignin peroxide activity was estimated by prepared reaction mixture containing 2 mM Veratryl alcohol (Km = 60 µM), 0.4 mM H_2O_2 (Km = 80 M), 50 mM tartaric acid and enough ligninase to give an absorbance change of 0.2/min.

One unit of enzyme activity is defined as the quality of enzyme required for the formation of 1 µM of Veratryl aldehyde per minute.

Manganese peroxidase: Manganese peroxidase activity estimated by prepared 50 mM sodium tartarate buffer (pH-4.5), added $MnSO_4$, H_2O_2 and phenol red at 0.2%, 0.1 mM and 0.0025% concentration respectively to the final volume of 5 ml reaction mixture. Read the change in absorbance at 431 nm. Maintained a heat killed enzyme source as control. One unit of enzyme activity is defined as the amount of enzyme required for 0.1 OD change at 431 nm/min.

2.4 Particle size analysis of coir pith by scanning electron microscope (SEM)
The surface particle size and pore size was studied using the Scanning Electron Microscope (VEGA3 TESCAN, JAPAN).

2.5 Composting
The experiment was conducted by mounting 5 kg of raw coir pith with *Pleurotus florida* and bacteria (*Azotobacter vinelandii*). The efficacy of growth of plants in coir pith compost is studied by laying sets of coir pith heaps in a shady place for composting, the heaps were moistened regularly to retain moisture.

3. Results and Discussion
3.1 Estimation of chemical properties
The biodegraded coir pith using *Pleurotus florida* in combination with *Azotobacter vinelandii* causes reduction in Lignin, Organic carbon and enhancement in the Nitrogen, Phosphorous and Potassium (NPK) content. The details of the chemical parameters given below.

Lignin: The investigations reveal that definite variations were observed in lignin content in the coir pith under various treatments with ligninolytic mushroom *Pleurotus florida* in combination with *Azotobacter vinelandii*. The periodical analysis of samples of coir pith drawn at regular intervals of 5 days from the experimental heaps shows the rate of decomposition on lignin in coir pith (20% to 18%) from the initial value of 32%, in the control (Raw coir pith). Biodegradation of coir pith using combination of *Pleurotus florida* and *Azotobacter vinelandii* the maximum lignin reduction (17%). The details are given in Figure 5.

Nitrogen: The percentage of nitrogen in the raw coir pith (Control) showed no change during the course of composting. The value of Nitrogen for Raw coir pith is 0.73 and the others show variations. The treatments with both the combinations shows the enhancement of nitrogen to 0.78% for *Pleurotus florida* and *Azotobacter vinelandii*. From the results, it is clear that all the treatments lead to the enhancement of Nitrogen. The studies of coir pith degradation with the combination of *Pleurotus florida* nitrogen fixing bacteria (*Azotobacter vinelandii* shows a definite enhancement in nitrogen content after the coir pith biodegradation (Figure 1).

Phosphorous: The periodical analysis of coir pith samples under the treatment with mushroom and fungal species indicated an increasing trend of phosphorous content during composting. The combinations used for composting of coir pith showed variation in phosphorous content ranging from 0.85% to 1.87% *Pleurotus florida* and *Azotobacter vinelandii*. The details of the amount of Phosphorous given in Figure 2.

Potassium: The values of Potassium in the biodegraded coir pith with the treatment with combination of mushroom and bacterial species were given in Figure 3. The potassium content in the coir pith maintained as control which shows no change throughout the composting process. However, the values under treatment with mushroom species showed variation in potassium content. The results obtained from the analysis of coir pith samples treated with the mushroom displayed the values of potassium in an increasing trend. The phosphorous content of the samples varied from 0.04% in raw coir pith to the maximum of 0.36 % in Biodegraded Coir pith Compost (BCC) using *Pleurotus florida* and *Azotobacter vinelandii*. Coir pith samples drawn out from the experimental heaps at regular intervals indicated increase in the potassium content (Figure 3).

Ammonia: Ammonia content of the compost was observed to be increased due to nitrogen fixation activity of Nitrogen fixing bacteria incorporated with *Pleurotus florida*. It is observed that the amount of ammonia increased at different intervals of decomposition. The raw coir pith accounts 1261.16 mg/kg which increased to 3016.27 mg/kg when treated with *Pleurotus florida* and *Azotobacter vinelandii*. The details have been given in Figure 4.

3.2 Enzyme activity
From the chemical analysis of Biodegraded coir pith it could be observed that coir pith contains three major constituents viz. lignin, cellulose and hemicelluloses. Almost all white rot fungi produce enzymes viz. Manganese Peroxidase (MnP) and laccase, but only some of them produce Lignin Peroxidase (LiP) [10, 11]. The enzymes produced by white rot fungi catalyze the initial polymerization of lignin are extracellular and unusually non-specific [12]. Lignin degradation by white rot fungi has been extensively studied and the results revealed that the three kinds of extracellular peroxidases viz. lignin peroxidase (LiP), Manganese peroxidase (MnP) and Laccase are responsible for initiating the depolymerization of lignin in coir pith. In our study, the activity of both the enzymes viz. MnP and LiP could be observed which leads to the degradation of lignin in the coir pith.

Lignin peroxidase: The enzyme assay was conducted for the enzyme produced by *Pleurotus florida*. Definite reduction of lignin is also observed by the action of these enzymes. A blank was also taken as control. Readings were taken using U.V - Visible spectrophotometer at different intervals. The details in the study are furnished in Figure 6.

Manganese peroxidase: Manganese peroxidase assay is based on the oxidation of phenol red during coir pith degradation. The principle function of manganese peroxidase (MnP) is to oxidize Mn^{2+} to Mn^{3+}, using H_2O_2 as oxidant [13]. Activity of the enzyme is stimulated by simple organic acids which stabilize the Mn^{3+}, thus producing diffusible oxidizing chelates. Figure 7 shows the variation in the enzyme activity by the organisms. The enzyme profile showed variation in activity with change in substrate, decomposition stage and method of composting.

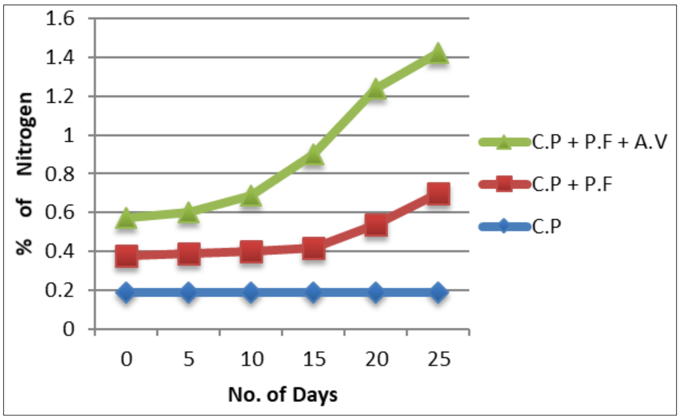

Fig 1: Nitrogen (%) during biodegradation of coir pith

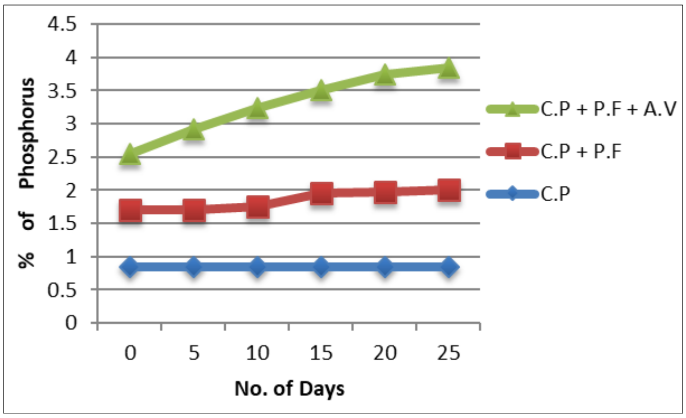

Fig 2: Phosphorus (%) during biodegradation of coir pith

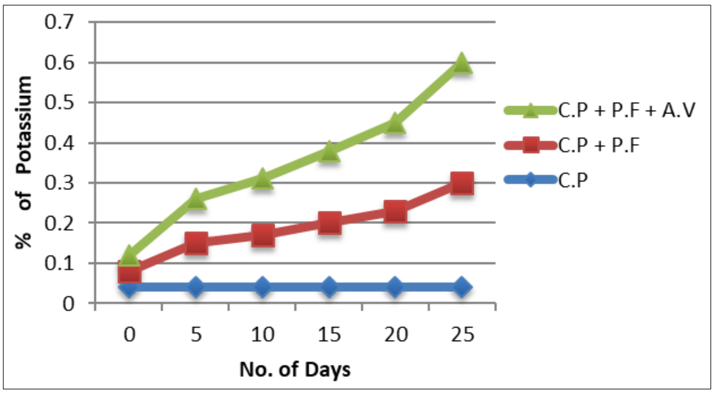

Fig 3: Potassium (%) during biodegradation of coir pith

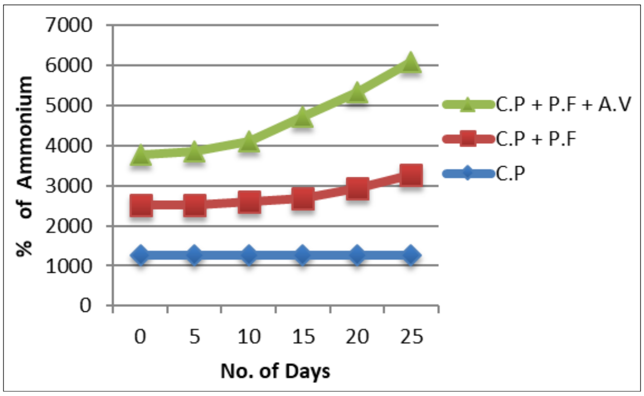

Fig 4: Ammonium (%) during biodegradation of coir pith

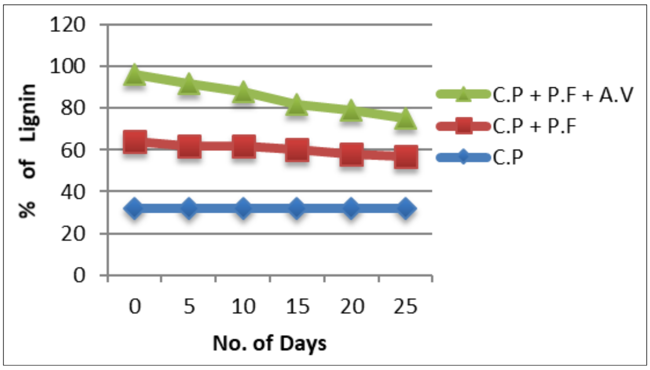

Fig 5: Reduction of Lignin during biodegradation of coir pith

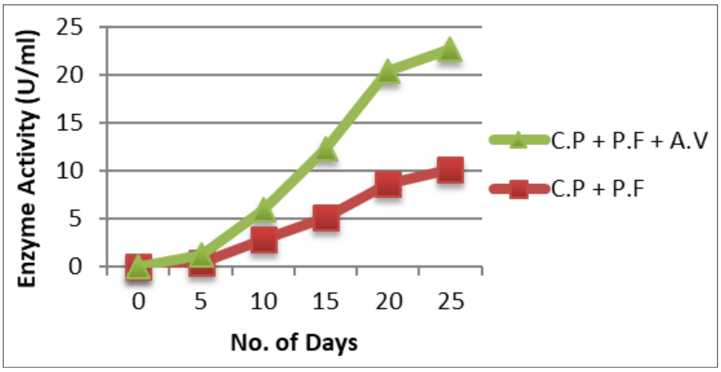

Fig 6: Enzyme activity (Lignin peroxidase)

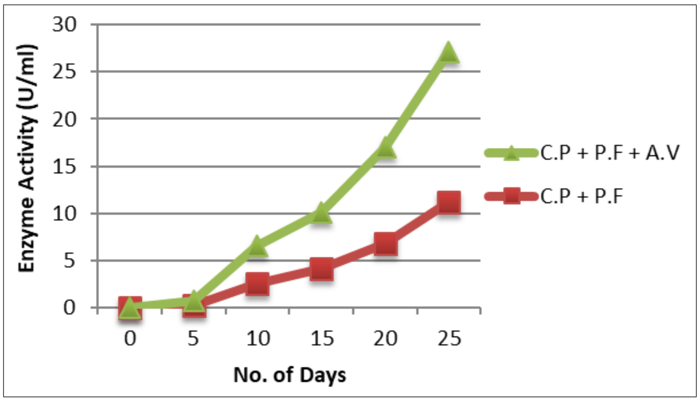

Fig 7: Enzyme activity (Manganese peroxidase)

3.3 Particle size analysis

Size and surface characteristics of biodegraded coir pith was determined by Scanning Electron Microscope (SEM). All the treated and raw coir pith was subjected to SEM imaging and the images are given in Figure 8 and Figure 9. From the images it is clear that the raw coir pith has greater size than after biodegradation thereby confirming that while degradation the size of the coir pith is reduced.

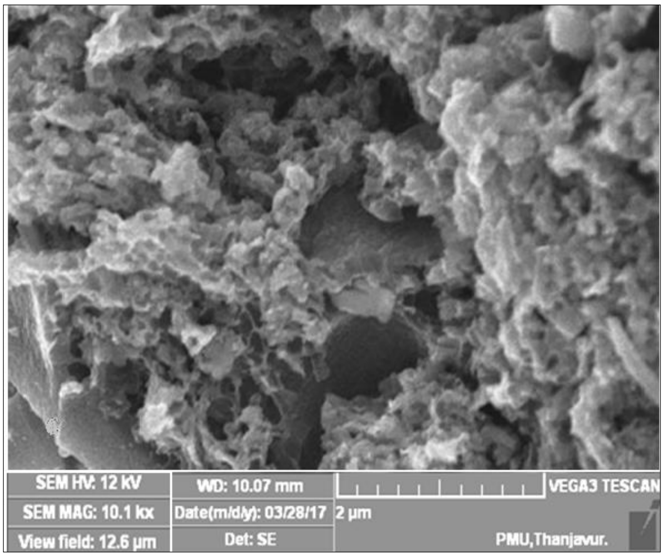

Fig 8: SEM image of raw coir pith (Single particle)

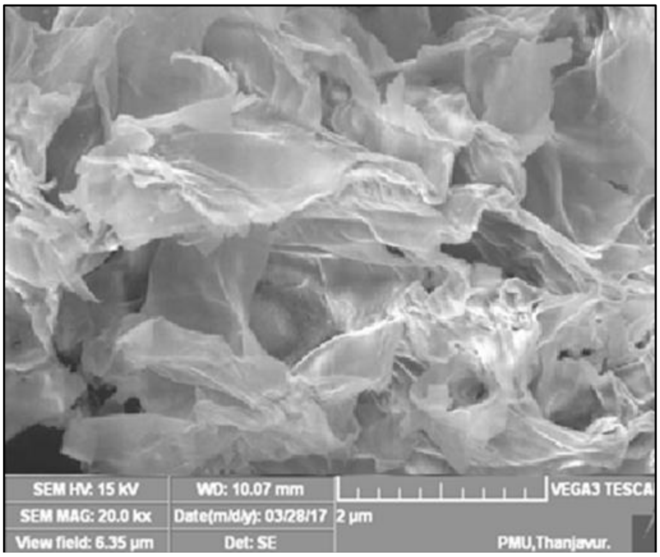

Fig 9: SEM image of biodegraded coir pith (Single particle)

3.4 Field Experiment

Experimental lots of coir pith composted using *P. florida* in combination with *Acetobacter vinelandii* and another one kept as untreated as raw coir pith itself were compared with control (Garden soil). Beans plant shows effective growth in pots containing higher content of biodegraded coir pith; using *Acetobacter* shows improved growth in medium with compost and soil. Thus, present study that composted coir pith with *P. florida* and Nitrogen fixing bacteria is an excellent potting media for medicinal plants; medium can be used as such or in combination with garden soil for their cultivation

Fig 10: Plant growth after 5 days

Fig 11: Plant growth after 15days

4. Conclusion

In our studies, we can conclude that coir pith can be converted to effective organic manure with biodegradation with *Pleurotus florida* and Nitrogen fixing bacteria *Azotobacter vinelandii*. The combined action of these organisms with the white rot fungi, there was a marked reduction in the lignocelluloses content and particle size, enhancement of NPK content and the resultant product can be used as effective manure for plants.

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