RESEARCH PAPER International Journal of Agricultural Sciences, Vol. 5 Issue 1, January to May, 2009 : 161-164

Phanerochaete chrysosporium mediated biocompost and its effect on growth in *Vigna radiata* L.

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ABSTRACT

Coir pith, an agro waste obtained from coconut husk contains high level of cellulose and lignin. These complex polymers need effective microorganisms for their degradation. One such lignocellulolytic fungus is *Phanerochaete chrysosporium,* which produce lignin peroxidase and cellulase which aids in the bioconversion of coir-pith into compost. The biocompost obtained was tested for its physio-chemical characteristics and growth promoting ability in 40 days old seedlings of *Vigna radiata.* The appealing results obtained were that the total carbon, protein and chlorophyll content of the test plants proved higher than that of the control.

Key words : Inoculation, *Azospirillum*, *Azotobacter*, Growth attributes, Yield attributes, Economics.

INTRODUCTION

Coir pith is an agro waste produced by coir fiber extraction from coconut husk. It is difficult to get degraded in soil but can be well degraded by microorganisms like *Phanerochaete chrysosporium, Humicola griea, Streptomyces* spp., *S.viridosporus, S.setonii, Nocardia* spp., *Micromonospora* spp. and *Arthobacter* spp. (Sheeba, 2005;Padmaja and Lavanya, 2006). *P. chrysoporium* is the common thermo-tolerant lingo-cellulose degrading fungus stored in wood chip piles.

Coir pith contains high level of cellulose and lignin and hence difficult to degrade. The cells are thin and empty cavities are rich in potash having low amounts of nitrogen and phosphorus. Coir pith waste causes pollution and disposal being a serious problem, can be utilized as organic manure after decomposing (Padmaja and Lavanya, 2006).

Cellulases produced by various fungi, hydrolyse cellulose into simple sugars to be used as a carbon source for their metabolism. Fungal cellulases are glycosylated which protects the enzyme from proteolysis and helps in secretion of proteins.Lignins are thermophilic and thermo-tolerant polymers composed of phenolic compounds and attacked most readily by fungi. The acid precipitable polymeric lignin (APPL) fraction released due to microbial decay is useful as an antioxidant surfactant and as a component of adhesives and resins (Crawford *et al.*, 1986).

Due to intimate association of cellulose with lignin it is not readily available as a carbon source and so delignification becomes necessary. Though chemical delignification is rapid it is not eco-friendly and so microbial delignification is preferred (Kirk and Farrell,1987).

P.chrysosporium releases two extra cellular enzymes lignin peroxidase and manganese peroxidase which are preferred for lignin decomposition. Nitrogen has a profound effect in degradation of lignin by *P.chrysosporium* (Kirk and Farrell, 1987; Reid, 1979; Yang *et al.*, 1980). Basidiomycetes colonizing ruminant dung in grassland ecosystem represent the ecological equivalents of white rot fungi *P.chrysosporium.*

Composting of coir pith helps in detoxifying phenolic compounds (Natarajan *et al.*, 1987). In the present investigation "Biodegradation of coir pith using *Phanerochaete chrysosporium"* the strain MTCC 787 was challenged for its cellulose and lignin degradation ability and biological assay of the enzymes responsible for degradation were undertaken.

MATERIALS AND METHODS

Collection of coir pith and its processing:

Coir pith collected in Pollachi, Tamilnadu, India from coir industry was allowed to degrade in a pot containing four alternative layers of soil and coir pith. Fresh and degraded coir pith was used for the following studies.

Standard plate count:

The garden soil utilized in study showed predominant population of *Aspergillus* spp., confirmed through macroscopic and microscopic tests. In addition the lignolytic fungi *Phanerochaete chrysosporium* was inoculated to enhance coir-pith degradation.

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Determination of pH:

The extract from coir-pith using deionised water was subjected to pH estimation using Elico pH meter.

Moisture content:

Using the weight loss, the moisture content was calculated using the formula

$$\text{Moisture \%} = \frac{\text{Initial weight (g)} - \text{final weight (g)}}{\text{Weight of sample (g)}} \times 100$$

Ash content:

After charring at 800°C, in a muffle furnace, the sample was used to analyze the ash content.

$$\text{Ash content} = \frac{\text{Initial weight (g)} - \text{final weight (g)} \times 100}{\text{Weight of sample (g)}} \text{ g}$$

Determination of organic carbon content:

Following the titration procedure by Walkely and Black (1934), the amount of organic carbon was calculated using the following formula.

$$\text{Organic carbon} = \frac{25 - (25 \times \text{titre value}) \times \frac{0.003}{0.1}}{\text{Bank value}} \times 100\%$$

Estimation of sodium and potassium:

The fluid under analysis is sprayed as a fine mist into a non-luminous flame which becomes colored according to the characteristic emission of the element. The characteristic emission of sodium and potassium were read at 589nm and 768nm, respectively using comparative standards.

Estimation of cellulose (Sadasivam and Manickam, 2003):

The cellulose undergoes acetolysis with acetic or citric reagent and form glucose molecule when treated with 67% H_2SO_4. It is dehydrated to form hydroxyl methyl furfuryl which forms green colored product with anthrone and the color intensity measured at 630nm.

Estimation of lignin (Sadasivam and Manickam, 2003):

Refluxing sample with acid detergent solution removes the material other than fibrous omponent. The left out material was weighed after filtration, dried, treated with 72% H_2SO_4 and filtered, dried and ashed. The loss of weight on ignition gives the acid detergent lignin.

$$\text{ADL \%} = \frac{\text{Weight of 72\% } H_2SO_4 \text{ washed fibre} - \text{ash}}{\text{Weight of sample}}$$

Enzyme assay :

Cellulase activity (Sadasivam and Manickam, 2003):

Cellulase was assayed by measuring reducing sugar as glucose by dinitrosalicilic acid method. The reaction mixture after incubation at 37°C for 2 hours was treated with 2ml Dinitrosalicylic acid. The tubes were kept to boil in a water bath for 15 minutes and 1ml of Rochelle salt solution was added. It was cooled under tap water and the color development was read at 575nm in a spectrophotometer using glucose as the standard solution. The enzyme activity was expressed as the mg of glucose released per minute per mg of protein.

Lignin peroxidase activity (Sadasivam and Manickam, 2003):

The assay for the enzyme was carried out with pyrogallol as substrate. The assay mixture contained 1ml of the crude enzyme sample, 0.2ml of 0.1M pyrogallol, 2ml of 0.1M phosphate buffer (pH6.5) with 0.1ml of 0.1M H_2O_2. The enzyme activity was determined by monitoring the absorbance at 436nm using spectrophotometer due to the formation of blue coloured end product purpurogallin. The enzyme activity was expressed in terms of units per litre.

Determination of total carbohydrate by Anthrone method (Sadasivam and Manickam, 2003):

Carbohydrates are first hydrolysed into simple sugar using dilute hydrochloric acid. In the hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630nm.

$$\text{Amount of carbohydrate present in 100 mg of sample} = \frac{\text{mg of glucose} \times 100}{\text{Volume of test sample}}$$

Protein estimation by Lowry's method (Sadasivam and Manickam, 2003):

The blue color developed by the reaction of the phosphomolybdic-phosphotungstic compounds in folin-ciocalteau reagent by the amino acid tyrosine and tryptophan present in the protein. The resulting blue color was measured at 660nm in a spectrophotometer.

Estimation of chlorophyll (Sadasivam and Manickam, 2003):

Chlorophyll extracted from leaf sample using 80%acetone and the absorption at 663nm and 645nm were read in a spectrophotometer. Using the absorption coefficience, the amount of total chlorophyll was calculated.

$$\text{mg total chlorophyll a/g tissue} = \frac{20.2\,(A_{645}) + 8.02\,(A_{663}) \times V}{1000 \times w}$$

where, A=absorbance at specific wavelength,

V=final volume of chlorophyll extract in 80% acetone,

W=fresh weight of tissue extracted.

RESULTS AND DISCUSSION

In the present investigation "bioconversion of coir pith to compost using cellulose and lignin peroxidase producing *Phanerochaete chrysosporium* MTCC 787 and its evaluation in growth promotion of *Vigna radiata* seedlings" the interesting results obtained were as follows (Table 1 and Fig. 1, 2, 3, and 4).

The cellulose, lignin, carbon, C:N ratio, reducing sugar and non-reducing sugar contents of compost obtained after the degradation of coir pith by *Phanerochaete chrysosporium* was decreased to 17.45%, 20.47%, 18.1%, 0.58mg/g, 0.42mg/g, 0.29mg/g and nitrogen content was increased to 1.28%, respectively. So the biodegraded coir waste can be used profitably as an organic manure substitute to promote the growth and yield of various crops were reported by Padmaja and Leena (2006).

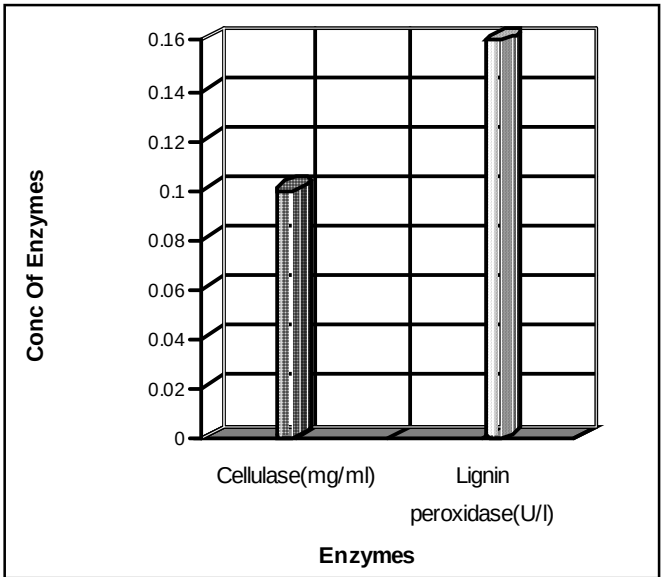

Fig. 1 : Showing the production of ligno cellulosic enzymes by *Phanerochaete chrysosorium*

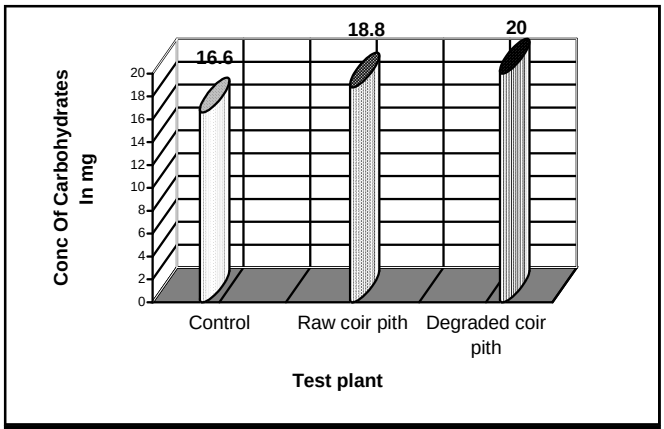

Fig. 2 : Showing the estimation of carbohydrates in test plants (*Vigna radiata* L.)

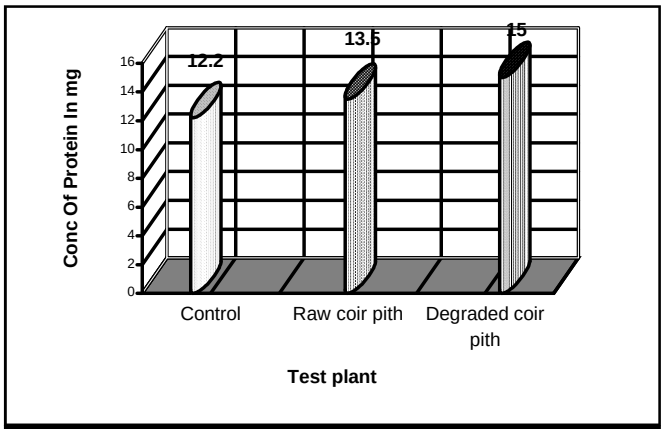

Fig. 3 : Showing the estimation of protin in test plants (*Vigna radiata* L.)

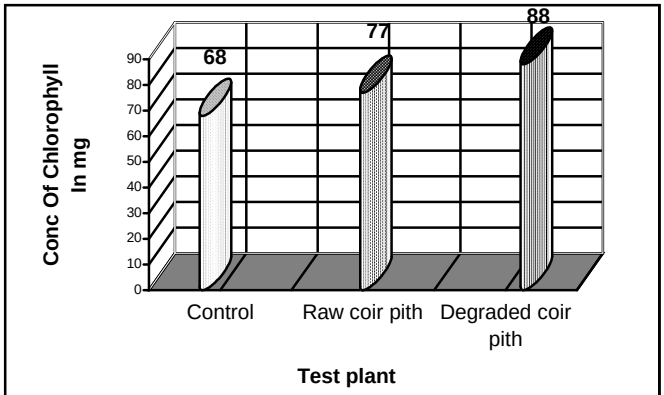

Fig. 4 : Showing the estimation of chlorophyll in test plants (*Vigna radiata* L.)

Table 1: Physio-chemical parameters of raw and degraded coir-pith

Parameters	Physical parameters		Chemical parameters			
	pH	Moisture (%)	Ash Content (%)	Carbon (%)	Sodium (milli equ/l)	Potassium (milli equ/l)
Raw coir-pith	6.5	43.1	3.16	32.25	230	200
Degraded coir-pith	7.5	53.2	4.13	13.5	320	300

The study by Vinodhini *et al.* (2005) revealed that 100% degraded coir-pith was ideally suited for the growth of medicinal plants grown.According to the present study which was carried out with carbon, potassium, sodium, cellulose, lignin, where in carbon 13.5%, potassium 300milli equ/l, sodium 320milli equ/l, cellulose 9.7mg, lignin 9.1mg which reveals the maximum degradation property by *Phanerochaete chrysosporium* and *Vigna radiata* L.

The decomposed coir pith which was used as fertilizer for *Vigna radiata* L. whereas their carbohydrate, protein and chlorophyll contents were measured and was compared with control and raw coir pith which showed growth promoting ability of degraded compost owing to its enriched macro and micro nutrients.

The concept of organic matter required for use in agriculture is becoming more popular than land-fill and incineration, since the availability of chemical fertilizers to the farmers at reasonable cost is fast declined. Management of solid waste has assumed paramount importance in recent years. Recycling of these waste or residues is necessary to prevent pollution and to conserve our scarce natural resources.

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Received : May, 2007; Accepted : November, 2008