# PROCESS IMPROVEMENT OF COIR PITH DEGRADATION INVOLVING WHITE ROT FUNGI BY SUBSTITUTION OF UREA WITH NITROGEN FIXING BACTERIA AND APPLICATION OF BIODEGRADED COIR PITH AS PLANT GROWTH MEDIUM

*Thesis submitted to the*

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*By*

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# *Process Improvement of Coir Pith Degradation Involving White Rot Fungi By Substitution of Urea With Nitrogen Fixing Bacteria and Application of Biodegraded Coir Pith as Plant Growth Medium*

*Ph D Thesis in the field of Environmental Biotechnology*

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***Cover page:*** *Plant cultivated in pots containing coir pith compost, coir pith hillocks accumulated in coir fibre extraction unit, Kattukada, Alappuzha and Big coir pith hilllock accumulated in Kalavoor, Alappuzha.*


*School of Environmental Studies, Cochin University of Science & Technology*
*Cochin – 682 022, India*

*April 2013*

Dedicated to

My beloved Parents

&

Loving Brother

# Certificate

*This is to certify that the research work presented in the thesis entitled "**Process improvement of coir pith degradation involving white rot fungi by substitution of urea with nitrogen fixing bacteria and application of biodegraded coir pith as plant growth medium**" is based on the original research work carried out by Mr. Abesh Reghuvaran under my guidance and supervision at School of Environmental Studies, Cochin University of Science & Technology, in partial fulfillment of the requirement for the degree of Doctor of Philosophy, and that no part thereof has been presented for the award of any other degree.*

***Dr. Anita Das Ravindranath***
*Supervising Guide*
*Senior Scientific Officer*
*Central Coir Research Institute*
*Kalavoor, Alappuzha*

# Declaration

*I hereby declare that the work presented in this thesis entitled "**Process improvement of coir pith degradation involving white rot fungi by substitution of urea with nitrogen fixing bacteria and application of biodegraded coir pith as plant growth medium**" is based on the original research carried out by me at the School of Environmental Studies, Cochin University of Science & Technology, Cochin under the guidance of Dr. Anita Das Ravindranath, Senior Scientific Officer, Central Coir Research Institute, Alappuzha, and the thesis or no part thereof has been presented for the award of any degree, diploma, associateship or other similar titles or recognition.*

*Cochin 22* **Abesh Reghuvaran**
*Date*

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***Abesh Reghuvaran***

# Contents

# Abbreviations

| | | |
|---|---|---|
| % | - | Percentage |
| ˚C | - | Degree Celcius |
| ˚F | - | Degree Fahrenheit |
| µg | - | Microgram |
| β | - | Beta |
| AGE | - | Agarose Gel Electrophoresis |
| APCC | - | Asian and Pacific Coconut Community |
| BLAST | - | Basic Local Allignment Search Tool. |
| Cm | - | Centimeter |
| BCC | - | Biodegraded Coir pith Compost. |
| CEC | - | Cation Exchange Capacity |
| DNA | - | Deoxyribonucleic Acid |
| DW | - | Distilled Water |
| EC | - | Electrical Conductivity |
| Fig | - | Figure |
| For | - | Forward |
| g | - | Gram |
| h | - | Hour |
| HCl | - | Hydrochloric Acid |
| $H_2SO_4$ | - | Sulphuric Acid |
| ha | - | Hectare |
| HPLC | - | High Performance Liquid Chromatography |
| $K_2SO_4$ | - | Potassium Sulphate |
| $K_2Cr_2O_7$ | - | Potassium Dichromate |
| Lac | - | Laccase |

| | | |
|---|---|---|
| LCW | - | Lignocellulosic Waste |
| LiP | - | Lignin Peroxidase |
| mg | - | Milligram |
| ml | - | Millilitre |
| mhos | - | Millimhos |
| mM | - | Millimolar |
| MTCC | - | Microbial Type Culture Collection |
| MnP | - | Manganese Peroxidase |
| MT | - | Metric Tons |
| N | - | Normality |
| NaOH | - | Sodium Hydroxide |
| nm | - | Nanometer |
| OD | - | Optical Density |
| ppt | - | parts per thousand |
| ppm | - | parts per million |
| PCR | - | Polymerase Chain Reaction |
| rDNA | - | Ribosomal DNA |
| *spp* | - | Species |
| sec | - | Second |
| U | - | Unit |
| UV | - | Ultra Violet |
| V | - | Volt |
| V/V | - | Volume/Volume |

Chapter 1

# GENERAL INTRODUCTION AND REVIEW OF LITERATURE

Contents



## 1. Introduction

### 1.1. Coconut.

Coconut is one of the oldest crops grown in India and presently covers 1.5 million hectares in this country with a total production of over 10,000 million nuts. India stands third in production in the world after Indonesia and Philippines, although has a potential of much higher production from the existing area. The potential is now being gradually exploited with the introduction of improved technology, as a result of which the productivity is showing marked increase since 1981-1982.

### 1.2. Coconut Cultivation in World.

Coconut is grown in more than 93 countries around the world in an area of 12.167 million ha producing 59,569 million nuts or 11.912 million tons of copra equivalent as on 2005. The member countries of the Asian and Pacific Coconut Community (APCC) occupy 10.691 million ha which is 87.9% of the total area under coconut. The production indicates that 83.3% (9.924 million tons) of copra equivalent is from the APCC countries. Over a period of 45 years, India, Indonesia

and Philippines had considerably increased the area under coconut, while Malaysia, Sri Lanka, Thailand and Vietnam among Asian countries and Fiji among Pacific countries showed a negative trend. Samoa had doubled the area. India ranks first in the productivity of coconut in the world with an annual productivity of 8303 nuts per ha. while in production India ranks second and in area, the country is in the third position (2009). Indonesia ranks first in area under coconut as well as in global coconut production. Malaysia imports coconut products equivalent to 1400 million nuts and exports coconut products equivalent to 1200 million nuts. (Press conference on 45 th Cocotech meeting, Kochi, 26 th June, 2012). Though there is an increase in production in some of the APCC member countries the production increases doesn't match with the additional area brought under this crop. The productivity in many countries remains below one ton of copra per ha depending upon the input management. Only a few countries such as India, Philippines, Malaysia, Solomon Islands, Vietnam and Thailand had exceeded the productivity of one ton copra per ha per year. However, it was observed that many farmers in India with optimum management are getting an average yield ranging between 150-200 nuts per palm per year. Coconut (*Cocos nucifera* L.) is socially, culturally, and religiously associated with millions of people around the world. Apart from food and drink, it provides sustainable income to millions who are involved in its cultivation and product utilization. In the major coconut growing countries coconut contributes to food security, nutritional security, employment security, social security and poverty alleviation. It is eco friendly and environmentally sustainable (Rethinam, P., 2007).

**Top 5 Coconut Producing Countries**

| Sl No. | Country | Production (2007) | Production (2010) | Percentage change |
|---|---|---|---|---|
| 1 | Indonesia | 19.625,000 m/t | 20,655,400 m/t | +5.25 % |
| 2 | Philippines | 14,852,900 m/t | 15,540,000 m/t | -4.421 % |
| 3 | India | 10,894,000 m/t | 10,825,100 m/t | +0.646 % |
| 4 | Brazil | 2,831,004 m/t | 2,705,860 m/t | +4.625 % |
| 5 | Sri Lanka | 2,180,680 m/t | 2,238,800 m/t | -4.779 % |

*Source : FAOSTAT data (last accessed on April, 2012.*

*List notes : Production is in metric tons (m/t) for the year 2010, Percentage change is from the year 2007.*

Coconut is grown mostly as a mono crop. It is essentially a small holder crop, expected to provide a basic, steady income to the farmer, round the year. The annual global production of coconut is estimated to be about 66 billion nuts, of which, the equivalent of at least 50% is estimated to enter the international trade, in the form of semi processed and value added products. For most of the major producing countries, coconut is an important foreign exchange earner. In most producing countries, coconut is also used for domestic dietary needs, mostly as coconut oil and coconut milk/cream, in daily household food preparations, providing in some instances, as much as 20% of the daily calorific requirements.

## 1.3. Coconut Cultivation in India.

The four states of India *viz.* Kerala, Tamil Nadu, Karnataka and Andhra Pradesh account for 89% of the total production of coconut in the country. The highest production of 13,299 million nuts was achieved in the year 1994-95. Thereafter, there has been a decline in the production to the present level of 12,832 million nuts and area under the plantation cultivation is around 1.78 million hectares in the country. The main factors responsible for the fall in production are the spread of mite infestation which had assumed epidemic proportions, the debilitating root wilt diseases in prime coconut growing areas, intermittent drought affecting the crop and the inability of the coconut farmers to develop necessary irrigation infrastructure due to the small and fragmented nature of holdings. Notwithstanding these constraints coconut production has shown up upward trend over a period of time. The production increased from 5,939 million nuts in 1981-82 to 12,832 million nuts in 2004-05. However, the annual growth rate has declined from 5.43% during the period between 1981-82 and 1991-92 to 1.88% during 1991-92 to 2004-05. The annual growth which was 18.29%, 7.12%. 3.42% and 2.95% respectively in the states of Andhra Pradesh, Tamil Nadu, Kerala and Karnataka during 1981-82 declined to 1.73%, 1.26%, 2.40% and (-) 0.11% respectively during the period between 1991-92 and 2004-05. The severe drought for three consecutive years from

2000-01 to 2003-04 in the major coconut producing states has been primarily responsible for this setback. The favorable monsoon from 2004-05 onwards had a positive impact on production particularly in the states of Tamil Nadu. The crop forecast done by the Central Plantation Crops Research Institute indicates a production of 14.30 billion nuts in the country during 2006-07.

**All India Estimates of area and production of Coconut**

| | 2007-2008 (Revised) | | | 2008-2009 (Final) | | |
|---|---|---|---|---|---|---|
| States /Union Territories | AREA ('000 Hectares) | Production (Million nuts) | Productivity (Nuts/ha) | AREA ('000 Hectares) | Production (Million nuts) | Productivity (Nuts/ha) |
| Andhra Pradesh | 101.32 | 1119.26 | 11047 | 104.00 | 970.00 | 9327 |
| Assam | 19.00 | 136.00 | 7158 | 18.80 | 147.10 | 7824 |
| Goa | 25.50 | 127.60 | 5004 | 25.61 | 128.18 | 5005 |
| Gujarat | 16.40 | 138.30 | 8433 | 15.98 | 157.42 | 9851 |
| Karnataka | 405.00 | 1635.00 | 4037 | 419.00 | 2176.00 | 5193 |
| Kerala | 818.80 | 5641.00 | 6889 | 787.77 | 5802.00 | 7365 |
| Maharashtra | 21.00 | 175.10 | 8338 | 21.00 | 175.10 | 8338 |
| Nagaland | 0.90 | 0.20 | 222 | 0.92 | 0.55 | 598 |
| Orissa | 51.00 | 275.80 | 5408 | 51.00 | 275.80 | 5408 |
| Tamil Nadu | 383.37 | 4968.20 | 12959 | 389.60 | 5365.00 | 13771 |
| Tripura | 5.80 | 11.40 | 1966 | 5.80 | 11.40 | 1966 |
| West Bangal | 28.60 | 355.50 | 12430 | 28.60 | 355.50 | 12430 |
| A & N Island | 21.60 | 80.60 | 3731 | 21.69 | 82.00 | 3781 |
| Lakshwadweep | 2.70 | 53.00 | 19630 | 2.70 | 53.00 | 19630 |
| Pondichery | 2.20 | 26.60 | 12091 | 2.10 | 30.70 | 14619 |
| All India | 1903.19 | 14743.56 | 7747 | 1894.57 | 15729.75 | 8303 |

***Source:*** *Directorate of Economics & Statistics, Ministry of Agriculture, Govt. of India.*

## 1.4. Coir Industry in Kerala.

Kerala is the largest producer of coconut in India, accounts for around 45% of the country's total production. Coir is a unique natural fibre used in diverse applications of great economic importance. This wonder fibre is extracted from coconut husk and is spun into coir yarn and a number of other value added products such as coir rope, mats, mattings, rugs carpets etc. are produced. Coir fibre is also used in combination with other natural or synthetic fibres or materials

such as Rubber or synthetic polymers for making products that are better suited for specific uses. Coir and coir fibre products sustain the livelihood of a significant segment of the population in the coastal belt of southern India, especially Kerala. In Kerala, the coconut kernel is an essential food ingredient that contributes to taste in a number of preparations. Coconut is also valued for the extractable edible oil contained in the kernel. Thus the fibre associated with coconut is often considered secondary and due to lack of an organized collection mechanism, a large part of this is utilized as fuel and only the remaining is utilized for fibre extraction. This happens even in the coconut oil industry where they concentrate on the oil and the oilcake. Enough attention is not paid for utilization of the fibrous part.

The coir industry in Kerala presently provides direct employment to about 3.60 lakhs persons including those who are employed for part of the year. It is a fact that a good percentage of this is women engaged in the spinning of coir. The indirect employment is also very significant. The potential of this industry for upgradation and expansion is high and if taken advantage of, this will have a significant impact on the coastal economy of the State. Recognizing this fact, the Government introduced a number of regulations for sustaining the industry including those intended to improve the availability of husk for the industry at reasonable cost. Thanks to increased level of mechanisation, utilisation of husk for production of fibre out of the total husk available in the region is expected to improve from the current level of 30 per cent to 50 per cent.

## 1.5. Coir Pith.

Coconut coir pith is an agro waste from coir industry and it is a renewable resource. The elastic cellular cork like pithy material forming the non fibrous tissue of the coconut husk generally accounts for 50-60 % of the total weight of husk. One ton of coir pith is produced for every 10,000 husks used in the coir industry. Accumulation of coir pith near coir factories in large quantities causes solid waste pollution problems. Since it is composed of lignocellulosic compounds the total

degradation of these compounds was once considered as an impossible task. Coir pith undergoes decomposition in a very slow rate only because of its low carbon (pentosan) to lignin ratio of less than 0.5 %, which is minimum required for the slow decomposition of organic matter in soil. The practical problem associated with direct application of raw coir pith as manure have been solved with earth worm (vermicomposting) or using Pith plus (a mushroom spawn). Nowadays, composted coir pith is being widely used along with organic supplements for many crops especially in Horticulture and Floriculture. Composted coir pith is highly beneficial in improving crop productivity in plants and it shows high conversion ratio. Moreover, its ability in the management of certain root diseases had also been reported.

### 1.5.1. Properties.

Coir pith is lignocellulosic in nature, brown coloured, light weight corky dust; particle size varies from 100-300 microns similar to peat moss. It has a porous structure and the pores are responsible for allowing good aeration around the roots of plants and retain water content in the pores for rewetting when dry. Chemical analysis reveals that coir pith contains three major constituents-cellulose, hemicelluloses and lignin. Cellulose is a polymeric chain of anhydrous glucose units and exists mainly in crystalline form. Hemicellulose is made up of mixed polymers of various pentose and hexose sugars and is amorphous in nature. Lignin is an amorphous polymer of phenyl propane, which surrounds the cellulose in the cell wall. Lignin exists in situ as large molecular weight component but indefinite in size. The major properties of coir pith is as follows

- High water holding capacity, *i.e*,. 6-8 times than its weight.
- Slow degradation due to high lignocellulose content.
- Excellent moisture retention even after drying.
- Porosity is high, stores and releases nutrients over extended periods of time.

- Acceptable electrical conductivity (EC), pH and Cation exchange capacity (CEC).
- Greater physical resiliency that withstands compression better.
- Being a poor conductor of heat, helps keep soil temperature under control.

**Table 1.** Chemical composition and physical properties of coir pith.

| Sl No | Constituents | Unretted coir pith | Retted coir pith |
|---|---|---|---|
| 1. | Lignin (%) | 38.50 | 30 |
| 2. | Cellulose (%) | 26.40 | 25.10 |
| 3. | Organic Carbon (%) | 29.50 | 29.0 |
| 4. | Nitrogen (%) | 0.24 | 0.26 |
| 5. | Phosphorous (%) | 0.01 | 0.01 |
| 6. | Potassium (%) | 0.71 | 0.76 |
| 7. | C:N ratio | 123:1 | 112:1 |
| 8. | Calcium (%) | 0.40 | 0.47 |
| 9. | Magnesium (%) | 0.36 | 0.41 |
| 10. | Copper (ppm) | 3.10 | 4.20 |
| 11. | Iron (ppm) | 0.07 | 0.08 |
| 12. | Manganese (ppm) | 12.50 | 17.00 |
| 13. | Zinc (ppm) | 7.50 | 9.80 |
| 14. | Moisture (%) | 20-30 | 60-80 |
| 15. | pH | 5.4-5.8 | 5.6-6 |
| 16. | EC (millimhos/cm) | 0.8-1.2 | 0.3-0.6 |
| 17. | Salinity (ppt) | 1 | 2-4 |
| 18. | CEC (Meq/100 g of sample) | 15-20 | 20-25 |

*Source: Coir News, 2007 (Coir Board)*

### 1.5.2. Uses.

As it has an excellent water holding capacity, it can be extensively used as potting medium for plants. The major uses of coir pith are as follows.

- Potting soil for commercial farming, in glass houses and also in the hobby market.

- Grow bags in glass houses, green houses for horticulture and floriculture.
- Briquettes used for house gardens and terrace gardens.
- In production of biogas and power.
- Water resistant boards and particle boards of different densities.
- As a light weight aggregate to produce low density concrete for thermal insulation of roof slabs.
- For production of light bricks and clays.
- Manufacture of rubber based coir pith gaskets.

### 1.5.3. Environmental Problems.

Coir pith degrades very slowly and it remains in the soil for a very long period of time, however due to the natural climatic effect of repeated washing in the rains leading to enhanced microbial activity which eventually degrades the pith. Coir pith is recalcitrant and accumulates in the environment forming hillocks posing environmental pollution in the areas close to coir fiber extraction units (Hume, 1949). As a result of its fluffy nature, its transportation will not be cost effective. Coir industries and fiber extraction units contribute considerably to the problems of environmental pollution, both land and water pollution. The high quantum of its production in the defibering units and the difficulties experienced in its disposal have tended to create a major problem of pollution of large areas of land and water in coir fiber extraction units. During monsoon rains leaching of tannins and salts from the hillocks of coir pith leads to ground water pollution and may be magnified up the food chain to levels that are harmful for wild life and possibly for humans because most of these pollutants are extremely difficult to remove by waste treatment methods. Therefore biodegradation of coir pith is essential to control the pollution caused by accumulation (Warrier and Moudil., 1947). Coir pith even though is a problematic waste, it is a potential wealth and can

be converted into valuable organic manure by microbial degradation. The industrial applications of coir pith have so far been very limited (Thomas *et al*., 1998)

### 1.5.4. Methods of Disposal.

As it contains Lignin and cellulose in a considerable level, it is resistant to biodegradation in normal natural conditions. Hence disposal problem also quite difficult in the case of coir pith. The unique ability of white rot basidiomycetes to degrade lignin has become a matter of high importance for effective biodegradation of coir pith and thereby eliminates environmental pollution. The biological decomposition of organic matter is mediated by a variety of biochemical process in which the enzymes play a key role. Conventionally a process has been standardized by Central Coir Research Institute (CCRI) for the preparation of organic manure/fertilizer out of coir pith. According to this process, one ton coir pith is composted by addition of 'Pith plus', an edible mushroom and urea over evenly spread out layer of pith. The heap of coir pith is moistened daily and left for about 30 days for composting. The organic manure thus obtained is rich in nitrogen, phosphorous and potassium (NPK). The present work is aimed to substitute urea with bacterial cultures for composting process.

## 1.6. Objectives.

1.6.1. To study the efficacy of different fungal species to degrade coir pith effectively.

1.6.2. Substitution of urea with fungi and nitrogen fixing bacteria for coir pith composting.

1.6.3. To study the efficacy of coir pith as growth medium for *Azotobacter vinelandii* and *Azospirillum brasilense*.

1.6.4. Isolation and characterization of nitrogen fixing bacteria from raw coir pith.

1.6.5. To study the efficacy of biodegraded coir pith for cultivation of medicinal plants.

# REVIEW OF LITERATURE

## 1.7. Coir Industry.

Coir is the only natural fibre that is not cultivated solely for coir where as jute and sisal are natural fibres cultivated only to produce the fibres and in turn, spun and woven into products.

The coir industry, which forms the main plank of the economy of the coastal region of Kerala, is one of the oldest and most traditional industries in the state. The geographical location of this area providing a salubrious climate for the large scale cultivation of coconut palms and the winding network of rivers, canals, lakes, lagoons and estuaries is an enormous inter connected, web of water ways. Virtual forests of coconut palm spread across these flat, green lands which is providing further a unique and distinct facility for the retting of coconut husk that constitute the basic raw material for the industry, have helped in concentrating this industry in and around coastal area. These natural facilities, which do not seem to exist as such anywhere in the other large coconut producing countries, have been fully made use of by the generations of men and women who inhabit this part of the country for exploiting the potential of the coconut husk for fibre extraction.

The golden textured Indian Coir Fibre, which earned it the unofficial brand name 'Golden fibre', captured European and world markets in no time. Since coir yarn and fibre could be most economically moved by well developed water transport to Alappuzha, a thinly populated, with communication facilities by road was sparse, but had facilities for shipping the product, with two canals connecting the port and backwaters. Coir industrial units came to be concentrated in and around Alappuzha due to the availability of cheap labour and the abundance of raw material.

Convinced by the potentiality of the fiber and yarn, Mr. Darragh, came to Alappuzha which was the chief port of the state and started a manufacturing unit with the establishment of the first coir factory in India at Alappuzha in 1859 by Mr. James

Darragh, an American of Irish origin. He enlisted the help of the foreign trader called Henry Smail and the factory known as 'Darragh Smail & Company'. From then on, there was no turning back. The big corporate of that era soon established coir factories in Alappuzha, Kollam, Kozhikode, Kochi and other parts of Kerala.

## 1.8. Coir Pith – A Natural Wealth.

Coconut is considered to play a vital role in the economy of many marginal farmers in India (Mathew. 2004).The coir pith accounts for 50-60 percent of the total weight of the husk (Jeyeseeli and Poul Raj, 2010) is the name given to dust left behind after the industrially valuable long fibre of coir has been extracted from the coconut husk. It is fluffy, spongy material with significant water holding capacity and is extremely compressible (Vijaya *et al*, 2008). Coconut husk is the basic raw material for the coir industry (Reghuvaran *et al*, 2009) and coir fibre is extracted from the husk and used for manufacture of mats, mattings, rubberized coir mattress and yarns in rope making. After extraction of coir fibre from husk, the remaining material (coir pith) is released into the sorroundings and total generation of coir pith in India is estimated to be around 0.5 million tons per year and the world production is around 3.6 million tons (Pillai *et al*., 1981). Therefore the coir industry face difficulty in the disposal of coir pith (Dan *et al*., 1993). Coir pith constitutes as much as 70% of the coconut husk and has a high water holding capacity of 8 times of its weight. It is estimated that 5.74 million metric tons of coconut are produced in the world (FAO 2001). India is reported to produce 0.77 million metric tons of coconut (*Cocos nucifera*) equivalent to the availability of 0.35 million metric tons of fibrous husk. Because of its high lignin content (48%) and amorphous, powdery nature, coir pith is one of the toughest biological materials, highly resistant to biological degradation. The barrier to the production and recovery of valuable materials from LCW (Lignocellulosic Wastes) is the structure of lignocelluloses which has evolved to resist degradation due to cross linking between the polysaccharides (cellulose and hemicelluloses) and the lignin

via ester and ether linkages (Yan and Shuya, 2006; Xiao *et al*., 2007). It is also stated that the lignin degradation not only helps the removal of environmental pollution, but also helps to obtain enriched protein biomass of fruiting bodies from white rot fungi (Rajarathnam. 1987)

Coir pith is easily blown by wind due to its light weight thereby creating air pollution. In comparison to other waste materials such as saw dust, rice husk and groundnut shell, coir pith is found to have a higher heat value (Krishnan, 1990; Sudhira and Jacob, 2000). Unfortunately, high levels of carbon dioxide and smoke are released from coir pith while burning due to its poor combustion properties. To overcome the problems associated with coir pith disposal, many innovative practices are being followed. In certain parts of India, coir pith finds its applications in making bricks (Dan, 1993) and particle boards (Viswanathan, 1998). A mixture of cow dung and coir pith at 4:1 ratio increases the biogas production (Pillai *et al*., 1981). Reports state that it is presently being successfully utilized as a soilless medium for vegetable crops such as bhendi (Abelmoschus esculentus) (Ross, 2002), Tomato (Lycopersicon lycopersicum)(Ramalakshmi, 2005) and an effective substrate for brinjal (Solanum melongena) (Ramaswamy *et al*., 1985)

The coir pith contains about 35% lignin 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, but mainly by beta-aryl ether bonds. It is also present in the fibers and is responsible for the stiffness of coir. It is thought that the lignin has its origin in carbohydrates. It is mainly a hard substance and is almost free from degradation in ordinary conditions. *Pleurotus sajor caju* belongs to a class of white rot fungi capable of preferentially degrading lignin when grown on lignocellulosics (Hatakka, 1994). A wide range of diverse cellulosic substrates are used for cultivation of *Pleurotus sajor caju.* Amongst various cereal straws, paddy straw was reported to be the best substrate for the cultivation of oyster mushrooms (Bano and Srivastava, 1962; Jandaik and

Kapoor, 1974; Khanna and Garcha, 1982). It has also reported that *Pleurotus spp*. are the third most commonly cultivated mushroom in the world and are known to have high nutritional values (Tshinyangu., 2006).

The study of coir pith degradation with bacteria is very limited due to its poor degrading ability to degrade the lignin present in it. Janshekar and Fiechter (1981) reported some bacterial cultures were tested for the degradation of lignin which has the ability to degrade numerous phenols with structural relationship to lignin. Several studies in this field state that; it takes decades for coir pith to decompose causing environmental pollution and disposal problems (Pazhanivel *et al*., 2011). The poor degradation of lignin was observed in all tested bacteria and it is also commented that the poor degradation does not seem to be influenced by medium composition and culture condition but is more probably due to the inability of the tested bacteria to degrade lignin to any considerable extent. For many years, the filamentous blue-green algae (Cyanobacteria) were believed to be primarily responsible for $N_2$ fixation in oceanic waters. There was a correlation of in situ nitrogen fixation with light intensity (Stewart, 1974), some Cyanobacteria were capable of degrading the coir pith. Some enhanced techniques show the use of coir pith based cyanobacterial biofertilizer in sustainable integrated agro ecosystems (Prabha *et al*, 2009). So, this method can be useful to promote the growth of plant and increase the quality and quantity of crop yield (Hume, 2007). Most recently, cellulolytic nitrogen fixing bacteria have been isolated in large numbers, apparently as a pure culture, from a specialized gland found in ship worms (Waterbury *et al*, 1983). The work conducted by Anandharaj (2007) also shows the coir pith can be partially decomposed through the action of cyanobacteria and can be used as biofertilizer for all varieties of food crops.

## 1.9. Composting.

Composting is the natural process of decomposition of organic waste that yields manure or compost, which is very rich in nutrients. Composting is a

biological process in which micro-organisms, mainly fungi and bacteria, convert degradable organic waste into humus like substance. This finished product, which looks like soil, is high in carbon and nitrogen and is an excellent medium for growing plants. The process of composting ensures the waste that is produced in the kitchens is not carelessly thrown and left to rot. It recycles the nutrients and returns them to the soil as nutrients. Apart from being clean, cheap and safe, composting can significantly reduce the amount of disposable garbage. The organic fertilizer can be used instead of chemical fertilizers and is better specially when used for vegetables. It increases the soil's ability to hold water and makes the soil easier to cultivate. It helped the soil retain more of the plant nutrients.

Composting is an aerobic exothermic waste treatment method where a mixed microbial population degrades heterogeneous organic matter. The main end products of composting are $CO_2$, biomass and humic substances (Crawford, 1983; Haug, 1993). The humified and stabilized end product can be used as a fertilizer (Crawford, 1983). During the composting process pH, temperature and structure of the organic matter alter along with the ongoing succession in the microbial population (Paatero *et al*., 1984). Addition of a bulking agent is usually needed, and water addition occasionally (Paatero *et al*., 1984; Haug, 1993). Either forced aeration or frequent turning ensures efficient oxygen transfer to the compost pile. Agitation is always needed to ascertain uniform degradation (Biddlestone and Gray, 1985; Haug, 1993).

### 1.9.1. Significance of Composting.

Composting microorganisms produce enzymes that are extracellular like a chemical aura outside the organism's body. They transform molecules of organic matter into less complex chemicals and energy. All organisms have intracellular enzymes to manage the diverse complexity of the life processes. Enzymes are unstable proteins or protein-containing compounds that, when present in small

amounts, promote a chemical reaction. The enzymes such as amylase, cellulase, lipase and protease are a few of the catalysts responsible for decomposition.

The solid, liquid and gaseous environment of the bioplex that forms a composting system provides a continuum of micro niches for the organisms and that affects the rate of biological transformation of organic matter. The speed of the composting process is influenced by several factors such as environmental factors, the composition and constituents of materials being composted, the health and number of the organisms which are using the materials as a food source and the management of the process by the operator.

The important parameters of composting are temperature, pH, moisture content and salinity, which are regulated by aeration, free air space and agitation. The main properties of feed materials include the C:N ratio, particle size, rigidity, nutrient and lignin content (Crawford, 1983; Paatero *et al.*, 1984; Biddlestone and Gray, 1985; Haug, 1993). Under optimal conditions, residence time is minimized and a good end product is achieved (Golueke, 1992).

Self heating commences and a sufficiently high temperature is reached when moisture content, nutrient level and particle size of the waste are adequate, but temperature can also be artificially kept at a high level for a longer time to intensify the degradation (Crawford, 1983, Horwath and Elliot. 1996a). A maximal degradation rate is achieved at temperatures from 45˚C to 60˚C, but excessive temperature suppresses decomposition (Waksman *et al*., 1939b; MacGregor *et al*., 1981; Strom 1985a). Control of pH is not normally needed. However, if the level of nitrogen in waste material is high, there may be excessive ammonia production, resulting in an odour problem (Eklind and Kirchmann, 2000).

Microorganisms need water to maintain their activity, but if moisture content is too high, the free air space is easily filled with water, and anaerobic conditions and a decreased degradation rate follow (Haug, 1993). The optimum moisture range in compost is usually 50-70%, but it is highly dependent on the feed

material (Haug, 1993). During composting the organic material dehydrates as heat together with vapour escapes the compost (MacGregor *et al.*, 1981). Dry unstable compost may behave like cooled, stabilized compost, but by re-wetting this "pseudostable" compost, reheating can be induced (MacGregor *et al.*, 1981; Haug, 1993; Itavaara *et al.*, 2002).

Aerobic conditions are essential for efficient degradation. One option is forced aeration, but turning or intermittent agitation is adequate for aeration in many cases. Air can reach the inner parts of the heap because the gaps between the particles constitute air spaces. Sufficient porosity is achieved by using a rigid, slowly degradable material, such as wood chips, bark or some other lignocellulosic substance as a bulking agent (Crawford, 1983; Paatero *et al.*, 1984; Haug, 1993). Microorganisms require a carbon source as a major energy source, macronutrients such as nitrogen, phosphorous, potassium and certain trace elements for their growth and lack of nitrogen could retard the composting process. The optimal C/N ratio has been reported to be 25-50, but this seems to have marked variation depending on the substrate, because nitrogen addition can increase (Inbar *et al.*, 1988), decrease (Horwath and Elliott, 1996b) or have no effect at all (Inbar *et al.*, 1988) on the degradation rate in compost. Thus the chemical composition of nitrogen in feed material and the structure of the waste have been suggested to be more important for composting performance than the C:N ratio (Crawford, 1983; Golueke, 1992; Horwath and Elliott, 1996b). Excess nitrogen is lost from compost as ammonia or other gaseous nitrogen compounds (Eklind and Kirchmann, 2000). During the decomposition of waste two thirds of carbon is released as carbon dioxide and the rest is accumulated as compost organic matter (Horwath and Elliott, 1996b; Eklind and Kirchmann, 2000).

Fungi, actinomycetes and unicellular bacteria from the majority of compost microorganisms and algae, viruses, protozoa and macroorganisms make up the minority (Finstein and Morris, 1975; Biddlestone and Gray, 1985). Bacteria are

mostly heterotrophic, but denitrifying and nitrogen-fixing bacteria, hydrogen oxidizing bacteria and sulphur oxidizing bacteria are also present (Diaz- Ravina *et al*., 1989; Beffa *et al*., 1996; Bess, 1999). Actinomycetes often grow extensively during the cooling and maturation phase (Biddlestone and Gray, 1985). Fungi grow in compost in all phases but may disappear temporarily during peak heating (Thambirajah *et al*., 1995). The percentage of fungi and actinomycetes relative to the whole population is lower in compost than in soil, while the actual density of these organisms is much higher (Diaz- Ravina *et al*., 1989). A small but significant number of anaerobic bacteria have also been found in a compost environment (Diaz- Ravina *et al*., 1989, Atkinson *et al*., 1996b., Ueno *et al*., 2001). Anaerobic microenvironments may develop, especially during the thermophilic phase, when oxygen is rapidly consumed (Haug, 1993). The isolated anaerobic bacteria have either been cellulolytic or saccharolytic (Akinson *et al*., 1996b; Ueno *et al*., 2001).

### 1.9.2. Chemistry of Composting.

Organic material where the decomposition is in presence oxygen is an "aerobic" process. When living organisms that use oxygen feed upon organic matter, they develop cell protoplasm from the nitrogen, phosphorus, some of the carbon, and other required nutrients. Carbon serves as a source of energy for organisms and is burned up and respired as carbon dioxide (CO2). Since carbon serves both as a source of energy and as an element in the cell protoplasm, much more carbon than nitrogen is needed. Generally, organisms respire about two-thirds of the carbon they consume as CO2, while the other third combines with nitrogen in the living cells.

Biological activity diminishes if the compost mix contains too much carbon in relation to nitrogen. Several cycles of microorganisms are required to burn the excess carbon, which is a complex chemical process. When organisms die, the stored nitrogen and carbon becomes available to other organisms. These new organisms form new cells which again need nitrogen to burn excess the carbon and

produce $CO_2$. Thus, the amount of carbon is reduced and the limited amount of nitrogen is recycled. Finally, when the ratio of available carbon to available nitrogen is low enough, nitrogen is released as ammonia. Under favorable conditions, some ammonia may oxidize to nitrates. Phosphorus, potash and various micronutrients are also essential for biological growth. These are normally present in more than adequate amounts in compostable materials. In nature, the aerobic process is most common in areas such as the forest floor, where droppings from trees and animals are converted into relatively stable organic matter.

### 1.9.3. Environmental Impacts of Composting.

Use of compost as a soil conditioner, a fertilizer, or a growth medium has, of course, significant environmental benefits. In addition to returning nutrients to the soil and thus permitting the reduction of artificial fertilizers, compost is waste that does not have to be land filled. When it is used as daily cover at landfills, it replaces other materials that would otherwise be used for that purpose.

However, there are also negative impacts on the environment associated with making and using compost. These impacts depend both on the technical approach used and the waste composition of the input streams. Gases released from improperly maintained compost piles are a negative effect associated with the composting process. When piles are not properly aerated, colonies of anaerobic bacteria flourish and produce methane gas. The decomposition process also releases carbon dioxide, volatile organic compounds, bacteria and fungi. The release of methane and carbon dioxide contributes to the problem of greenhouse gases in the atmosphere. Poorly operated composting facilities also cause unpleasant odours. Other air emissions are generated by the combustion engines used to power windrow turning machines and grinders.

Leachate production is also common. Leachate from water runoff and condensation at compost facilities occasionally contains levels of biological oxygen demand (BOD) and phenols (a byproduct of the decomposition of the

lignin in leaves) that may exceed acceptable discharge limits, but pose few problems if absorbed into the ground or passed through a sand filter. High concentrations of BOD in runoff to surface water is a bigger problem, as this can reduce the amount of dissolved oxygen in lakes and streams that is available for aquatic life. Sound practice here is to avoid discharge to water and to capture or direct all leachate to absorption in sand or soil.

## 1.10. Lignocellulose Degradation.

Grasses, wood and most of the plant litter represent the major part of the biomass in the nature which is collectively called Lignocellulose (Kuhad *et al*., 1997). It mainly composed of cellulose, hemicelluloses and lignin (Sjostrom, 1993). It is estimated that there is 2.5-4×$10^{11}$ tons of cellulose and 2-3×$10^{11}$ tons of lignin in the earth, representing 40% and 30% of organic matter carbon, respectively, with other polysaccharide comprising 26% (Fengel and Wegener, 1989; Argyropoulose and Menachem, 1997) as cellulose, hemicelluloses and lignin are closely associated in plants, isolating these compounds to a pure state is virtually impossible. They are not uniformly distributed in the plant cell wall; the S2- layer of the secondary wall has the highest percentage of cellulose, and the middle lamella has the highest percentage of lignin, but all three compounds can be found in every layer of the cell wall. Distribution in the different parts of the plant is not uniform either (Kuhad *et al.,* 1997). Some grasses contain considerable amount of pectin in the middle lamella, where as wood contains only small quantities of extractives, inorganic compounds and pectin compounds (Fengel and Wegener, 1989; Hatfield, 1989; McDougall *et al*., 1993; Kuhad *et al*., 1997).

Photosynthesis and degradation of Lignocellulose are essential for the global carbon cycle (Brown, 1985; Colberg, 1988). The degradation rate of the lignocellulose is governed by temperature, moisture content and the type of lignocellulose (Rayner and Boddy, 1988; Kuhad *et al*., 1997). Rayner and Boddy (1988) observed that a warm and wet other than a cold and dry environment which

enhances degradation. Wood and various grass lignocelluloses are the main raw materials of pulp. The proportional amount of carbohydrates and lignin in the end product vary due to different pulping and bleaching processes. Chemical pulping with bleaching removes lignin almost completely, while mechanical pulping leaves the composition of the pulp close to that of wood (Gullichson, 2000).

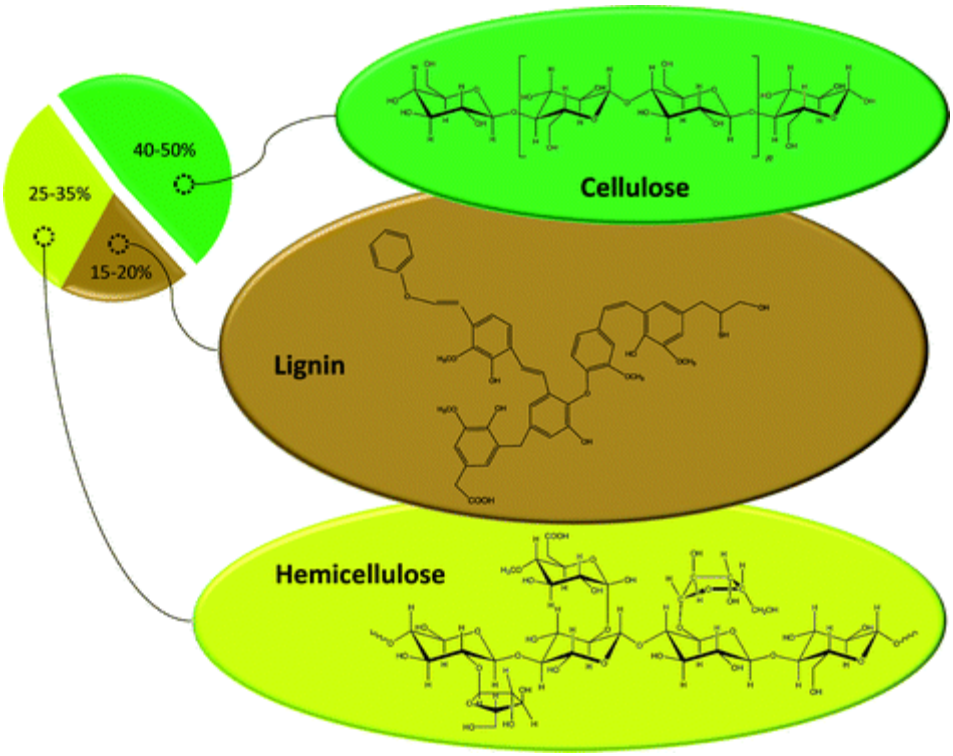



**Fig. I.** Lignocellulose

Lignin is considered as a composite material in all vascular plants, providing the plant with strength and rigidity (Brown, 1985; Argyropoulose and Menachem, 1997). By decreasing water permeation across the cell wall, lignin renders the plant resistant to biodegradation as well as to environmental stresses (Eriksson *et al.*, 1990; Argyropoulose and Menachem, 1997). Lignin is an amorphous, aromatic, water insoluble, heterogenous, three dimensional and cross-linked polymer with low viscosity (Fengel and Wegener, 1989; Sjostrom, 1993;

Brunow, 2001). The molecular mass of lignin is high (600-1000 KDa), although not uniform, varying greatly within isolated samples (Kirk and Farrell, 1987; Fengel and Wegener, 1989; Brunow, 2001). The molecular mass of lignin is thus difficult to determine, and use of a conventional formula is not possible (Brunow, 2001). Lignin is highly reduced and its carbon content is 50% higher than that of polysaccharides, which makes lignin energy rich (Brown, 1985). Lignin is distributed throughout the cell wall, although, again, not uniformly the highest lignin content is in middle lamella, however the greatest amount of lignin is in the secondary wall (Fengel and Wegener, 1989; Eriksson *et al*., 1990).

The primary precursors for p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units of lignin are p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol respectively (Fengel and Wegener, 1989; Brunow, 2001). The monomers couple with the phenolic end groups of the growing lignin polymer, not with each other (Sjostrom, 1993). Peroxidase and laccase catalyse the random polymerization (Brown, 1985; Eriksson *et al*., 1990; Argyropoulose and Menachem, 1997). Lignin has no single repeating bond; phenylpropanoid units are linked together by more than ten different types of aryl ether and carbon-carbon linkages (Brown, 1985; Sjostrom, 1993; Brunow, 2001). The predominant type of linkage in wood lignin is the arylglycerol-β-aryl ether (β-O-4) bond (45-60% of all bonds), and the next most common bonds are β-5 (6-12%), and 5-5 (4.5-17%). Recently a new type of linkage in softwoods forming dibenzodioxocin moity was discovered by G. Brunow's group (Karhunen *et al*., 1995a, 1995b, Brunow, 2001). The lignin content of wooden plants, especially softwoods is higher than that of gramineous plants (Fengel and Wegener, 1989; McDougall *et al*., 1993). The G-type of lignin dominates in softwoods and the middle lamella of hardwoods, the G-S type in hardwoods, and the G-S-H type in gramineous plants (Brown 1985; Besle *et al*., 1989, Sjostrom, 1993; Brunow, 2001) for precursors. In addition to methoxyl groups that distinguish various lignin types, lignin has other functional groups, including phenyl hydroxyl, benzyl alcohol and carbonyl groups (Sjostrom, 1993, Brunow, 2001).

## 1.11. Lignin Degrading Microorganisms.

The most efficient lignin degraders are basidiomycetous white and brown rot fungi which are taxonomically so close to each other that both types may sometimes appear in the same genus (Hatakka, 2001). However, less than 10% of wood degrading basidiomycetous fungal species are brown rot fungi (Eaton and Hale, 1993; Dix and Webster, 1995). Wood rotting Ascomycotena and Deuteromycotena, i.e. microfungi are considered to be soft rot fungi, while Basidiomycotena are either white rot or brown rot fungi (Daniel and Nilsson, 1998; Hatakka, 2001).

Lignin degradation by white rot fungi has been studied intensively to safeguard the commercial interests of the pulp and paper industry (Akhtar *et al*., 1997; Scott and Akhtar, 2001). Degradation of lignin requires unspecific and extracellular enzymes because of the random structure and high molecular mass of the lignin molecule (Kirk and Farrell, 1987). Lignin usually cumulates during the degradation of lignocellulose, and white rot fungi are the only organisms that are able to degrade lignin selectively (Blanchette, 1995). Lignin is probably degraded by an array of microorganisms in nature, although abiotic degradation may also occure in special environments, such as those due to alkaline chemical spills (Blanchette *et al*., 1991) or UV radiation (Vahatalo *et al*., 1999). The environment governs which lignocellulose utilizing organisms dominate, and thus, the speed of the degradation (Blanchette, 1995). In aqueous or other anaerobic environments, polymeric lignin is not degraded, and wood may persist in non degraded form for several hundred or thousand years (Blanchette, 1995).

## 1.12. Nitrogen Fixing bacteria.

Nitrogen-fixing bacteria are microorganisms capable of transforming atmospheric nitrogen into fixed nitrogen, inorganic compounds usable by plants. More than 90 percent of all nitrogen fixation is effected by them. Two kinds of nitrogen fixers are recognized: free-living (non-symbiotic) bacteria, including the

Cyanobacteria (or blue-green algae) like *Anabaena*, *Nostoc* and such genera *viz*. *Azotobacter, Beijerinckia,* and *Clostridium*; and mutualistic (symbiotic) bacteria such as *Rhizobium,* associated with leguminous plants, and *Spirillum lipoferum,* associated with cereal grasses.

The symbiotic nitrogen-fixing bacteria invade the root hairs of host plants, where they multiply and stimulate formation of root nodules, enlargements of plant cells and bacteria in intimate association. Within the nodules the bacteria convert free nitrogen to nitrates, which the host plant utilizes for its development. To ensure sufficient nodule formation and optimum growth of legumes (*e.g.,* alfalfa, beans, clovers, peas, soybeans), seeds are usually inoculated with commercial cultures of appropriate *Rhizobium* species, especially in soils poor or lacking in the required bacterium.

Microorganisms which pass independent life and fix atmospheric nitrogen are known as free living diazotrophs. There are two groups of such micro-organisms: bacteria and Cyanobacteria (blue-green algae). Based on the mode of nutrition (carbon, nitrogen and oxygen and requirement of reducing groups) bacteria are divided into *(i)* aerobic bacteria *(Azonomas, Azotobacter, Beijerinckia, Mycobacterium, Methylomonas), (ii)* facultative anaerobic bacteria (*Bacillus, Enterobacter, Klebsiella,* etc.), *(iii)* anaerobic bacteria (*Clostridium, Desulfovibrio,* etc.) and *(iv)* photosynthetic bacteria *(Rhodomicrobium, Rhodopseudomonas, Rhodospirillum, Chromatium, Chlorobium,* etc.).

### 1.12.1. *Azotobacter vinelandii.*

*Azotobacter vinelandii* is an aerobic soil-dwelling organism with a wide variety of metabolic capabilities which include the ability to fix atmospheric nitrogen by converting it to ammonia. Like *Klebsiella pneumoniae* it fixes nitrogen in the free-living state and does not enter into symbioses with plants; a process typified by the symbiosis between members of the genus *Rhizobium* and a variety of leguminous plants. Two features of the biology of *Azotobacter* make it

of particular interest to scientists studying the nitrogen fixation process. *Azotobacter vinelandii* is capable of synthesising not only the molybdenum-containing nitrogenase enzyme that typifies most diazotrophs including *Klebsiella pneumoniae* and *Rhizobium leguminosarum*, but also two alternative nitrogenases; one in which vanadium replaces molybdenum and a second which contains neither transition metal but only iron (*http://www.jic.ac.uk/SCIENCE/molmicro/Azot.html*). This ability to carry out the chemistry of nitrogen reduction at sites that do not contain molybdenum is of particular importance to chemists and biochemists investigating the mechanism of biological nitrogen fixation. The alternative nitrogenases are encoded by distinct structural genes, *vnfHDGK* and *anfHDGK*: the *vnfG* and *anfG* genes encoding an extra small subunit not found in molybdenum nitrogenase. However many of the same ancillary genes e.g. *nifUSVWZ* and *nifM* are used in biosynthesis of all three enzymes.

Synthesis of the alternative nitrogenases is regulated by availability of the appropriate metals i.e. molybdenum or vanadium, and expression of each set of genes is controlled by a specific regulatory protein, the products of the *nifA*, *vnfA* and *anfA* genes. Interest in this regulation has focussed research on the mechanisms whereby *Azotobacter* transports molybdate into the cell and distinguishes it from similar molecules such as sulphate. This has led to the dissection of the molybdate transport genes, *modEABC* and *modG* of *Azotobacter* that have homologues in many other bacteria (*http://www.jic.ac.uk/SCIENCE/molmicro/Azot.html*).

*Azotobacter* has evolved a number of physiological mechanisms to allow it to fix nitrogen aerobically despite the inherent oxygen-sensitivity of nitrogenase (Joao, C., 2009). It has uniquely high rates of respiration coupled with specific cytochromes to ensure that nitrogenase experiences an essentially anoxic environment despite the fact that energy is being derived from aerobic metabolism. It can also synthesize a protective 2Fe-2S protein which can bind to

nitrogenase in conditions of oxygen stress to form an oxygen-stable complex that is inactive but protected from damage.

### 1.12.2. *Azospirillum brasilense.*

*Azospirillum brasilense,* a nitrogen-fixing bacterium found in the rhizosphere of various grass species, was investigated to establish the effect on plant growth of growth substances produced by the bacteria (Tien *et al*., 1979). *Azospirillum brasilense* and *Azospirillum lipoferum* are the most commonly studied organisms among the Azospirilla. They are potential nitrogen fixers and nitrogen fixing is associated closely with roots and rhizospheres of many economically important plants and grasses (Baldani, V.L.D and Dobereiner, J. 1980; Baldani *et al*., 1997; Baldani *et al*., 1996; Baldani, 1996; Hartman *et al*., 1995; Olivares *et al*., 1996; Weber *et al*., 1995).

Chapter 2

# EFFICACY OF DIFFERENT MUSHROOM SPECIES FOR THE DEGRADATION OF COIR PITH

Contents



## 1. Introduction.

Essentially coir pith is a Lignocellulosic substrate that decomposes very slowly in soil as it contains 30%-35% of Lignin. The 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. It degrades the phenolic group, as observed by the decrease in methoxy content and also causes an oxidative shortening of the 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 therefore essential to study the degradation of lignin in the biodegradation process with effective mushroom species.

## 2. Methodology.

### 2.1. Sample Collection.

Coir pith was collected from the coir industries located in Kattukada, Alappuzha district. There are a number of coir based industries working in this place. These coir fiber extraction units have large areas which are accumulated

with coir pith lying unutilized for past several years. Samples from these natural dumps of coir pith have been taken for investigation.

## 2.2. Test Organism and their Maintenance.

Four fungal cultures *viz*. *Pleurotus sajor caju, Pleurotus florida, Pleurotus eous* and *Calocybe indica* are the test cultures used. All the cultures were procured from different research institutions. *Pleurotus sajor caju* was obtained from Central Coir Research Institute (Coir Board), Kalavoor, Alappuzha and the other three organisms obtained from the Regional Agricultural Research Station, Kerala Agricultural University, Kumarakom. All the cultures were maintained on PDA slants and stored under refrigeration at 15˚C. The fungi were mass cultured on sterilized media consisting of sorghum mixed with 0.2% calcium carbonate as carbon source in polythene bags to adjust pH. Fully grown packets of mushrooms after 15 days incubation were used for carrying out experimental studies on composting of coir pith. All experiments and biochemical analysis were carried out at the Rajiv Gandhi Chair in Contemporary Studies, CUSAT and Central Coir Research Institute, Alappuzha. Experimental studies on composting of coir pith were carried out in open areas as multilayered heaps with incorporation of different mushroom species *viz., Pleurotus sajor caju, Pleurotus florida, Pleurotus eous* and *Calocybe indica*, which is the standard procedure for composting and bioconversion of coir pith into compost.

## 2.3. Experimental Set Up

The efficacy of different mushroom species for the biodegradation of coir pith were studied by laying different sets of coir pith heaps in a shady place. Care was taken to prevent the heaps from any disturbances. Each of the experiment was conducted by mounting one kg heap of coir pith supplemented with 4 gms of four different mushroom species and 10 g of urea as nitrogen supplement. Five sets were designed for four different mushroom species and an uninoculated control

(without the organism) was maintained simultaneously. All the heaps were moistened regularly to retain 200% moisture.

## 2.4. Physical Properties of Raw and Biodegraded Coir Pith.

### 2.4.1. Moisture.

Moisture of the coir pith samples were determined by oven dry method using A7D – MS 70 analyzer (Gupta 2001).

### 2.4.2. Temperature.

Temperature was recorded using a laboratory thermometer at five points on the composting heap and the average recorded.

### 2.4.3. pH.

Calibrated METLER TOLEDO pH meter was used for the measurement of pH (Muthuvel and Udayasoorian, 1992).

### 2.4.4. Electrical Conductivity.

Electrical conductivity was measured using deluxe conductivity meter – Model 601 (Muthuvel and Udayasooriyan, 1992).

### 2.4.5. Salinity.

A calibrated laboratory salinometer was used for the measurement of salinity as per the method described by Lenore *et al*., 1989.

## 2.5. Estimation of Chemical Properties.

### 2.5.1. Lignin.

The Klason lignin in coir pith was determined by the following method (Stephen and Carlton, 1992). Approximately one gram of coir pith was weighed out in triplicates in tarred weighing bottles and dried at 105˚C for one hour. The material was weighed thrice till a constant weight (A) was obtained. The material was then carefully transferred to a clean filter paper and rolled to pack the contents.

The three packed samples were transferred into the reflux unit of the soxhlet extraction apparatus for the first extraction with ethanol: benzene (2:1 v/v) for 4 hour. The samples were then allowed to dry and the contents transferred to a 400 ml beaker and refluxed in 200 ml distilled water for 4 hours.

The cooled samples were then transferred into a 100 ml beaker to which 3 ml of 72% Sulphuric acid was added carefully with the help of a glass rod. Another 22 ml of 72% $H_2SO_4$ was added to make the total volume to 25 ml. The sample was then carefully macerated to form a fine paste and kept covered at room temperature for 2 hours. The sample was then diluted with distilled water (575 ml) in a one litre beaker and heated on a water bath for 4 hours. After cooling the sample was filtered carefully through a clean tarred G-4 sintered Gooch crucible. The residue was washed till free of acid, dried at 105˚C and weighed till constant reading obtained (B). The net weight recorded and the percentage of lignin calculated as per the formula

% of lignin = B/A × 100.

**2.5.2. Nitrogen**

Nitrogen in the coir pith samples were estimate by Alkalline permanganate method using Kjeldahl distillation Unit (Vogel, 1961). The method includes digestion, distillation and titration. The digestion process required following chemicals for every 0.2 gm coir pith samples; Conc. $H_2SO_4$ (10 ml), 3 gms of catalyst mixture ($K_2SO_4$ & $CuSO_4$ mixture in 50 and 10 gm proportions). Adjusted the temperature to 420˚C and digestion time set to 1.5 hours. Distillation process was done with 40% NaOH and 4% Boric acid. After 9 minutes, the colour in the conical flask changes to pink colour to green. This is the end point of distillation. Then take conical flask for titration, with 1 N $H_2SO_4$, till the colour changes from green to permanent pale pink colour. The nitrogen content is calculated as follows.

$$\% \text{ of Nitrogen} = \frac{14 \times \text{titrant value} \times \text{Normality of Acid} \times 100}{1000 \times \text{sample weight.}}$$

**2.5.3. Phosphorus.**

Phosphorous was estimated by Vanado Molybdo phosphoric yellow colour method. Dried and powdered coir pith sample (0.5 g) is taken in boiling tube. 15 ml tri acid is added (mixture of Conc. $HNO_3$ and Perchloric acid in the ratio 10:1:4) and digested overnight. The digested sample is quantitatively transferred to a 100 ml volumetric flask and makeup to 100 ml with distilled water. 5 ml of the diluted solution is taken in a 25 ml volumetric flask and 5 ml of Vanado molybdate reagent is added and made up to 25 ml with distilled water. Shake thoroughly and the absorbance read in spectrophotometer at a wavelength of 430 nm after 10 min. A standard curve is prepared and the reading obtained for the sample extrapolated to evaluate the phosphorous content.

$$\text{Percentage of Phosphorous} = \frac{100 \times 25 \times \text{ppm (dilution)}}{0.5 \times 5 \times 10{,}000}$$

**2.5.4. Potassium.**

Potassium was estimated by Flame photometry. The estimation done by triple acid extract is diluted suitably to contain approximately 4-7 ppm of K and read in the flame photometer after calibrating with standard (100 ppm, 50 ppm and 20 ppm) solution.

$$\text{Percentage of Potassium} = \frac{\text{Reading} \times 100}{0.5 \times 10{,}000}$$

**2.5.5. Organic Carbon.**

Weigh exactly 0.5 g of compost (passed through 0.2 mm sieve) and transfer it to a 500 ml conical flask. Add 100 ml of 1N $K_2Cr_2O_7$ and mix well by swirling the flask and then add 20 ml of conc. $H_2SO_4$ and mix by gentle rotation for one minute to ensure complete contact of the reagent with the compost. Allow the contents to stand for 20-30 minutes. After 30 minutes, add 200 ml of water. Then add 10 ml of phosphoric acid and 1 ml of diphenylamine indicator. Titrate the solution with 0.5N ferrous ammonium sulphate. The colour is dull green at the

beginning and then shifts to a turbid blue as the titration proceeds. The end point is very sharp. At the end point the colour sharply shifts to a bright green colour. Simultaneously conduct a blank titration (without compost) and note down the volume of 0.5N ferrous ammonium sulphate consumed.

$$\text{Percentage of Organic Carbon} = \frac{(x-y)\times 0.003\times 100}{\text{Weight of the sample}}$$

The results of biodegradation revealed that, all the mushroom species were potent in biodegradation of coir pith and definite reductions in lignin content were observed. With a view to develop an efficient method for the biodegradation of coir pith with four species of white rot fungi *viz*., *Pleurotus sajor caju, Pleurotus florida, Pleurotus eous* and *Calocybe indica* were used. The Urea was also used as a nitrogen supplement and was added to the coir pith. The study was completed in 30 days. The composted coir pith was observed to be black in colour and its volume reduced by about 40%. The composted mass of coir pith also shows increased NPK (Nitrogen, Phosphorous and Potassium) and variable loss in carbon content. The Temperature of the pith heap shows variations, as the degradation causes increases in the temperature of the system due to the activity of microorganisms. The pH shows slight variation and the electrical conductivity decreases considerably with different fungal biodegradation. The details of the results are as follows.

# Result

## 3.1. Physical Properties of Coir Pith.

### 3.1.1. Moisture.

From the experiments, it is clear that there was variation in moisture content in coir pith during the course of microbial biodegradation. The moisture content during composting is kept high and constant for facilitating decomposition. The moisture content in raw coir pith was found to be around 20% and during the composting process it increased to higher levels. The higher moisture content shows increased levels of biodegradation (Table II b).

### 3.1.2. Temperature.

Temperature of the compost heaps is dynamic. It was periodically measured and was observed to be 25˚C initially and which was increased to 35˚C on biodegradation proceeds.

### 3.1.3. pH.

The biodegradation with different species of mushrooms on coir pith showed variations in pH. Initially the pH was just below the neutral values but as the composting proceeds, the pH fluctuated to slightly acidic level due to the formation of phenolic compounds (Table II b).

### 3.1.4. Electrical Conductivity (EC).

Electrical conductivity was observed to decrease during biodegradation. Raw coir pith, which was maintained as control indicated a value of 0.095 millimhos/cm, which tends to decrease during composting. All the four organisms causes the reduction on electrical conductivity and among the four organisms tested, biodegradation with *Pleurotus sajor caju* was observed to be more effectively reducing the Electrical Conductivity. The details are given in Table II b.

### 3.1.5. Salinity.

Salinity of coir pith also decreased during composting and ranged from 6 ppt to 2 ppt.

## 3.2. Chemical Properties.

The biodegradation of coir pith with different mushroom species causes reduction in Lignin, Organic carbon and enhancement in Nitrogen, Phosphorous and Potassium (NPK) content. The details of the chemical parameters are given below.

### 3.2.1. Lignin.

The investigations reveal that definite variations were observed in the lignin content of coir pith under various treatments using ligninolytic mushrooms viz., *Pleurotus sajor caju, Pleurotus florida, Pleurotus eous* and *Calocybe indica*. The periodical analysis of samples of coir pith drawn at regular intervals from the experimental heaps show the rate of decomposition of lignin in coir pith by reduction from 32% in the control to 20% & 18%. Biodegradation of coir pith with *Calocybe indica* exhibited the maximum value for lignin reduction (18%) and the least was observed by *Pleurotus florida*. The details are given in Table II a.

### 3.2.2. Nitrogen.

The investigations reveal that the treatment of coir pith with different mushroom species lead to changes in the Nitrogen content. The percentage of nitrogen in the raw coir pith kept as control displays no change and is observed to be 0.73%. Treatments with different mushroom species show enhancement in nitrogen content to 0.86%, 0.89%, 0.84% and 0.96% for *Pleurotus sajor caju, Pleurotus florida, Pleurotus eous* and *Calocybe indica* respectively. From the results, it is clear that addition of the mushroom species are lead to enhancement in the Nitrogen content in coir pith.

### 3.2.3. Phosphorous.

The Phosphorous content in coir pith also show enhancement with the treatment of all the four mushroom species. The periodical analysis of coir pith samples under the treatment with various mushroom species indicated an increasing trend of phosphorous content during composting. The mushroom

species used for composting of coir pith showed variation in phosphorous content ranging from 0.24% to 0.41%. The details are furnished in table II a.

### 3.2.4. Potassium.

The values of Potassium in the samples of biodegraded coir pith with different mushroom species have been furnished in table II a. 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 all the mushrooms displayed the values of potassium in an increasing trend. The phosphorous content of the samples varied from 0.28% for raw coir pith to the maximum of 0.41% for Biodegraded Coir pith Compost (BCC) with *Pleurotus sajor caju* and minimum in *Pleurotus eous*. Coir pith samples drawn out from the experimental heaps at regular intervals indicated increase of potassium content in coir pith.

### 3.2.5. Organic Carbon.

The Organic Content (OC) value obtained in raw and Biodegraded coir pith Compost (BCC) is given in Table II a. The use of mushroom species for the decomposition was found to reduce the OC content from 6.28% (raw coir pith) to 6.06%. The results indicating a decreasing trend in the carbon content of coir pith treated with different mushroom species. The carbon content of the raw coir pith (untreated) kept as control did not show any variation.

**Table II a.** Chemical properties of biodegraded coir pith with different fungal species

| Sample | Lignin (%) | | | Organic Carbon (%) | | | Nitrogen (%) | | | Phosphorous (%) | | | Potassium (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Raw Coir pith | 32 | 32 | 32 | 6.28 | 6.28 | 6.28 | 0.73 | 0.73 | 0.73 | 0.24 | 0.24 | 0.24 | 0.28 | 0.28 | 0.28 |
| Coir pith treated with *Pleurotus sajor* | 32 | 28 | 20 | 6.28 | 6.17 | 6.06 | 0.73 | 0.84 | 0.86 | 0.24 | 0.29 | 0.48 | 0.28 | 0.42 | 0.41 |
| Coir pith treated with *Pleurotus florida* | 32 | 31 | 30 | 6.28 | 6.11 | 6.10 | 0.73 | 0.86 | 0.89 | 0.24 | 0.28 | 0.36 | 0.28 | 0.39 | 0.38 |
| Coir pith treated with *Pleurotus eous* | 32 | 30 | 26 | 6.28 | 6.09 | 6.09 | 0.73 | 0.81 | 0.84 | 0.24 | 0.25 | 0.29 | 0.28 | 0.32 | 0.48 |
| Coir pith treated with *Calocybe indica* | 32 | 26 | 18 | 6.28 | 6.12 | 6.11 | 0.73 | 0.93 | 0.96 | 0.24 | 0.28 | 0.41 | 0.28 | 0.37 | 0.38 |

**Table II b.** Physical properties of biodegraded coir pith with different fungal species

| Sample | Moisture (%) | | | pH | | | Temperature (°C) | | | Salinity (ppt) | | | Electrical Conductivity (millimhos/cm²) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Raw Coir pith | 73 | 74 | 77 | 6.5 | 6.5 | 6.3 | 27 | 25 | 28 | 6 | 6 | 5 | 0.91 | 0.83 | 0.76 |
| Coir pith treated with *Pleurotus sajor caju* | 74 | 75 | 79 | 6.4 | 6.3 | 6.0 | 24 | 27 | 29 | 6 | 5 | 2 | 0.91 | 0.84 | 0.80 |
| Coir pith treated with *Pleurotus florida* | 84 | 83 | 88 | 6.6 | 6.5 | 6.1 | 23 | 28 | 30 | 5 | 5 | 3 | 0.89 | 0.85 | 0.84 |
| Coir pith treated with *Pleurotus eous* | 74 | 75 | 77 | 6.7 | 6.5 | 6.2 | 22 | 25 | 30 | 5 | 5 | 2 | 0.90 | 0.87 | 0.83 |
| Coir pith treated with *Calocybe indica* | 74 | 78 | 79 | 6.4 | 6.5 | 6.2 | 26 | 26 | 28 | 6 | 4 | 3 | 0.89 | 0.87 | 0.85 |

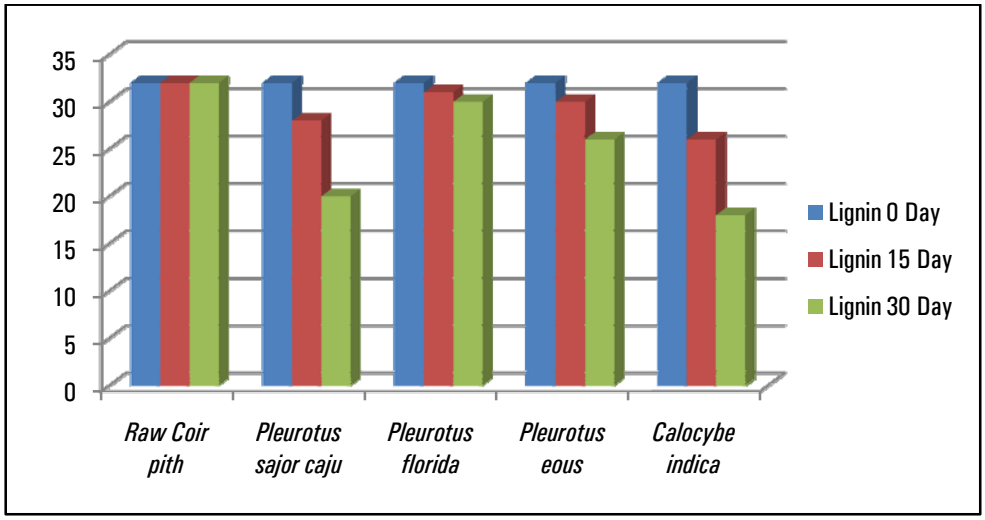



**Fig. II A.** Variation in Lignin content during composting of coir pith using different fungal species.

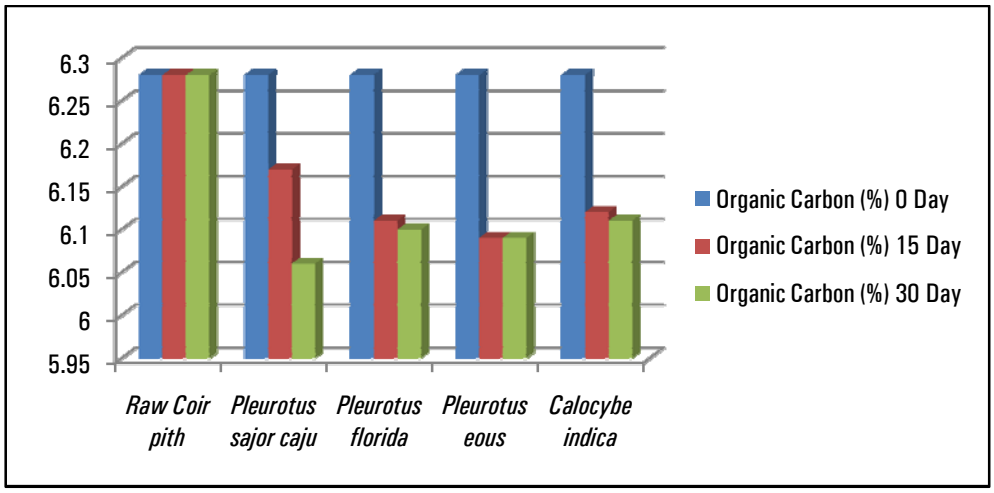



**Fig. II B.** Variation in Organic Carbon (OC) content during composting of coir pith using different fungal species.

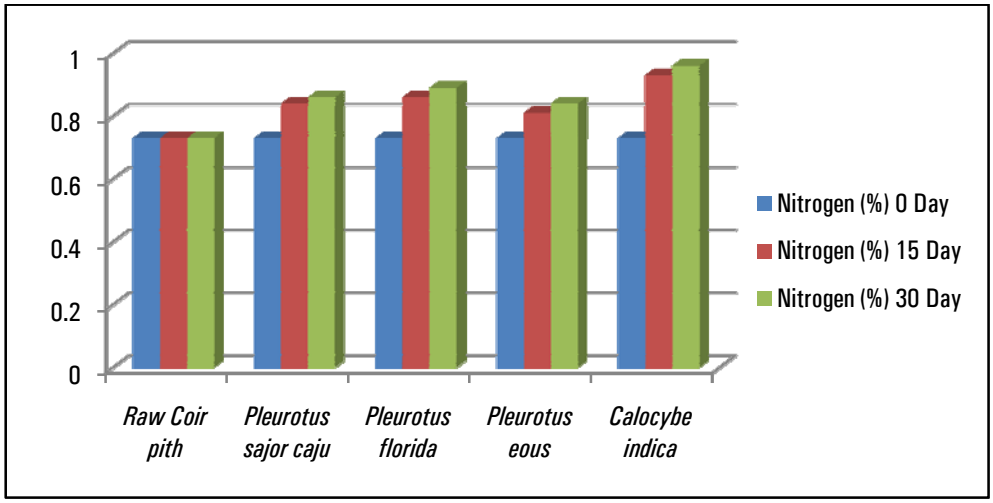



**Fig. II C.** Variation in Nitrogen content during composting of coir pith using different fungal species.

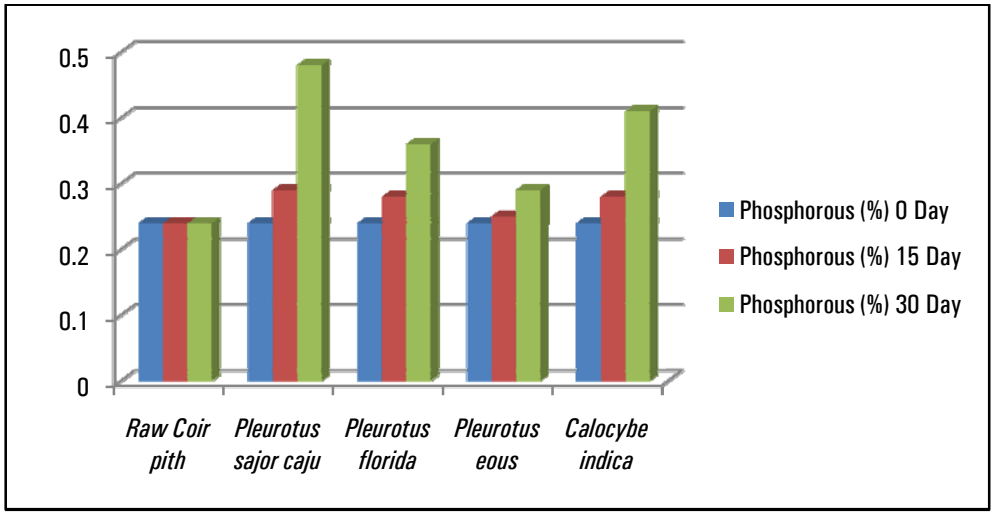



**Fig. II D.** Variation in Phosphorous content during composting of coir pith using different fungal species.

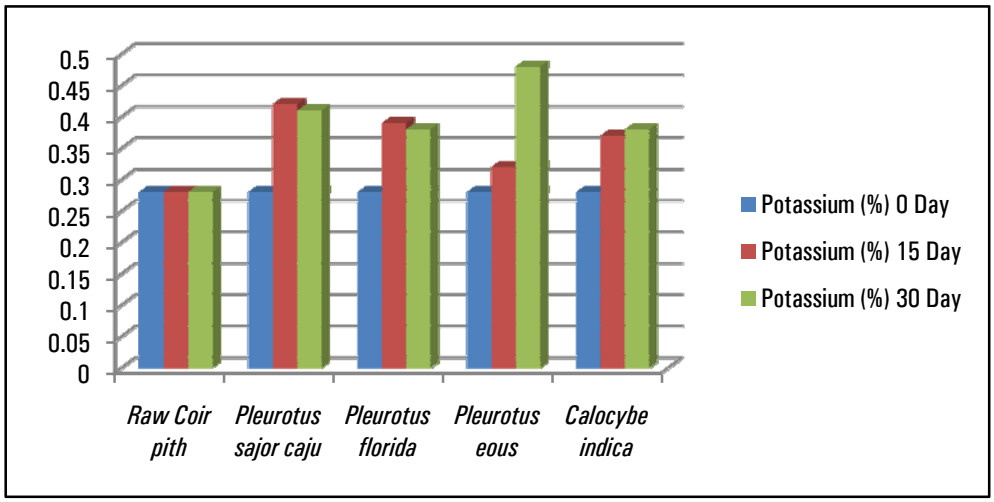



**Fig. II E.** Variation in Potassium content during composting of coir pith using different fungal species.

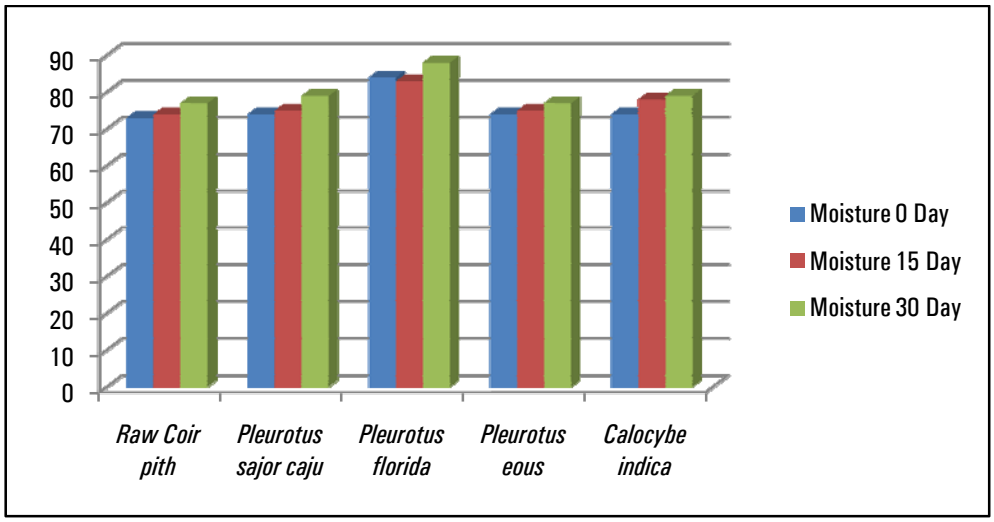



**Fig. II F.** Variation in Moisture content during composting of coir pith using different fungal species.

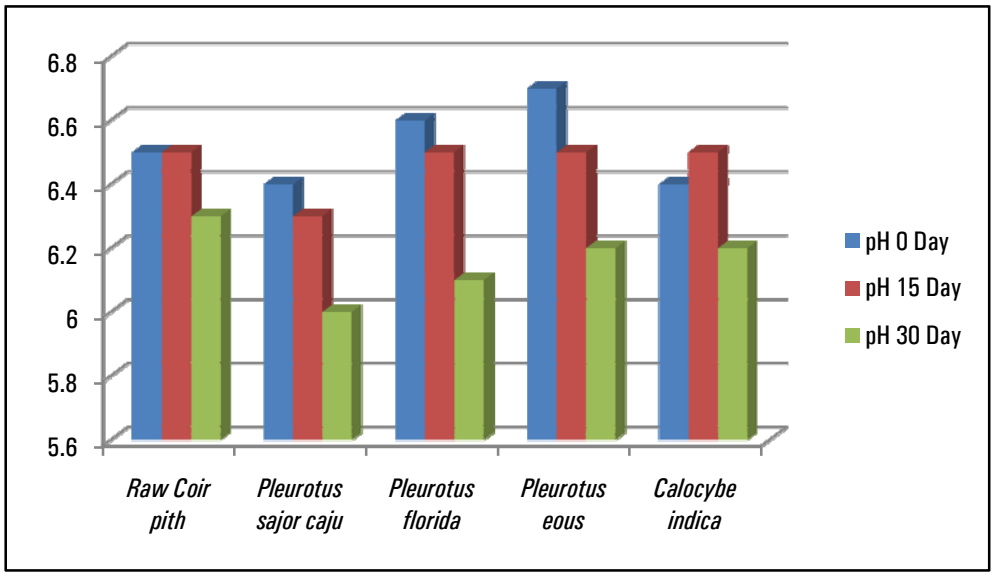



**Fig. II G.** Variation in pH during composting of coir pith using different fungal species.

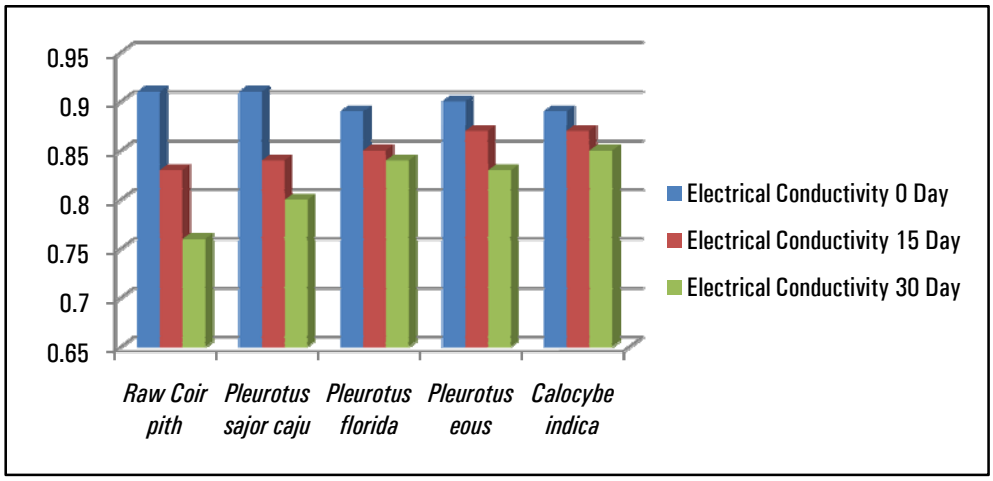



**Fig. II H.** Variation in Electrical Conductivity during composting of coir pith using different fungal species.

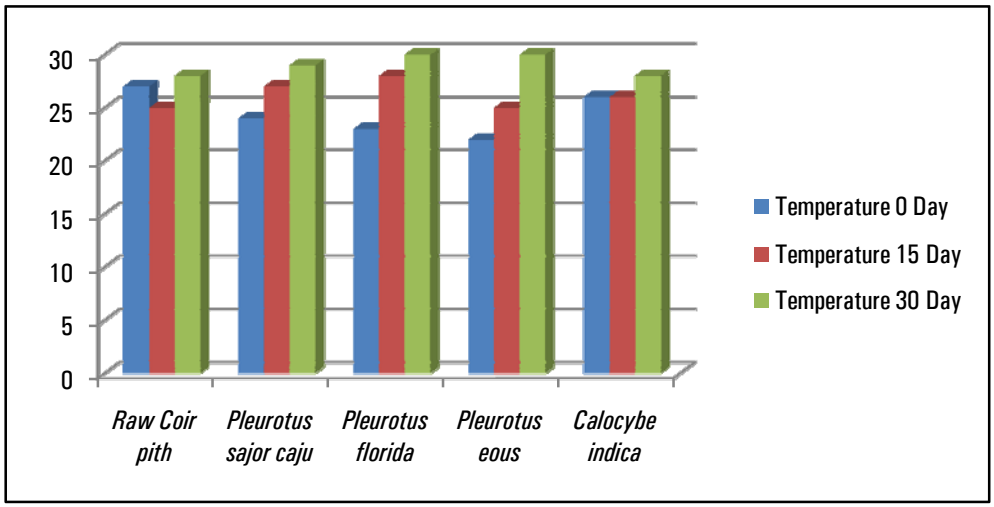



**Fig. II I.** Variation in Temperature during composting of coir pith using different fungal species.

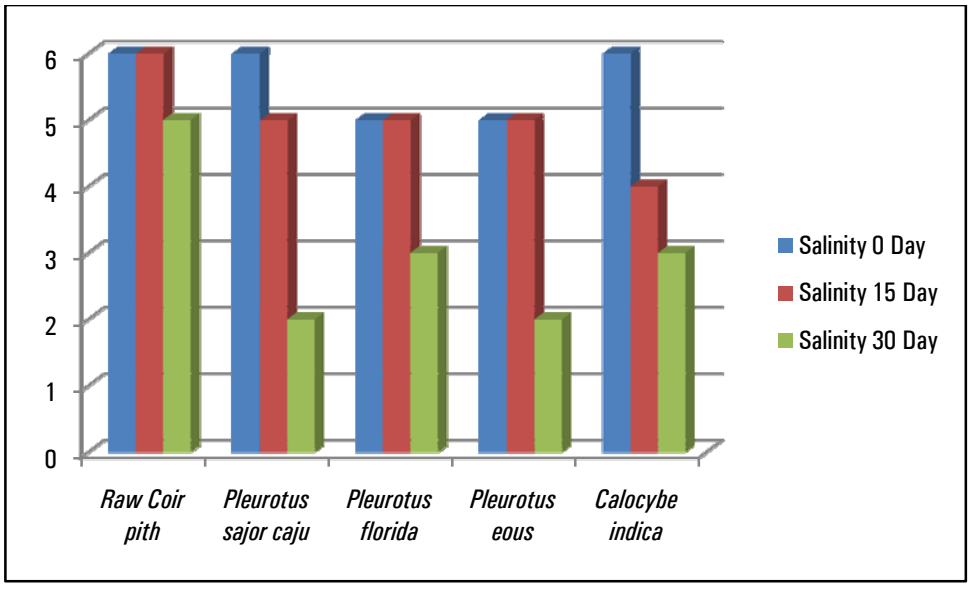



**Fig. II J.** Variation in Salinity during composting of coir pith using different fungal species.

## 3.3. Discussion

The aim of the present work is to study the efficacy of the different fungal species *viz. Pleurotus sajor caju, Pleurotus florida, Pleurotus eous* and *Calocybe indica* to degrade coir pith. The Physico-chemical parameters such as Moisture, pH, Salinity, Electrical conductivity, Temperature, Lignin, Organic carbon, and NPK before and after decomposition by the different fungal species was studied and details furnished in Tables II a and II b. 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 was observed to be 32 % which varied when coir pith was treated with different fungal species. The decomposition ranged from 18 to 30.1%. The maximum decomposition was evident when the pith was treated with *Pleurotus florida.* The pH ranged between 6.4 and 6.8 during the composting in all the sets. After 30 days, a marginal increase in values was observed in all the sets. The organic carbon content of treated samples drawn at different time intervals showed variation. The maximum reduction was observed in the sets treated with *Pleurotus sajor caju*.

Nitrogen, phosphorus and potassium (NPK) content of raw and fungus treated coir pith are presented in Table II a. The nitrogen content in the control for 30 days was observed to be constant (0.73%), but after 15 days of treatment with the different species of fungus, the nitrogen content in the samples increase to the range 0.81 to 0.93 %. In the samples treated with *Calocybe indica*, the values observed were between 0.84 and 0.96 % and it could be confirmed that the *C. indica* was more effective in enhancing nitrogen content during the decomposition process. The study on the phosphorous content in the samples treated 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 %). After 30 days of biodegradation 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 fungus, has already been established.

The potassium content of coir pith did not show any change in controls. However, the values under treatments with mushroom species showed variation. After 15 days, the potassium content ranged between 0.30 and 0.40%, but the values increased after 30 days to a range between 0.32 and 0.41 %. *P. sajor caju* was found to be effective in enhancing the potassium content followed *by P. florida* and *C. indica*. Table. II a presents the results of the 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 %). However in the sets where the chemical fertilizer, urea was added as an additional component along with *P.sajor caju* and *P. florida*, the results revealed that NPK content was greater in coir pith treated with *P.sajor caju.*

Coir pith has been reported to have a C: N ratio of 112:1 (Nagarajan *et al*., 1985). White rot fungi which degrade cellulose and hemi cellulose as well as lignin are widely used to increase the digestibility of agro-residues (Kurtzman, 1981; Neelakantan, 1987; Zabrazil, 1987). Lignin is the most abundant aromatic polymer on earth, being produced by plants. The first step in lignin degradation is depolymerization, catalyzed by lignolytic enzymes. Degradation of lignin is carried out by a group of basidiomycetes categorized as white rot fungi (Kamitsuji *et al*., 2004). High content of lignin in coir pith causes very slow decomposition following which it is used as raw organic manure for crops (Vinodhini *et al*, 2005). The degrading capacity of the combined consortium is more than that by the individual organisms. Even, white rot fungi which are the most effective basidiomycetes for biological pretreatment of lignocellulosic materials (Sun and Cheng, 2002), when added with appropriate bacterial species, interesting results could be obtained.

Thus this chapter confirms that the individual mushroom species *viz*. *Pleurotus sajor caju, Pleurotus florida, Pleurotus eous* and *Calocybe indica* influence lignin degradation and enhance the NPK content of coir pith to make it suitable as a plant nutrient substrate.

## 3.4. Conclusion

The resistance of the lignocellulose constituents in coir pith to biodegradation results in the accumulation of the same as huge hillock in the coir fibre production units. This causes problem of disposal and management of the coir pith. Lignin degrading mushroom *Pleurotus sajor caju* has been proved to degrade coir pith and convert it into organic manure. This study on the application of lignin degrading mushroom species of *Pleurotus sajor caju, Pleurotus florida, Pleurotus eous* and *Calocybe indica* on the coir pith was a comparative study on the efficiency of different fungal species to degrade the lignin in coir pith. From the study it could be concluded that all the fungal cultures could degrade the lignin in coir pith or enhance its nutrient status. *Pleurotus sajor caju* was observed to be more efficient in lignin degradation as compared to others. The study could emphasize the fact that lignin degrading mushroom species could be used for finding a solution to the problem of accumulation of the biological waste 'coir pith' and also convert it into a value added eco-friendly fertilizer for different type of plants.

Chapter 3

# ISOLATION AND STUDY OF THE MOLECULAR CHARACTERISTICS OF INDIGENOUS NITROGEN FIXING BACTERIA IN COIR PITH.

Contents



## 1. Introduction

Nitrogen fixation can be considered as one of the most interesting microbial activity as it makes the recycling of nitrogen on earth possible and gives a fundamental contribution to nitrogen homeostasis in the biosphere (Aquilantia *et al*., 2004). It is the reduction of $N_2$ (atmospheric nitrogen) to $NH_3$ (ammonia). Free living prokaryotes with the ability to fix atmospheric dinitrogen (diazotrophs) are ubiquitous in soil. In natural ecosystems, biological nitrogen fixation is the most important source of nitrogen. The capacity for nitrogen fixation is widespread among bacteria. The estimated contribution of free living N-fixing prokaryotes to the nitrogen input of soil ranges from 0-60 kg/ha/year (Burgmann *et al*., 2003). In recent years, many studies have addressed the importance and contribution of biological nitrogen fixation in ecologically unique terrestrial and aquatic habitats by focusing on the diversity of *nif*H sequences (Zehr *et al*., 2003). Such studies have provided a rapidly expanding database of *nif*H sequences and revealed a wide diversity of uncultured diazotrophs (Tan *et al*., 2003). Plant associated nitrogen fixing bacteria have been considered as one of the possible alternatives for

inorganic nitrogen fertilizer for promoting plant growth and yield (Ladha and Reddy, 2000). A variety of nitrogen fixing bacteria like *Acetobacter, Arthrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Beijerinckia, Derxia, Enterobacter, Herbaspirillum, Klebsiella, Pseudomonas* and *Zoogloea* have been isolated from the rhizosphere of various crops. (Barraquio *et al*., 2000). A significant reduction in the use of nitrogen-fertilizer could be achieved if biological nitrogen fixation is made available to crop plants (Dawe, 2000)

Nitrogenous fertilizers are one of the most widely used chemical fertilizers, as deficiency of nitrogen in the soil often limits crop yield. Consumption of nitrogen fertilizer in Asia has increased from 1.5 to 47 million tons (mt) during the last 35 years (Dawe, 2000). Only less than 50% of the added nitrogen is available to the plants. The enzymatic reduction of nitrogen to ammonia replenishes the loss of nitrogen from soil-plant ecosystems and is achieved through biological nitrogen fixation. Diazotrophs in the soil are the main source of nitrogen input in primary production ecosystems (Cleveland, 1999).

Nitrogen fixers in the environment are diverse. Bacteria of the genus *Azospirillum* are well-known examples of so-called associative nitrogen fixers, which are widespread in the soils of tropical, subtropical and temperate regions. These bacteria develop close relationships with the roots of various wild and agricultural plants (Tyler *et al*, 1979; Steenhoubt and Vanderleyden., 2000). Studies on these microorganisms carried out over the last few decades have primarily been aimed at gaining insight into the molecular nature of plant-microbial interactions in order to develop efficient modern agricultural biotechnology (Burdman *et al*, 2001; Fedonenko *et al*, 2001). Associative nitrogen fixing bacteria such as *Azospirillum brasilense, Herbaspirillum seropedicae* and *Acetobacter diazotropicus* may benefit their host plants as nitrogen biofertilizers and plant growth promoters. The latter two organisms were the first nitrogen-fixing bacteria suggested to be endophytes (Baldani *et al*., 1997; James *et al*., 1997).

*A.lipoferum* and *A.brasilense* were for long the only known members of the genus *Azospirillum (*Tarrand *et al*., 1978).

*Azotobacter* is used as a biofertilizer in the cultivation of most crops. It naturally fixes atmospheric nitrogen in the rhizosphere. Besides nitrogen fixation, *Azotobacter* also produces Thiamin, Riboflavin, Indole acetic acid and gibberellins. The seed germination is improved to a considerable extent when *Azotobacter* is applied to seeds and it also controls plant diseases due to the hormones produced (Kader *et al*., 2002). Nitrogen fixation in *A. vinelandii* is complicated by the presence of three biochemically and genetically distant nitrogenase enzymes, each of which is synthesized under different conditions of metal supply. The regulation of conventional molybdenum nitrogenase, whose subunits are encoded by the Nif-HDK genes, is similar to the enzyme purified from a number of other nitrogen fixing organisms. (Sabra *et al*., 2000).

A large number of *nif*H primers have been designed to study the diversity of diazotrophs. (Poly *et al*, 2001., Rosch *et al*, 2002., Widmer *et al*, 1999 and Shaffer *et al*, 2000). The *nif*H gene has been largely studied by culture independent approaches. The structure of the *nif*H gene pool was investigated by RFLP analysis of the *nif*H gene, which has been amplified from DNA directly extracted from soil samples (Poly *et al*., 2001) and other techniques, such as PCR cloning (Zehr *et al*., 1995; 1998). The *nif*H genes are very diverse, some of them are characteristic of an ecological niche (Chelius & Lepo., 1999; Shaffer *et al*., 2000).

## 2. Methodology.

### 2.1 Sample Collection.

Samples of coir pith were collected from the accumulated heaps in Kattukada in the Alappuzha district of Kerala in India. The samples were randomly collected in sterile plastic bags and stored at four degrees Celsius in the laboratory for further experiments.

## 2.2. Isolation of Nitrogen Fixing Bacteria.

General plating techniques were followed for screening and isolation. Individual colonies were picked, purified and assayed as pure cultures for nitrogenase activity using N-deficient medium. Pure cultures of nitrogen fixing isolates were obtained by repeated sub culturing and confirmed by gram staining and biochemical tests.

**Composition of Nitrogen Free Media (Jensen's medium)**

| Ingredients | | Gms / Litre |
|---|---|---|
| $KH_2PO_4$ | - | 0.2g |
| $K_2HPO_4$ | - | 0.8g |
| $MgSO_4.7H_2O$ | - | 0.2g |
| $CaSO_4.2H_2O$ | - | 0.1g |
| $FeCl_3$ | - | Trace |
| $Na_2MoO_4$ | - | Trace |
| Yeast Extract | - | 0.5g |
| Sucrose | - | 20.0g |
| Agar | - | 15.0g |
| Distilled Water | - | 1.0 L |
| Adjust pH to 7.2 | | |

## 2.3. Extraction and Analysis of DNA.

Genomic DNA was obtained by using standard procedure for isolation of bacterial DNA (Sambrook *et al*., 1989). The DNA stock samples were quantified using UV spectrophotometer at 260 and 280 nm using the convention that one absorbance unit at 260 nm wavelength equals 50µg DNA per ml. The absorbance in the UV range of 260 and 280 nm were studied for determination of DNA concentration and purity. Purity of DNA was confirmed on the basis of optical density ratio at 260/280 nm. The quality of DNA was further confirmed using agarose gel electrophoresis (Maniatis *et al*., 1982). 16S rDNA fragment was amplified by PCR from the bacterial genomic DNA using 16S rDNA universal primers.

## 2.4. PCR Amplification of the *nif*H Gene Fragment.

One hundred nanogram of DNA were used as template in the PCR. Selected primers *Nif*H for-5' TAYGGNAARGGNGGHATYGGYATC and *Nif*H rev -5'

ATRTTRTTNGCNGCRTAVABBGCCATCAT were used for amplification (Poly *et al*., 2001; Paul, M K., 2012)). PCR was carried out in a final reaction volume of 25µl in 200µl capacity thin wall PCR tubes. The PCR tubes containing the mixture were tapped gently and centrifuged at 10,000 rpm. The PCR tubes with all the components were transferred to the thermal cycler. The thermo cycling conditions consisted of an initial denaturation step at 94ºC for 3 minutes, 30 amplification cycles of 45 sec at $94^0$C,30 sec at $55^0$C,60 sec at $72^0$C and a final extension step at 72ºC for 5 minutes with Gene Amp PCR system (Perkin-Elmer Co., Norwalk, Conn.).

### 2.5. Analysis of DNA Amplification by AGE.

Commercially available 100bp ladder was used as standard molecular weight DNA. Analysis of the PCR products was carried out by electrophoresis. Loaded 5µl of PCR product with 4µl bromophenol blue (Loading Dye) in 1.5% agarose gels. Ran the gel at a constant voltage of 100V and current of 45A for a period of 1 hr 20 min till the bromophenol blue travelled 6 cms from the wells. Viewed the gels on UV transilluminator.

### 2.6. Purification & DNA Sequencing of Samples.

The kit used for the purification of DNA was Sigma Aldrich, Bangalore. Amplified PCR product was purified using column purification as per manufacturer's guidelines and further used for sequencing reaction.

### 2.7. Sequencing of Purified 16S rDNA Gene Segment.

The concentration of the purified DNA was determined and was subjected to automated DNA sequencing on ABI3730xl Genetic Analyzer (Applied Biosystems, USA).

### 2.8. 16S rRNA Sequence Analysis.

Each nucleic acid sequence was edited manually to correct falsely identified bases and trimmed to remove unreadable sequences at the 3'and 5' ends (considering peak and Quality Values for each base) using the sequence analysis tools. The edited sequences (16S rDNA) were then used for similarity search using BLAST (Basic Local Alignment Search Tool) programme in the NCBI GenBank (www.ncbi.nlm.nih.gov)

DNA database for identifying the bacterial strains. The phylogenetic tree was constructed by the methods implemented in the TREECONW software package.

### 2.9. Sequence Deposition.

The 16s rRNA, *nifH* gene fragments of the strain NF4, NF7, NF12 and NF18 have been deposited in the GenBank.

## 3. Result.

### 3.1 Isolation of Nitrogen Fixing Bacteria.

Nineteen different colonies with different physiological and biochemical characters were obtained after primary screening using nitrogen deficient medium.

### 3.2. Extraction and Analysis of DNA.

High molecular weight genomic DNA were obtained from the strains which was observed in agarose gel electrophoresis evaluation 16S rDNA fragment amplified by PCR from bacterial genomic DNA using 16S rDNA universal primers

### 3.3. PCR Amplification of the *nif* H Gene Fragment.

Successfully amplified *nifH* gene fragment could be viewed as clear bands in agarose gel (Fig III b).

### 3.4. Purification & DNA Sequencing Of Samples

Column purification yielded contaminant free PCR product. Automated sequence analyser gives sequence data for similarity search.

### 3.5. Sequencing of Purified 16SrDNA Gene Segment

BLAST results indicated that four cultures have greater similarity *with Lysinibacillus sp.,Ochrobactrum., Paenibacillus.,*and *Clostridium sp*

### 3.6. Sequence Deposition.

The 16s rDNA, *nif*H gene fragments of the strain NF4, NF7, NF12 and NF18 have been deposited in the GenBank with the accession numbers JN230510, JN230511, JN230512 and JN230513 respectively

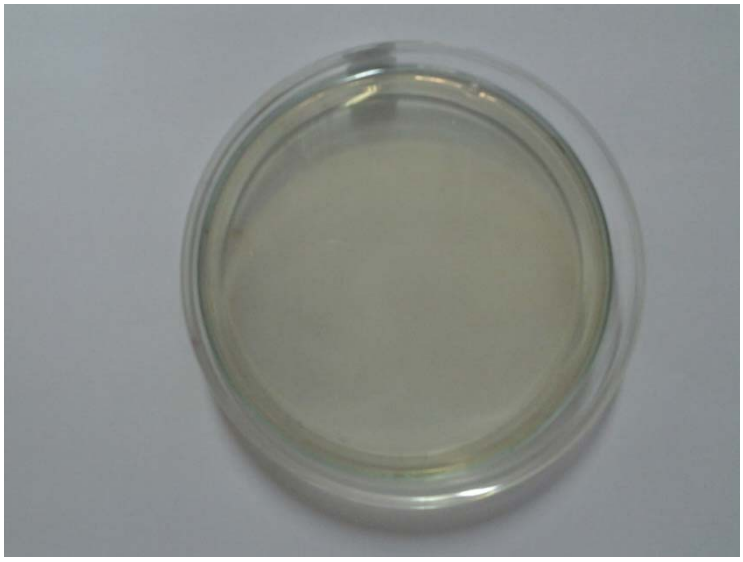


**Fig. III**. Nitrogen fixing bacterial colonies deficient on nitrogen deficient medium (Control)

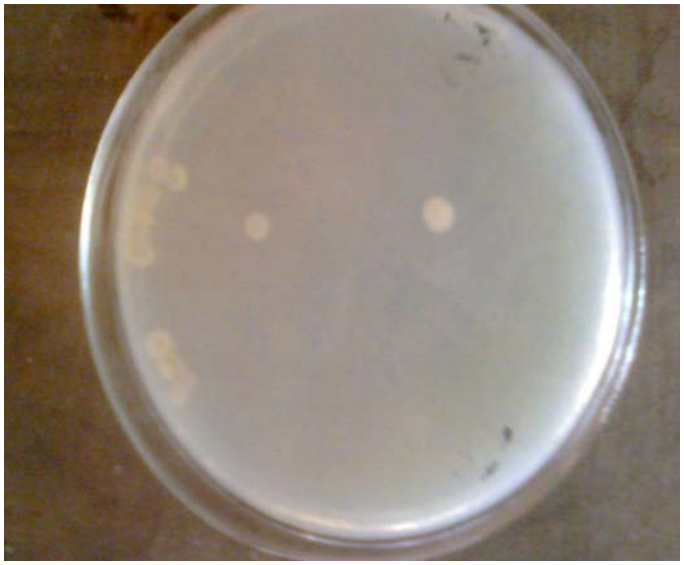


**Fig. III a**. Nitrogen fixing bacterial colonies growing on nitrogen deficient medium

**Table I**. Result of biochemical Estimation

| Sl.No | Characterization | *Clostridium spp* | *Paenibacillus spp* | *Ochrobactrum spp* | *Lysinibacillus spp* |
|---|---|---|---|---|---|
| 1 | Gram staining | + | + | - | + |
| 2 | Indole | - | + | - | + |
| 3 | Methyl red | - | - | - | - |
| 4 | VP | - | + | + | + |
| 5 | Glucose | + | + | - | + |
| 6 | Fructose | + | + | + | + |
| 7 | Maltose | + | + | + | + |
| 8 | Mannitol | - | + | + | + |
| 9 | Galactose | - | + | - | + |
| 10 | Glycerol | + | - | + | + |
| 11 | Gelatin | + | v | - | v |
| 12 | Salicin | v | + | + | + |
| 13 | Catalase | - | + | - | + |
| 14 | Urease | + | - | - | v |
| 15 | H2S | + | - | - | - |
| 16 | Nitrate reduction | + | + | + | + |
| 17 | Acetylene reduction | + | + | + | + |

\+ Positive,

\- Negative,

V Variable reaction

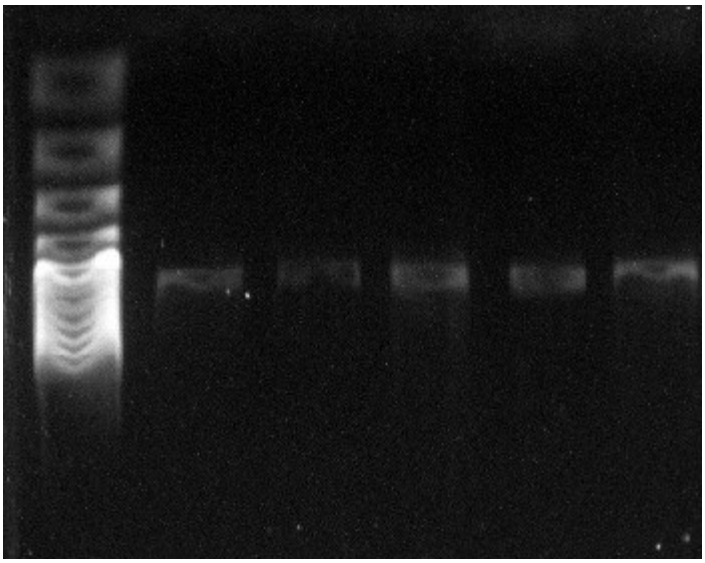
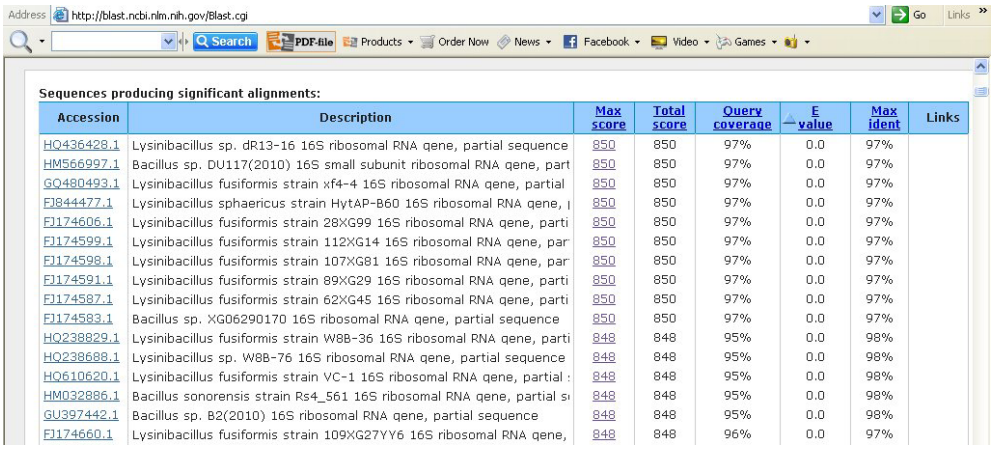


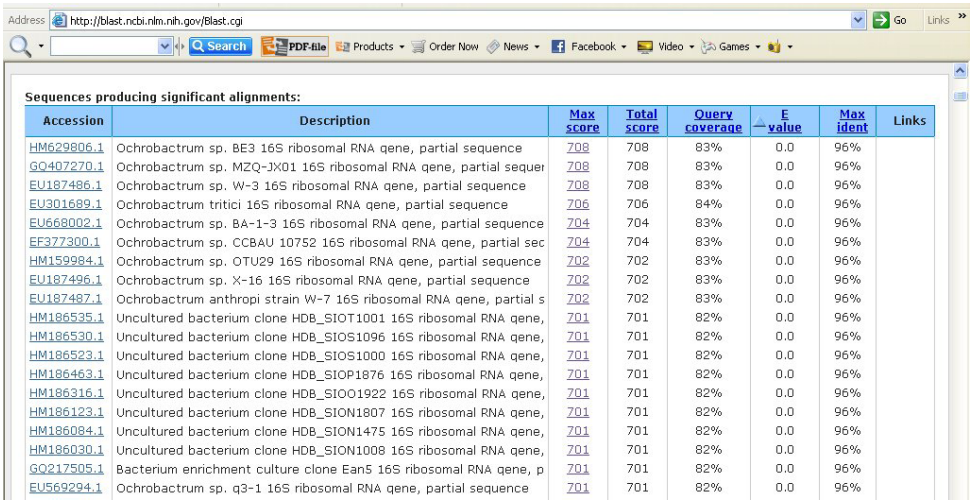
**Fig. III b.** Amplification of bacterial DNA(Isolated from coir pith samples) with *nifH* primers. Lane I, 100 bp Molecular weight marker, Lane 2, NF-4; Lane 3, NF-7; Lane 4, NF-12 and Lane 5, NF-18.



Sequences producing significant alignments:

| Accession | Description | Max score | Total score | Query coverage | E value | Max ident | Links |
|---|---|---|---|---|---|---|---|
| HQ436428.1 | Lysinibacillus sp. dR13-16 16S ribosomal RNA gene, partial sequence | 850 | 850 | 97% | 0.0 | 97% | |
| HM566997.1 | Bacillus sp. DU117(2010) 16S small subunit ribosomal RNA gene, part | 850 | 850 | 97% | 0.0 | 97% | |
| GQ480493.1 | Lysinibacillus fusiformis strain xf4-4 16S ribosomal RNA gene, partial | 850 | 850 | 97% | 0.0 | 97% | |
| FJ844477.1 | Lysinibacillus sphaericus strain HytAP-B60 16S ribosomal RNA gene, | 850 | 850 | 97% | 0.0 | 97% | |
| FJ174606.1 | Lysinibacillus fusiformis strain 28XG99 16S ribosomal RNA gene, parti | 850 | 850 | 97% | 0.0 | 97% | |
| FJ174599.1 | Lysinibacillus fusiformis strain 112XG14 16S ribosomal RNA gene, par | 850 | 850 | 97% | 0.0 | 97% | |
| FJ174598.1 | Lysinibacillus fusiformis strain 107XG81 16S ribosomal RNA gene, par | 850 | 850 | 97% | 0.0 | 97% | |
| FJ174591.1 | Lysinibacillus fusiformis strain 89XG29 16S ribosomal RNA gene, parti | 850 | 850 | 97% | 0.0 | 97% | |
| FJ174587.1 | Lysinibacillus fusiformis strain 62XG45 16S ribosomal RNA gene, parti | 850 | 850 | 97% | 0.0 | 97% | |
| FJ174583.1 | Bacillus sp. XG06290170 16S ribosomal RNA gene, partial sequence | 850 | 850 | 97% | 0.0 | 97% | |
| HQ238829.1 | Lysinibacillus fusiformis strain W8B-36 16S ribosomal RNA gene, parti | 848 | 848 | 95% | 0.0 | 98% | |
| HQ238688.1 | Lysinibacillus sp. W8B-76 16S ribosomal RNA gene, partial sequence | 848 | 848 | 95% | 0.0 | 98% | |
| HQ610620.1 | Lysinibacillus fusiformis strain VC-1 16S ribosomal RNA gene, partial : | 848 | 848 | 95% | 0.0 | 98% | |
| HM032886.1 | Bacillus sonorensis strain Rs4_561 16S ribosomal RNA gene, partial s | 848 | 848 | 95% | 0.0 | 98% | |
| GU397442.1 | Bacillus sp. B2(2010) 16S ribosomal RNA gene, partial sequence | 848 | 848 | 95% | 0.0 | 98% | |
| FJ174660.1 | Lysinibacillus fusiformis strain 109XG27YY6 16S ribosomal RNA gene, | 848 | 848 | 96% | 0.0 | 97% | |

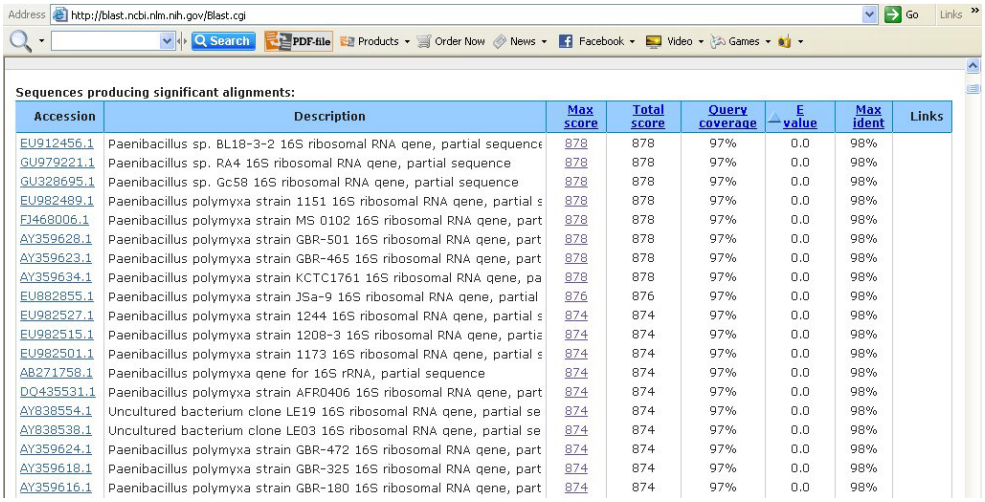
**Fig. III c.** Blast result of Culture 4. Based on the 16s rDNA analysis, the culture 4 showed 97% similarity with *Lysinibacillus sp.* (Accession No: HQ436428.1)



Sequences producing significant alignments:

| Accession | Description | Max score | Total score | Query coverage | E value | Max ident | Links |
|---|---|---|---|---|---|---|---|
| HM629806.1 | Ochrobactrum sp. BE3 16S ribosomal RNA gene, partial sequence | 708 | 708 | 83% | 0.0 | 96% | |
| GQ407270.1 | Ochrobactrum sp. MZQ-JX01 16S ribosomal RNA gene, partial sequer | 708 | 708 | 83% | 0.0 | 96% | |
| EU187486.1 | Ochrobactrum sp. W-3 16S ribosomal RNA gene, partial sequence | 708 | 708 | 83% | 0.0 | 96% | |
| EU301689.1 | Ochrobactrum tritici 16S ribosomal RNA gene, partial sequence | 706 | 706 | 84% | 0.0 | 96% | |
| EU668002.1 | Ochrobactrum sp. BA-1-3 16S ribosomal RNA gene, partial sequence | 704 | 704 | 83% | 0.0 | 96% | |
| EF377300.1 | Ochrobactrum sp. CCBAU 10752 16S ribosomal RNA gene, partial sec | 704 | 704 | 83% | 0.0 | 96% | |
| HM159984.1 | Ochrobactrum sp. OTU29 16S ribosomal RNA gene, partial sequence | 702 | 702 | 83% | 0.0 | 96% | |
| EU187496.1 | Ochrobactrum sp. X-16 16S ribosomal RNA gene, partial sequence | 702 | 702 | 83% | 0.0 | 96% | |
| EU187487.1 | Ochrobactrum anthropi strain W-7 16S ribosomal RNA gene, partial s | 702 | 702 | 83% | 0.0 | 96% | |
| HM186535.1 | Uncultured bacterium clone HDB_SIOT1001 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| HM186530.1 | Uncultured bacterium clone HDB_SIOS1096 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| HM186523.1 | Uncultured bacterium clone HDB_SIOS1000 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| HM186463.1 | Uncultured bacterium clone HDB_SIOP1876 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| HM186316.1 | Uncultured bacterium clone HDB_SIOO1922 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| HM186123.1 | Uncultured bacterium clone HDB_SION1807 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| HM186084.1 | Uncultured bacterium clone HDB_SION1475 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| HM186030.1 | Uncultured bacterium clone HDB_SION1008 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| GQ217505.1 | Bacterium enrichment culture clone Ean5 16S ribosomal RNA gene, p | 701 | 701 | 82% | 0.0 | 96% | |
| EU569294.1 | Ochrobactrum sp. q3-1 16S ribosomal RNA gene, partial sequence | 701 | 701 | 82% | 0.0 | 96% | |

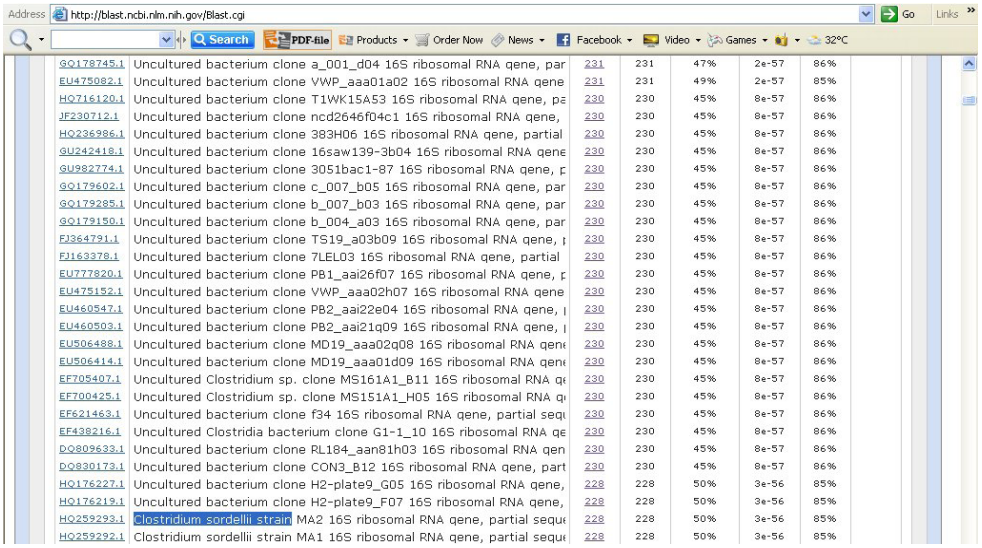
**Fig. III d.** Blast result of Culture 7. Based on the 16s rDNA analysis, the culture 7 showed 96% similarity with *Ochrobactrum sp.* (Accession No: HM629806.1)



Sequences producing significant alignments:

| Accession | Description | Max score | Total score | Query coverage | E value | Max ident | Links |
|---|---|---|---|---|---|---|---|
| EU912456.1 | Paenibacillus sp. BL18-3-2 16S ribosomal RNA gene, partial sequence | 878 | 878 | 97% | 0.0 | 98% | |
| GU979221.1 | Paenibacillus sp. RA4 16S ribosomal RNA gene, partial sequence | 878 | 878 | 97% | 0.0 | 98% | |
| GU328695.1 | Paenibacillus sp. Gc58 16S ribosomal RNA gene, partial sequence | 878 | 878 | 97% | 0.0 | 98% | |
| EU982489.1 | Paenibacillus polymyxa strain 1151 16S ribosomal RNA gene, partial s | 878 | 878 | 97% | 0.0 | 98% | |
| FJ468006.1 | Paenibacillus polymyxa strain MS 0102 16S ribosomal RNA gene, part | 878 | 878 | 97% | 0.0 | 98% | |
| AY359628.1 | Paenibacillus polymyxa strain GBR-501 16S ribosomal RNA gene, part | 878 | 878 | 97% | 0.0 | 98% | |
| AY359623.1 | Paenibacillus polymyxa strain GBR-465 16S ribosomal RNA gene, part | 878 | 878 | 97% | 0.0 | 98% | |
| AY359634.1 | Paenibacillus polymyxa strain KCTC1761 16S ribosomal RNA gene, pa | 878 | 878 | 97% | 0.0 | 98% | |
| EU882855.1 | Paenibacillus polymyxa strain JSa-9 16S ribosomal RNA gene, partial | 876 | 876 | 97% | 0.0 | 98% | |
| EU982527.1 | Paenibacillus polymyxa strain 1244 16S ribosomal RNA gene, partial s | 874 | 874 | 97% | 0.0 | 98% | |
| EU982515.1 | Paenibacillus polymyxa strain 1208-3 16S ribosomal RNA gene, partia | 874 | 874 | 97% | 0.0 | 98% | |
| EU982501.1 | Paenibacillus polymyxa strain 1173 16S ribosomal RNA gene, partial s | 874 | 874 | 97% | 0.0 | 98% | |
| AB271758.1 | Paenibacillus polymyxa gene for 16S rRNA, partial sequence | 874 | 874 | 97% | 0.0 | 98% | |
| DQ435531.1 | Paenibacillus polymyxa strain AFR0406 16S ribosomal RNA gene, part | 874 | 874 | 97% | 0.0 | 98% | |
| AY838554.1 | Uncultured bacterium clone LE19 16S ribosomal RNA gene, partial se | 874 | 874 | 97% | 0.0 | 98% | |
| AY838538.1 | Uncultured bacterium clone LE03 16S ribosomal RNA gene, partial se | 874 | 874 | 97% | 0.0 | 98% | |
| AY359624.1 | Paenibacillus polymyxa strain GBR-472 16S ribosomal RNA gene, part | 874 | 874 | 97% | 0.0 | 98% | |
| AY359618.1 | Paenibacillus polymyxa strain GBR-325 16S ribosomal RNA gene, part | 874 | 874 | 97% | 0.0 | 98% | |
| AY359616.1 | Paenibacillus polymyxa strain GBR-180 16S ribosomal RNA gene, part | 874 | 874 | 97% | 0.0 | 98% | |

**Fig. III e.** Blast result of Culture 12. Based on the 16s rDNA analysis, the culture 12 showed 98% similarity with *Paenibacillus sp.* (Accession No: EU912456.1)



| | | | | | | |
|---|---|---|---|---|---|---|
| GQ178745.1 | Uncultured bacterium clone a_001_d04 16S ribosomal RNA gene, par | 231 | 231 | 47% | 2e-57 | 86% |
| EU475082.1 | Uncultured bacterium clone VWP_aaa01a02 16S ribosomal RNA gene | 231 | 231 | 49% | 2e-57 | 85% |
| HQ716120.1 | Uncultured bacterium clone T1WK15A53 16S ribosomal RNA gene, pa | 230 | 230 | 45% | 8e-57 | 86% |
| JF230712.1 | Uncultured bacterium clone ncd2646f04c1 16S ribosomal RNA gene, | 230 | 230 | 45% | 8e-57 | 86% |
| HQ236986.1 | Uncultured bacterium clone 383H06 16S ribosomal RNA gene, partial | 230 | 230 | 45% | 8e-57 | 86% |
| GU242418.1 | Uncultured bacterium clone 16saw139-3b04 16S ribosomal RNA gene | 230 | 230 | 45% | 8e-57 | 86% |
| GU982774.1 | Uncultured bacterium clone 3051bac1-87 16S ribosomal RNA gene, p | 230 | 230 | 45% | 8e-57 | 86% |
| GQ179602.1 | Uncultured bacterium clone c_007_b05 16S ribosomal RNA gene, par | 230 | 230 | 45% | 8e-57 | 86% |
| GQ179285.1 | Uncultured bacterium clone b_007_b03 16S ribosomal RNA gene, par | 230 | 230 | 45% | 8e-57 | 86% |
| GQ179150.1 | Uncultured bacterium clone b_004_a03 16S ribosomal RNA gene, par | 230 | 230 | 45% | 8e-57 | 86% |
| FJ364791.1 | Uncultured bacterium clone TS19_a03b09 16S ribosomal RNA gene, p | 230 | 230 | 45% | 8e-57 | 86% |
| FJ163378.1 | Uncultured bacterium clone 7LEL03 16S ribosomal RNA gene, partial | 230 | 230 | 45% | 8e-57 | 86% |
| EU777820.1 | Uncultured bacterium clone PB1_aai26f07 16S ribosomal RNA gene, p | 230 | 230 | 45% | 8e-57 | 86% |
| EU475152.1 | Uncultured bacterium clone VWP_aaa02h07 16S ribosomal RNA gene | 230 | 230 | 45% | 8e-57 | 86% |
| EU460547.1 | Uncultured bacterium clone PB2_aai22e04 16S ribosomal RNA gene, p | 230 | 230 | 45% | 8e-57 | 86% |
| EU460503.1 | Uncultured bacterium clone PB2_aai21q09 16S ribosomal RNA gene, p | 230 | 230 | 45% | 8e-57 | 86% |
| EU506488.1 | Uncultured bacterium clone MD19_aaa02q08 16S ribosomal RNA gene | 230 | 230 | 45% | 8e-57 | 86% |
| EU506414.1 | Uncultured bacterium clone MD19_aaa01d09 16S ribosomal RNA gene | 230 | 230 | 45% | 8e-57 | 86% |
| EF705407.1 | Uncultured Clostridium sp. clone MS161A1_B11 16S ribosomal RNA g | 230 | 230 | 45% | 8e-57 | 86% |
| EF700425.1 | Uncultured Clostridium sp. clone MS151A1_H05 16S ribosomal RNA g | 230 | 230 | 45% | 8e-57 | 86% |
| EF621463.1 | Uncultured bacterium clone f34 16S ribosomal RNA gene, partial sequ | 230 | 230 | 45% | 8e-57 | 86% |
| EF438216.1 | Uncultured Clostridia bacterium clone G1-1_10 16S ribosomal RNA ge | 230 | 230 | 45% | 8e-57 | 86% |
| DQ809633.1 | Uncultured bacterium clone RL184_aan81h03 16S ribosomal RNA gen | 230 | 230 | 45% | 8e-57 | 86% |
| DQ830173.1 | Uncultured bacterium clone CON3_B12 16S ribosomal RNA gene, part | 230 | 230 | 45% | 8e-57 | 86% |
| HQ176227.1 | Uncultured bacterium clone H2-plate9_G05 16S ribosomal RNA gene, | 228 | 228 | 50% | 3e-56 | 85% |
| HQ176219.1 | Uncultured bacterium clone H2-plate9_F07 16S ribosomal RNA gene, | 228 | 228 | 50% | 3e-56 | 85% |
| HQ259293.1 | Clostridium sordellii strain MA2 16S ribosomal RNA gene, partial sequ | 228 | 228 | 50% | 3e-56 | 85% |
| HQ259292.1 | Clostridium sordellii strain MA1 16S ribosomal RNA gene, partial sequ | 228 | 228 | 50% | 3e-56 | 85% |

**Fig. III f.** Blast result of Culture 18. Based on the 16s rDNA analysis, the culture 18 showed 85% similarity with *Clostridium sordellii.* (Accession No: HQ259293.1)

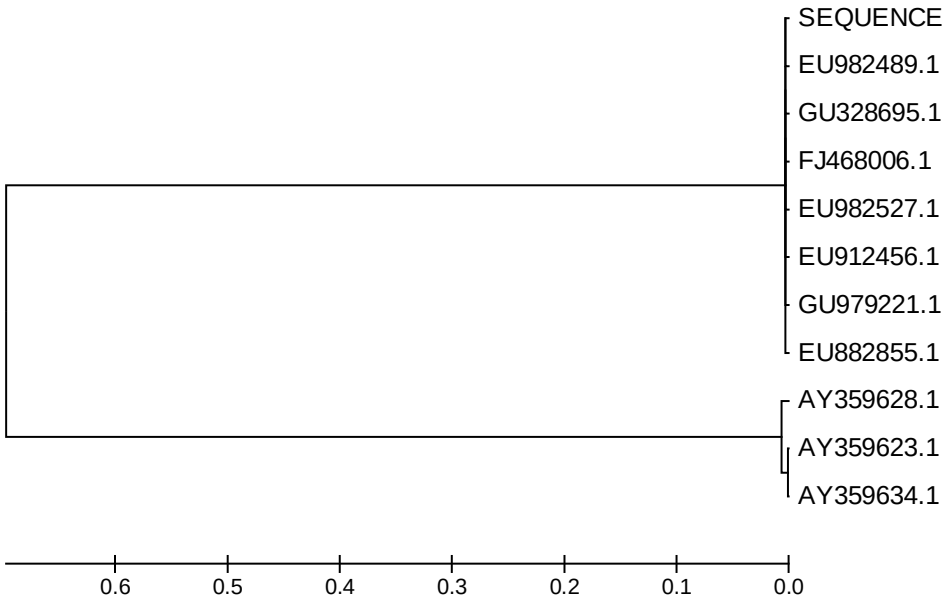



**Figure III g.** (Sample 4) Evolutionary relationships of 11 taxa (linearized)

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.04149565 is shown. The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages [2]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [3] and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 500 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [4].

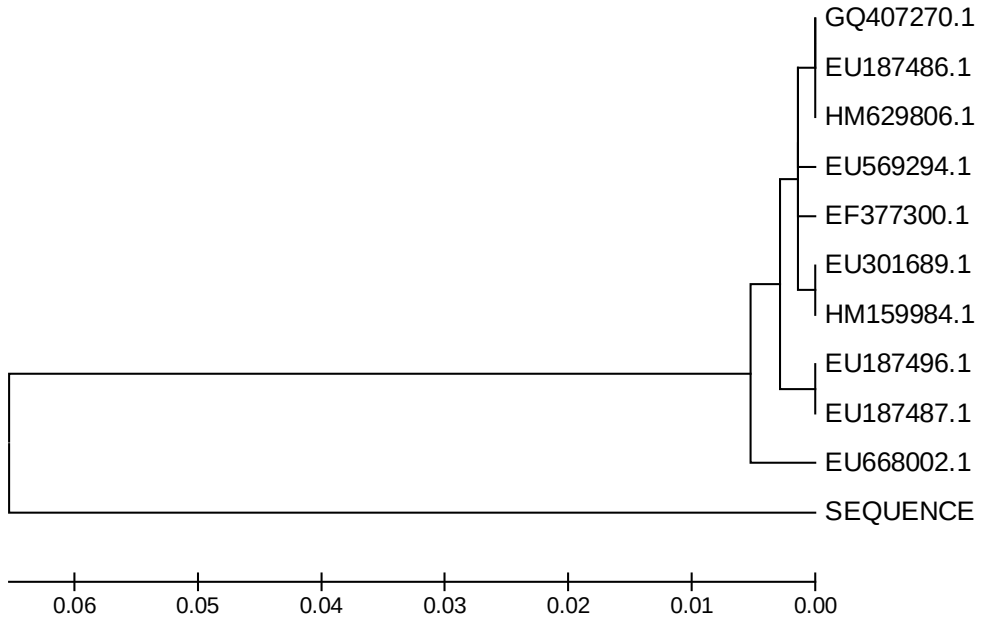



**Figure III g.** (Sample 7) Evolutionary relationships of 11 taxa (linearized)

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.14439229 is shown. The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages [2]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [3] and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 500 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [4].

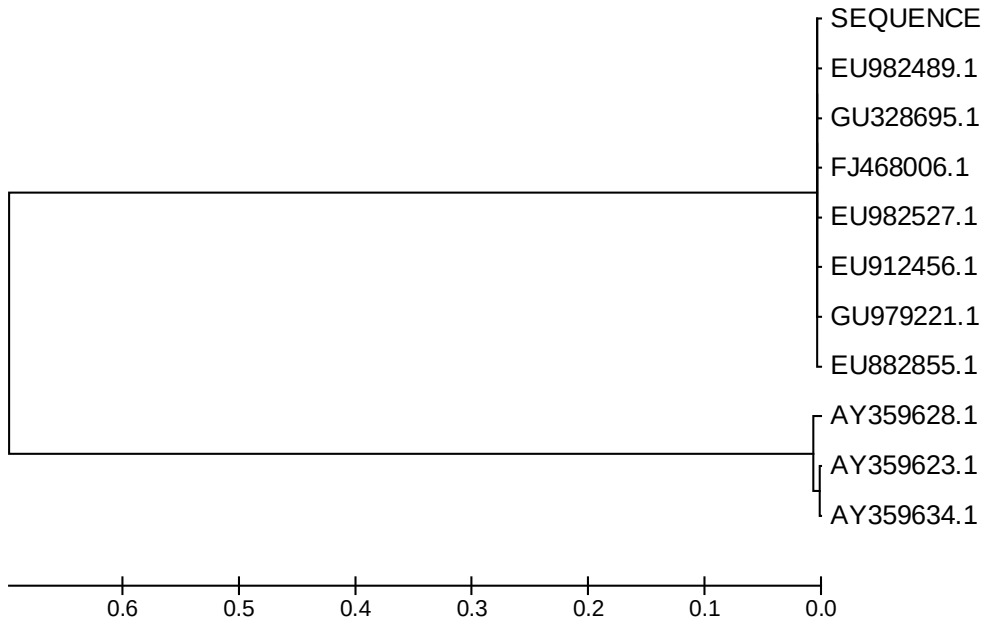



**Figure III h.** (Sample Ab) Evolutionary relationships of 11 taxa (linearized)

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 1.41433670 is shown. The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages [2]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [3] and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 484 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [4].

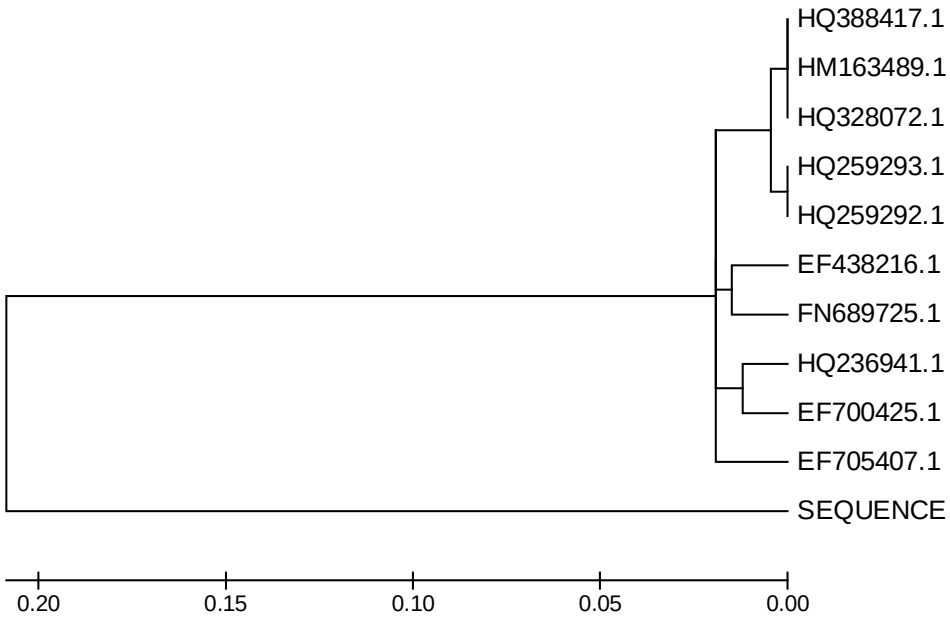



**Figure III i.** (Sample As) Evolutionary relationships of 11 taxa (linearized)

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.51014890 is shown. The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages [2]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [3] and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 345 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [4].

## 4. Discussion

In the present study, an attempt was made to isolate indigenous microorganisms in the coir pith samples drawn from accumulated heaps of coir pith in coir fibre extraction units stored for the past 3 years. Four nitrogen fixing bacteria with different physiological and biochemical characters could be isolated and sequenced. Out of the 19 isolated colonies, four strains were amplified with *nifH* gene *viz.* NF-4 (*Lysinibacillus sp.*), NF-7 (*Ochrobactrum sp*), NF-12 (*Paenibacillus sp*) and an uncultured bacterial clone was isolated which shows only 50% similarity to NF-18 (*Clostridium sp.*) (Reghuvaran *et al.*, 2012). By repeated plating on to nitrogen deficient agar media, pure cultures of the four bacterial colonies could be obtained. High molecular weight DNA was observed in agarose gel evaluation. The 16s rDNA fragment was amplified by PCR from genomic DNA using 16S rDNA universal primer and column purification yielded contaminant free PCR product. A large number of *nifH* primers were designed to study the diversity of diazotrophs (Poly *et al.*, 2001; Rosch *et al.*, 2002; Widmer *et al.*, 1999; Shaffer *et al.*, 2000). Here, the design of the appropriate primers was done with utmost priority for the novel primers, as the use of highly generated primers in combination with low stringency amplification conditions could result in biased conclusions. In the present study, predesigned degenerated primers were used for the isolates from coir pith *viz. Nif*H for-5' TAYGGNAARGGNGGHATYGGYATC and NifH rev -5' ATRTTRTTNGCNGCRTAVABBGCCATCAT (Edwards *et al.*, 1989). The *nif*H primers were designed from the available *nif*H sequences of different organisms from NCBI GenBank (www.ncbi.nlm.nih.gov). After the amplification of microbial DNA from coir pith with *nif* H primers (Figure III b), the edited sequences (16s rDNA) were then subjected to the similarity searches using BLAST programme (Table III c to III f). The BLAST results show that the four cultures have greater similarity with *Lysinibacillus sp.*, *Ochrobactrum sp.*, *Paenibacillus sp.* and *Clostridium sp.* The results have been furnished in Figures III c to III f. Coir pith is degraded very slowly due to

the presence of lignin and accumulates in coir fiber extraction units. The microflora inhabiting coir pith is therefore limited, as the lignocellulose complex resists biodegradation. Herein, an effort is made to isolate the microorganisms in coir pith which possess the nitrogen fixing ability. The DNA isolated from the natural microflora in coir pith showed similarities with *Lysinibacillus sp*., *Ochrobactrum sp*., *Paenibacillus sp*. and *Clostridium sp*. These bacteria are important nitrogen fixing species and most of them have other applications too. It is observed that activities that have been found to be associated with *P. polymyxa* treatment on plants in field experiments include nitrogen fixation, soil phosphorous solubilization, production of antibiotics, auxins, cytokinins, chitinase and hydrolytic enzymes, as well as the promotion of increased soil porosity (Timmusk and Wagner, 1999; Timmusk *et al*., 1999). All these activities might be of importance for plant growth promotion. Timmusk *et al*. (2009) reported *P. polymyxa* B2, B5 and B6 antagonistic mechanisms against the well characterized model of oomycetic pathogens *Phytophthora palmivora* and *Pythium aphanidermatum. P. polymyxa* (previously *Bacillus polymyxa*; Ash *et al*., 1994) is a common soil bacterium belonging to plant growth promoting rhizobacteria(PGPR) which has also present in the coir pith sample. The activities associated with *P. polymyxa* include nitrogen fixation (Heulin *et al*., 1994), soil phosphorous solubilization (Jisha and Alagawadi, 1996), as well as promotion of increased soil porosity (Gouzou *et al.,* 1993; Timmusk and Wagner, 1999; Timmusk *et al*., 1999). Besides, it produces antimicrobial substances active against fungi and bacteria (Rosado and Seldin, 1993; Picard *et al*., 1995; Kajimura and Kaneda, 1996). *P. polymyxa* also has been used for the control of plant sample was *Ochrobactrum sp*., which has several properties of importance. The genus *Ochrobactrum* was described first by Homes *et al*. (1988) and belongs to the α-2 subclass of the Proteobacteria (De Ley, 1992). *Ochrobactrum tritici* was identified as Bacterial strain 5bv11, isolated from a chromium-contaminated waste water treatment plant, which is resistant to a broad range of antibiotics and metals viz. Cr (VI), Ni (II), Co (II), Cd (II) and Zn (II) (Branco *et al.,* 2004). Holmes *et al*. (1988) proposed *Ochrobactrum anthropi* as a sole and type

species of *Ochrobactrum*, but they observed heterogeneities in geno- or phenotypic characters within the tested *O. anthropi* collection. *O. anthropi* strains have been isolated from samples originating from different continents. *Ochrobactrum sp*. contains root associated bacteria that enter bivalent interactions with plant and human hosts. Several members of these genera show plant growth promotion as well as excellent antagonistic properties against plant pathogens and therefore were utilized for the development of biopesticides (Weller, 1988; Whipps, 2001). Most available *O. anthropi* isolates are from human clinical specimens (Lebuhn *et al*., 2000). *O. anthropi* LMG 5140 has also been isolated from arsenical cattle dipping fluid (Holmes *et al*., 1988). Moreover, there are some reports on the presence of *O. anthropi* in soil, on wheat roots and in internal root tissues of different plants (Anguillera *et al*., 1993; McInroy and Kloepper, 1994; Sato and Jiang, 1996). More also, NF-4(*Lysinibacillus* sp.) could be isolated from the coir pith samples. It is described by Ahmed *et al*., (2007) as spore forming, Gram positive, motile, rod shaped and boron-tolerant. A large number of Bacillus strains, including *B. fusiformis* capable of degrading different hydrocarbons, have been isolated from oil contaminated soils (Bento *et al.,* 2003).

## 5. Conclusion

The indigenous properties of coir pith such as its porous nature which retains moisture makes it suitable for application as a soil additive. This study aimed at isolating nitrogen fixing bacteria in coir pith which could add to its nutrient status. The nitrogen fixing bacteria *viz. Lysinibacillus sp*., *Ochrobactrum sp., Paenibacillus sp*. and *Clostridium sp*. could be isolated and their molecular characteristics studied. This could confirm that even raw coir pith is rich in nutrients which acts as an efficient growing medium for nitrogen fixing bacteria. Also it can be concluded that introduction of nitrogen fixing bacteria to coir pith makes the composting more effective and makes the compost rich in nitrogen.

Chapter 4

# SUBSTITUTION OF UREA WITH NITROGEN FIXING BACTERIA FOR FOUR DIFFERENT MUSHROOM SPECIES.

Contents



## 1. Introduction

Studies on bacterial degradation of coir pith are limited due to poor degrading ability of bacteria to degrade the lignin present. Janshekar and Fiechter (1981) reported some bacterial cultures with the ability to degrade numerous phenols with structural relationship to lignin. Several studies indicate that it takes decades to decompose coir pith, due to its high lignin content, causing environmental pollutions and disposal problems (Pazhanivel *et al*., 2011). The filamentous blue-green algae (Cyanobacteria) were believed to be primarily responsible for nitrogen fixation in oceanic waters because low or negligible in situ rates were observed in their absence and there was a correlation of in situ $N_2$ fixation with light intensity (Stewart, 1974), some Cyanobacteria were capable of degrading the coir pith. Some enhanced techniques show the use of coir pith based Cyanobacterial biofertilizer in sustainable integrated agro ecosystems (Prabha *et al*, 2009). Therefore such techniques could be useful to promote the growth of plant and increase the quality and quantity of crop yield (Hume, 2007). Most recently, cellulolytic nitrogen fixing bacteria have been isolated in large numbers, apparently as a pure culture, from a specialized gland found in ship worms (Waterbury *et al*, 1983). The work conducted by Anandharaj (2007) also shows the coir pith can be

partially decomposed through the action of Cyanobacteria and can be used as bio-fertilizer for all varieties of food crops.

Nitrogen fixation is an important source of nitrogen for biological productivity in the environment. Newton and Cavins, 2003 studied the nitrogen fixation (acetylene reduction) and ammonia liberation was studied in a facultative heterotrophic Cyanobacterium. Nitrogeneous fertilizers are one of the most widely used chemical fertilizers, as deficiency of nitrogen in the soil often limits crop yields (Sarita *et al*, 2008). Consumption of nitrogen fertilizer in Asia has increased from 1.5 to 47 million tonnes (mt) during the last 35 years (Dawe, 2000). Plant associated nitrogen-fixing bacteria have been considered as one of the possible alternatives for inorganic nitrogen fertilizer for promoting plant growth and yield (Ladha & Reddy, 2000). Nitrogen fixation is the reduction of atmospheric nitrogen to $NH_3$ (ammonia). Free living prokaryotes with the ability to fix atmospheric dinitrogen (diazotrophs) are ubiquitous in soil. The free living diazotrophs are subclassified. Aerobic diazotrophs, of which there are over 50 genera, including *Azotobacter*, methane-oxidizing bacteria and cyanobacteria require oxygen for growth and fix nitrogen when oxygen is present. *Azotobacter*, some related bacteria and some Cyanobacteria fix nitrogen in ordinary air but most members of this group fix nitrogen only when the oxygen concentration is low. Free living diazotrophs, which fix nitrogen only when oxygen is absent or vanishingly low, are wide spread. But our knowledge of their ecological importance and their diversity remains incomplete. In natural ecosystems, biological $N_2$ fixation is most important source of N. The contribution of free-living N-fixing prokaryotes estimated to the nitrogen input of soil ranges from 0-60 kg/ha /year (Burgmann *et al*., 2003). It is also evident that the dinitrogen ($N_2$)-fixing microorganisms (diazotrophs) play important roles in ocean biogeochemistry and plankton productivity (Church *et al*., 2005). Besides the nitrogen fixation, *Azotobacter* also produces Thiamine, Riboflavin, Indole acetic acid and gibberellins. When *Azotobacter* is applied to seeds, seed germination is improved to a considerable extent, so also it controls

plant diseases due to above substances produced by *Azotobacter* (Kader *et al*., 2002). These organisms, characterized by high nitrogen fixing ability, are found in abundant numbers in the rhizosphere as well as in the intercellular spaces of the roots of certain cereals and other plants (Bashan and Holguin, 1997). 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 *Azotobacter vinelandii and Azospirillum brasilense*. A consortium using the above in combination this with appropriate fungal species for composting would make coir pith a perfect soil conditioner and bio organic manure.

## 2. Methodology.

### 2.1 Sample Collection.

Coir pith was collected from the coir fibre extraction unit in Kattukada, Alappuzha district. There are a number of coir based industries working in this place. The samples of coir pith were transported in sterile container & stored under refrigeration for further studies.

### 2.2 Test Organism and their Maintenance.

Four fungal cultures *viz*. *Pleurotus sajor caju, Pleurotus florida, Pleurotus eous* and *Calocybe indica,* procured from different research institutions have been used for the present study. *Pleurotus sajor caju* was obtained from Central Coir Research Institute (Coir Board), Kalavoor, Alappuzha and the other three organisms from the Regional Agricultural Research Station, Kerala Agricultural University, Kumarakom. All the cultures were maintained in PDA slants and stored under refrigeration at 4˚C. The fungi were mass cultured on sterilized media consisting of sorghum mixed with calcium carbonate to adjust pH. Fully grown packets of mushrooms after 15 days incubation at 15˚C were used for carrying out experimental studies on composting of coir pith. All experiments and biochemical

analysis were carried out at the Rajiv Gandhi Chair in Contemporary Studies, CUSAT and the Central Coir Research Institute, Alappuzha. Composting of coir pith was carried out in open areas as multilayered heaps with the addition of different mushroom species *viz., Pleurotus sajor caju, Pleurotus florida, Pleurotus eous* and *Calocybe indica*, keeping untreated controls.

The bacterial species such as *Azotobacter vinelandii* and *Azospirillum brasilense* incorporated in lignite were procured from Agrobiotech limited, Kotayam as Bacterial formulations.

## 2.3 Experimental Set Up

The efficacy of different mushroom species for the biodegradation of coir pith were studied by laying different sets of coir pith heaps in a shady place. Care was taken for the heaps from stray animals. Each of the experiments were conducted by mounting 1 kg heaps of coir pith supplemented with four different mushroom species and 10 g of urea as nitrogen supplement. There are 5 sets were designed for 4 mushroom species and a control (without the organism). All the heaps were moistened regularly to keep 200% moisture by adding 0.375 litres of water.

## 2.4 Physical Properties of Raw and Biodegraded Coir pith.

### 2.4.1 Moisture.

Moisture of the coir pith samples were determined by Moisture analyzer using AND – MS 70 analyzer (Gupta 2001).

### 2.4.2Temperature.

Temperature was recorded using a laboratory thermometer at five points on the composting heap and taken the average.

### 2.4.3pH.

Calibrated METLER TOLEDO pH meter was used for the measurement of pH (Muthuvel and Udayasoorian, 1992).

**2.4.4Electrical Conductivity.**

Electrical conductivity was measured using Deluxe conductivity meter – Model 601 (Muthuvel and Udayasooriyan, 1992).

**2.4.5Salinity.**

A calibrated laboratory salinometer was used for the measurement of salinity as per the method described by Lenore *et al*., 1989.

## 2.5 Estimation of Chemical Properties.

**2.5.1 Lignin.**

The lignin in coir pith was determined by Klason lignin method (Stephen and Carlton, 1992).

**2.5.2 Nitrogen**

Nitrogen in the coir pith samples were estimate by Alkaline permanganate method using Kjeldahl distillation Unit (Vogel, 1961).

**2.5.3 Phosphorus.**

Phosphorous was estimated by Vanado Molybdo phosphoric yellow colour method.

**2.5.4 Potassium.**

Potassium was estimated by Flame photometry.

**2.5.5 Organic Carbon.**

Organic carbon was estimated by Walkey and Black method

## 3. Results

The results show that the biodegradation of coir pith is more effective with the use of nitrogen fixing bacteria in combination with fungi. Definite reductions in lignin was observed by composting coir pith with the four different mushrooms

(*Pleurotus sajor caju, Pleurotus florida, Pleurotus eous* and *Calocybe indica*.) in addition with two nitrogen fixing bacteria (*Azotobacter vinelandii* and *Azospirillum brasilense*). Nitrogen, Phosphorous and Potassium content was also observed to be enhanced. The Temperature showed variation, but the pH does not show much variation and the electrical conductivity was observed to decrease considerably.

## 3.1 Physical Properties of Coir pith.

### 3.1.1 Moisture.

From the experiments, it is clear that there was variation in moisture content in coir pith during the course of microbial biodegradation. As the moisture of composting ained as 200% as reported by Central Coir Research Institute (CCRI) method, moisture content raw coir pith was around 20% and during the composting process it increased to higher levels. The high moisture content favour increased levels of biodegradation (Table IV a to IV h).

### 3.1.2 Temperature.

Temperature in the compost heaps was observed to be dynamic and was observed to be 25˚C initially and which increased to 35˚C as biodegradation proceeds.

### 3.1.3 pH.

Biodegradation with different species of mushrooms on coir pith showed variations in pH. Initially the pH was observed to be below the neutral values. But as the composting proceeds, the pH lowered to slightly acidic levels. This could be attributed to the formation of phenolic compounds (Table IV a to IV h).

### 3.1.4 Electrical Conductivity (EC).

Electrical conductivity also decreased during biodegradation. Raw coir pith, which was maintained as control indicated a value of 0.095 millimhos/cm, which tends to decrease during composting. All the four organisms causes the

reduction on electrical conductivity, among the four organisms tested, biodegradation with *Pleurotus sajor caju* was observed to be more effective in reducing Electrical Conductivity. The details given in Table IV a to IV h.

### 3.1.5 Salinity.

Salinity of coir pith also decreased during composting as detailed in IV a to IV h.

## 3.2 Chemical Properties.

The biodegraded coir pith with different mushroom species displayed reduction in Lignin, Organic carbon content and enhancement in the case of Nitrogen, Phosphorous and Potassium (NPK). The details of the chemical parameters given below.

### 3.2.1 Lignin.

The lignocellulosic biomass, coir pith possess 32%. The investigations reveal that definite variations were observed in the lignin content in the coir pith under various treatments in combinations with ligninolytic mushrooms viz., *Pleurotus sajor caju, Pleurotus florida, Pleurotus eous* and *Calocybe indica* and Nitrogen fixing bacteria. The periodical analysis of samples of coir pith drawn at regular intervals from the experimental heaps shows the rate of decomposition of lignin in coir pith (20% to 18%) from the initial value of 32%. The details are given in Table IV i to IV p.

### 3.2.2 Nitrogen.

The investigations reveal that the treatment of coir pith with different mushroom species and nitrogen fixing bacteria show compatible and novel changes in the Nitrogen content. The percentage of nitrogen in the raw coir pith kept as control exhibited no change during the course of composting. The value of Nitrogen in raw coir pith was observed to be 0.73% and the treated samples show

variations. From the results, it is clear that activity of all the mushroom species causes enhancement of Nitrogen content with the addition of both the nitrogen fixing bacteria.

### 3.2.3 Phosphorous.

The Phosphorous content in coir pith also show enhancement with the treatment of fungus and nitrogen fixing bacteria. The periodical analysis of coir pith samples from different treatments using various mushroom species indicated an increasing trend in phosphorous content during composting. The details of the amount of Phosphorous are furnished in table IV i to IV p.

### 3.2.4 Potassium.

The values of Potassium in the biodegraded coir pith with the treatment with different mushroom species were given in table IV i to IV p. The potassium content in the coir pith maintained as control which shows no change throughout the composting process. However, the values in samples under treatment with mushroom species showed variation in potassium content. The results obtained from the analysis of coir pith samples treated with all the mushrooms displayed the values of potassium in an increasing trend. Coir pith samples drawn out from the experimental heaps at regular intervals indicated increase of potassium content in coir pith.

### 3.2.5 Organic Carbon.

The Organic Content (OC) in the raw and Biodegraded coir pith Compost (BCC) is given in table IV I to IV p. The use of combined action of mushroom species and bacteria for the decomposition could reduce the OC content from 6.28 (raw coir pith) to 6.08. The results indicating a decreasing trend in the carbon content of biodegraded coir pith. The carbon content of the raw coir pith (untreated) kept as control did not show any variation.

**Table IV a.** Physical Properties of Biodegraded coir pith with *Pleurotus sajor caju* and *Azotobacter vinelandii*.

| Composting Combinations | Moisture (%) | | | pH | | | Temperature (°C) | | | Salinity (ppt) | | | Electrical Conductivity (mmhos/cm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *P. sajor caju* + *A. vinelandii*(5%) | 60, 61, 60 | 77, 75, 78 | 81, 82, 80 | 6.8, 6.8, 6.6 | 6.5, 6.4, 6.6 | 6.3, 6.3, 6.2 | 26, 24, 24 | 27, 27, 28 | 33, 34, 34 | 6, 5, 6 | 4, 3, 5 | 2, 2, 2 | 0.90, 0.89. 0.92 | 0.88, 0.88, 0.82 | 0.76, 0.75, 0.74 |
| Coir pith + *P. sajor caju* + *A. vinelandii*(10%) | 66, 64, 62 | 80, 80, 81 | 82, 83, 80 | 6.8, 6.6, 6.8 | 6.7, 6.7, 6.6 | 6.5, 6.5, 6.4 | 26, 26, 25 | 28, 27, 28 | 33, 34, 34 | 6, 5, 6 | 3, 3, 2 | 3, 2, 2 | 0.90, 0.91, 0.92 | 0.80, 0.81, 0.80 | 0.66, 0.61, 0.65 |
| Coir pith + *P. sajor caju* + *A. vinelandii*(15%) | 60, 61, 62 | 70, 74, 75 | 84, 84, 86 | 6.8, 6.7, 6.6 | 6.5, 6.5, 6.5 | 6.5, 6.3, 6.5 | 26, 25, 26 | 29, 28, 28 | 34, 34, 35 | 6, 6, 5 | 4, 4, 4 | 2, 2, 1 | 0.90, 0.90, 0.91 | 0.75, 0.75, 0.74 | 0.54, 0.53, 0.51 |
| Coir pith + *P. sajor caju* + *A. vinelandii*(20%) | 62, 63, 64 | 77, 78, 75 | 83, 84, 81 | 6.8, 6.7, 6.6 | 6.6, 6.6, 6.4 | 6.4, 6.3, 6.3 | 26, 26, 25 | 30, 31, 31 | 33, 33, 34 | 6, 5, 4 | 5, 4, 4 | 2, 2, 1 | 0.90, 0.88, 0.91 | 0.56, 0.56, 0.54 | 0.34, 0.32, 0.32 |
| Coir pith + *P. sajor caju* + *A. vinelandii*(25%) | 65, 66, 65 | 78, 78, 79 | 85, 86, 86 | 6.8, 6.7, 6.8 | 6.5, 6.5, 6.4 | 6.2, 6.2, 6.1 | 26, 26, 25 | 29, 29, 26 | 38, 38, 34 | 5, 4, 4 | 3, 3, 2 | 0, 1, 0 | 0.90, 0.91, 0.90 | 0.56, 0.56, 0.54 | 0.22, 0.21, 0.28 |
| Coir pith + *P. sajor caju* + Urea (Control) | 62, 61, 61 | 77, 74, 77 | 76, 75, 75 | 6.8, 6.8, 6.6 | 6.7, 6.6, 6.6 | 6.8, 6.8, 6.7 | 26, 26, 25 | 28, 27, 25 | 28, 27, 25 | 6, 4, 6 | 4, 3, 3 | 3, 2, 2 | 0.90, 0.91, 0.93 | 0.87, 0.81, 0.86 | 0.78, 0.76, 0.78 |

**Table IV b.** Physical Properties of Biodegraded coir pith with *Pleurotus sajor caju* and *Azospirillum brasilense*

| Composting Combinations | Moisture (%) | | | pH | | | Temperature (°C) | | | Salinity (ppt) | | | Electrical Conductivity (mmhos/cm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *P. sajor caju* + *A. brasilense*(5%) | 62, 63, 64 | 72, 71, 72 | 84, 84, 81 | 6.6, 6.4, 6.3 | 6.5, 6.4, 6.3 | 6.3, 6.2, 6.2 | 25, 22, 24 | 27, 26, 26 | 33, 33, 31 | 6, 4, 4 | 4, 4, 3 | 2, 2, 1 | 0.91, 0.91, 0.90 | 0.88, 0.87, 0.88 | 0.76, 0.75, 0.80 |
| Coir pith + *P. sajor caju* + *A. brasilense*(10%) | 64, 60, 61 | 86, 85, 82 | 89, 86, 88 | 6.7, 6.5, 6.5 | 6.6, 6.6, 6.4 | 6.5, 6.4, 6.5 | 26, 25, 22 | 27, 27, 25 | 33, 32, 34 | 6, 6, 5 | 4, 3, 3 | 3, 2, 2 | 0.90, 0.90, 0.90 | 0.84, 0.84, 0.85 | 0.66, 0.66, 0.68 |
| Coir pith + *P. sajor caju* + *A. brasilense*(15%) | 61, 62, 61 | 77, 71, 73 | 94, 92, 91 | 6.8, 6.7, 6.6 | 6.6, 6.2, 6.5 | 6.5, 6.2, 6.4 | 28, 22, 23 | 29, 25, 29 | 34, 33, 32 | 7, 6, 7 | 5, 4, 5 | 1, 1, 1 | 0.91, 0.92, 0.90 | 0.74, 0.74, 0.72 | 0.55, 0.52, 0.50 |
| Coir pith + *P. sajor caju* + *A. brasilense*(20%) | 68, 67, 66 | 87, 87, 81 | 86, 86, 82 | 6.7, 6.6, 6.8 | 6.6, 6.6, 6.7 | 6.4, 6.4, 6.2 | 26, 26, 28 | 31, 32, 30 | 33, 31, 32 | 6, 5, 6 | 6, 5, 5 | 2, 1, 2 | 0.90, 0.92, 0.92 | 0.56, 0.62, 0.59 | 0.34, 0.32, 0.36 |
| Coir pith + *P. sajor caju* + *A. brasilense*(25%) | 64, 64, 61 | 78, 70, 72 | 95, 94, 93 | 6.8, 6.5, 6.7 | 6.4, 6.2, 6.1 | 6.2, 6.2, 6.1 | 27, 21, 26 | 29, 28, 26 | 38, 36, 36 | 5, 5, 5 | 3, 2, 1 | 1, 2, 1 | 0.90, 0.90, 0.91 | 0.53, 0.52, 0.56 | 0.24, 0.21, 0.20 |
| Coir pith + *P. sajor caju* + Urea (Control) | 62, 60, 60 | 77, 76, 71 | 66, 66, 61 | 6.8, 6.8, 6.7 | 6.7, 6.7, 6.7 | 6.8, 6.8, 6.6 | 26, 25, 27 | 29, 29, 28 | 28, 28, 26 | 6, 6, 5 | 4, 3, 3 | 3, 3, 1 | 0.90, 0.91, 0.92 | 0.87, 0.86, 0.82 | 0.78, 0.76, 0.75 |

**Table IV c.** Physical Properties of Biodegraded coir pith with *Pleurotus florida* and *Azotobacter vinelandii.*

| Composting Combinations | Moisture (%) | | | pH | | | Temperature (°C) | | | Salinity (ppt) | | | Electrical Conductivity (mmhos/cm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *P. florida* + *A. vinelandii* (5%) | 72, 71, 72 | 75, 75, 72 | 84, 83, 84 | 6.6, 6.6, 6.5 | 6.5, 6.4, 6.5 | 6.3, 6.2, 6.1 | 25, 22, 24 | 27, 27, 26 | 33, 32, 35 | 6, 7, 6 | 5, 4, 5 | 2, 2, 1 | 0.90, 0.91, 0.90 | 0.88, 0.82, 0.84 | 0.76, 0.75, 0.72 |
| Coir pith + *P. florida* + *A. vinelandii* (10%) | 74, 74, 76 | 86, 84, 82 | 89, 86, 89 | 6.6, 6.5, 6.6 | 6.6, 6.5, 6.6 | 6.3, 6.2, 6.3 | 28, 28, 25 | 27, 26, 28 | 33, 30, 36 | 6, 6, 7 | 4, 3, 4 | 3, 2, 3 | 0.90, 0.89, 0.90 | 0.86, 0.86, 0.82 | 0.66 0.62, 0.61 |
| Coir pith + *P. florida* + *A. vinelandii* (15%) | 71, 69, 68 | 79, 78, 74 | 94, 82, 86 | 6.8, 6.6, 6.7 | 6.5, 6.6, 6.6 | 6.5, 6.4, 6.4 | 28, 26, 20 | 29, 29, 31 | 34, 36, 36 | 5, 6, 7 | 5, 4, 3 | 1, 2, 1 | 0.91, 0.90, 0.91 | 0.74, 0.81, 0.70 | 0.55, 0.50, 0.50 |
| Coir pith + *P. florida* + *A. vinelandii* (20%) | 78, 68, 76 | 83, 82, 83 | 86, 89, 89 | 6.7, 6.6, 6.6 | 6.5, 6.5, 6.4 | 6.4, 6.4, 6.2 | 23, 20, 22 | 31, 30, 24 | 33, 35, 32 | 6, 5, 4 | 6, 5, 4 | 2, 1, 1 | 0.92, 0.91, 0.92 | 0.56, 0.50, 0.52 | 0.37, 0.35, 0.38 |
| Coir pith + *P. florida* + *A. vinelandii* (25%) | 74, 73, 71 | 76, 74, 72 | 95, 94, 92 | 6.8, 6.7, 6.8 | 6.5, 6.5, 6.7 | 6.2, 6.1, 6.1 | 26, 25, 22 | 29, 28, 33 | 38, 30, 36 | 5, 5, 6 | 3, 2, 3 | 1, 2, 2 | 0.90, 0.91, 0.91 | 0.53, 0.52, 0.48 | 0.28, 0.27, 0.21 |
| Coir pith + *P. florida* + Urea (Control) | 62, 70, 66 | 73, 70, 70 | 66, 68, 78 | 6.8, 6.6, 6.7 | 6.7, 6.7, 6.6 | 6.8, 6.8, 6.5 | 26, 25, 22 | 30, 30, 32 | 28, 27, 32 | 6, 7, 6 | 4, 3, 3 | 3, 3, 2 | 0.90, 0.90, 0.91 | 0.88, 0.86, 0.82 | 0.78, 0.80, 0.71 |

**Table IV d.** Physical Properties of Biodegraded coir pith with *Pleurotus florida* and *Azospirillum brasilense*.

| Composting Combinations | Moisture (%) | | | pH | | | Temperature (°C) | | | Salinity (ppt) | | | Electrical Conductivity (mmhos/cm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *P. florida* + *A. brasilense* (5%) | 72, 68, 65 | 75, 74, 71 | 84, 82, 86 | 6.6, 6.9, 6.6 | 6.5, 6.6, 6.4 | 6.3, 6.2, 6.3 | 26, 23, 26 | 27, 26, 28 | 33, 33, 36 | 6, 6, 7 | 4, 5, 4 | 2, 2, 2 | 0.92, 0.90, 0.92 | 0.88, 0.86, 0.84 | 0.76, 0.78, 0.76 |
| Coir pith + *P. florida* + *A. brasilense* (10%) | 73, 70, 72 | 84, 80, 82 | 89, 91, 92 | 6.7, 6.6, 6.9 | 6.6, 6.5, 6.5 | 6.3, 6.1, 6.1 | 28, 25, 25 | 28, 25, 25 | 33, 36, 30 | 5, 6, 6 | 4, 4, 5 | 3, 2, 1 | 0.90, 0.90, 0.91 | 0.85, 0.84, 0.87 | 0.66, 0.65, 0.66 |
| Coir pith + *P. florida* + *A. brasilense* (15%) | 70, 68, 69 | 72, 71, 69 | 95, 90, 96 | 6.8, 6.7, 6.7 | 6.5, 6.4, 6.5 | 6.5, 6.3, 6.4 | 26, 26, 27 | 29, 30, 28 | 34, 34, 36 | 5, 5, 6 | 3, 4, 4 | 1, 1, 1 | 0.91, 0.91, 0.86 | 0.79, 0.80, 0.85 | 0.55, 0.54, 0.50 |
| Coir pith + *P. florida* + *A. brasilense* (20%) | 78, 76, 75 | 84, 81, 81 | 86, 87, 85 | 6.7, 6.9, 7.0 | 6.7, 6.6, 6.7 | 6.4, 6.4, 6.4 | 23, 21, 23 | 32, 30, 31 | 33, 32, 31 | 6, 7, 7 | 5, 3, 5 | 2, 1, 2 | 0.91, 0.87, 0.90 | 0.56, 0.70, 0.61 | 0.37, 0.38, 0.39 |
| Coir pith + *P. florida* + *A. brasilense* (25%) | 74, 70, 70 | 78, 80, 82 | 95, 90, 91 | 6.8, 7.1, 6.9 | 6.5, 6.5, 6.5 | 6.0, 6.2, 6.4 | 23, 22, 21 | 29, 32, 32 | 38, 37, 35 | 5, 5, 5 | 2, 3, 4 | 1, 2, 3 | 0.90, 0.89, 0.89 | 0.52, 0.51, 0.56 | 0.28, 0.29, 0.32 |
| Coir pith + *P. florida* + Urea (Control) | 62, 62, 61 | 73, 71, 78 | 83, 82, 82 | 6.8, 6.9, 6.9 | 6.7, 6.6, 6.7 | 6.8, 6.7, 6.4 | 26, 26, 25 | 30, 29, 28 | 28, 30, 36 | 6, 6, 5 | 3, 2, 2 | 3, 3, 1 | 0.90, 0.90, 0.91 | 0.84, 0.81, 0.82 | 0.78, 0.70, 0.76 |

**Table IV e.** Physical Properties of Biodegraded coir pith with *Pleurotus eous* and *Azotobacter vinelandii*

| Composting Combinations | Moisture (%) | | | pH | | | Temperature (°C) | | | Salinity (ppt) | | | Electrical Conductivity (mmhos/cm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *P. eous* + *A. vinelandii*(5%) | 69, 64, 68 | 73, 70, 74 | 35, 86, 85 | 6.6, 6.8, 6.6 | 6.5, 6.4, 6.3 | 6.3, 6.2, 6.2 | 25, 24, 22 | 27, 26, 28 | 33, 35, 36 | 6, 6, 5 | 4, 6, 4 | 2, 1, 1 | 0.90, 0.91, 0.89 | 0.84, 0.86, 0.86 | 0.78, 0.75, 0.71 |
| Coir pith + *P. eous* + *A. vinelandii*(10%) | 72, 71, 69 | 85, 80, 86 | 38, 87, 86 | 6.6, 6.7, 6.9 | 6.3, 6.3, 6.5 | 6.3, 6.3, 6.1 | 26, 25, 23 | 27, 27, 26 | 33, 31, 32 | 6, 7, 6 | 4, 4, 3 | 3, 2, 2 | 0.90, 0.90, 0.91 | 0.86, 0.81, 0.80 | 0.66, 0.62, 0.61 |
| Coir pith + *P. eous* + *A. vinelandii*(15%) | 73, 68, 67 | 75, 74, 70 | 34, 86, 89 | 6.7, 6.7, 6.7 | 6.5, 6.5, 6.5 | 6.5, 6.4, 6.5 | 28, 26, 27 | 28, 28, 28 | 34, 36, 31 | 5, 5, 5 | 4, 4, 1 | 1, 2, 1 | 0.92, 0.90, 0.91 | 0.74, 0.74, 0.72 | 0.55, 0.52, 0.51 |
| Coir pith + *P. eous* + *A. vinelandii*(20%) | 72, 72, 71 | 83, 80, 86 | 37, 89, 86 | 6.7, 6.8, 6.7 | 6.5, 6.2, 6.3 | 6.4, 6.2, 6.1 | 25, 22, 24 | 31, 30, 30 | 33, 29, 31 | 6, 4, 6 | 3, 2, 2 | 2, 1, 2 | 0.92, 0.92, 0.91 | 0.58, 0.56, 0.55 | 0.37, 0.36, 0.36 |
| Coir pith + *P. eous* + *A. vinelandii*(25%) | 74, 71, 70 | 79, 70, 79 | 95, 91, 92 | 6.8, 6.6, 6.6 | 6.6, 6.4, 6.4 | 6.2, 6.2, 6.2 | 26, 25, 25 | 29, 29, 28 | 37, 36, 35 | 5, 6, 5 | 3, 1, 3 | 0, 1, 0 | 0.90, 0.91, 0.92 | 0.53, 0.53, 0.54 | 0.26, 0.28, 0.29 |
| Coir pith + *P. eous* + Urea (Control) | 62, 64, 68 | 73, 72, 72 | 66, 61, 68 | 6.8, 6.7, 6.7 | 6.7, 6.6, 6.7 | 6.6, 6.6, 6.2 | 26, 26, 27 | 31, 26, 28 | 28, 29, 33 | 6, 6, 6 | 4, 3, 2 | 3, 2, 2 | 0.91, 0.90, 0.91 | 0.88, 0.81, 0.79 | 0.78, 0.79, 0.76 |

**Table IV f.** Physical Properties of Biodegraded coir pith with *Pleurotus eous* and *Azospirillum brasilense*.

| Composting Combinations | Moisture (%) | | | pH | | | Temperature (°C) | | | Salinity (ppt) | | | Electrical Conductivity (mmhos/cm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *P. eous* + *A. brasilense*(5%) | 79, 74, 76 | 73, 73, 72 | 85, 86, 89 | 6.5, 6.6 6.5, | 6.5, 6.4, 6.5 | 6.4, 6.4, 6.5 | 25, 25, 27 | 28, 27, 26 | 33, 30, 31 | 6, 5, 6 | 4, 4, 5 | 2, 1, 2 | 0.91, 0.92, 0.90 | 0.84, 0.83, 0.81 | 0.76, 0.74, 0.75 |
| Coir pith + *P. eous* + *A. brasilense*(10%) | 72, 71, 72 | 84, 84, 83 | 88, 89, 90 | 6.8, 6.5, 6.4 | 6.3, 6.3, 6.4 | 6.3, 6.3, 6.2 | 26, 24, 23 | 27, 26, 24 | 33, 32, 32 | 6, 4, 5 | 3, 3, 4 | 3, 3, 3 | 0.90, 0.91, 0.92 | 0.86, 0.81, 0.80 | 0.66, 0.61, 0.66 |
| Coir pith + *P. eous* + *A. brasilense* (15%) | 78, 76, 75 | 75, 74, 75 | 84, 84, 86 | 6.8, 6.6, 6.6 | 6.5, 6.5, 6.4 | 6.5, 6.4, 6.2 | 28, 26, 24 | 29, 29, 28 | 34, 34, 31 | 5, 5, 6 | 4, 3, 4 | 1, 1, 2 | 0.92, 0.91, 0.89 | 0.75, 0.82, 0.86 | 0.55, 0.54, 0.51 |
| Coir pith + *P. eous* + *A. brasilense*(20%) | 72, 72, 71 | 83, 81, 83 | 88, 88, 86 | 6.7, 6.7, 6.4 | 6.5, 6.6, 6.6 | 6.4, 6.4, 6.5 | 25, 24, 26 | 31, 29, 30 | 33, 31, 30 | 6, 7, 6 | 4, 4, 4 | 2, 2, 3 | 0.92, 0.86, 0.89 | 0.58, 0.56, 0.57 | 0.37, 0.36, 0.34 |
| Coir pith + *P. eous* + *A. brasilense*(25%) | 75, 74, 73 | 76, 75, 74 | 95, 92, 91 | 6.8, 6.6, 6.5 | 6.5, 6.5, 6.3 | 6.2, 6.2, 6.1 | 26, 25, 24 | 27, 28, 28 | 37, 36, 32 | 5, 6, 5 | 4, 3, 2 | 0, 1, 0 | 0.90, 0.91, 0.90 | 0.59, 0.56, 0.57 | 0.26, 0.24, 0.25 |
| Coir pith + *P. eous* + Urea (Control) | 62, 62, 62 | 68, 69, 71 | 66, 68, 69 | 6.8, 6.7, 6.4 | 6.7, 6.3, 6.4 | 6.6, 6.5, 6.4 | 25, 25, 27 | 31, 30, 30 | 28, 27, 26 | 6, 4, 4 | 4, 3, 1 | 3, 2, 1 | 0.91, 0.90, 0.90 | 0.88, 0.81, 0.86 | 0.78, 0.78, 0.76 |

**Table IV g.** Physical Properties of Biodegraded coir pith with *Calocybe indica* and *Azotobacter vinelandii*.

| Composting Combinations | Moisture (%) | | | pH | | | Temperature (°C) | | | Salinity (ppt) | | | Electrical Conductivity (mmhos/cm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *C. indica* + *A. vinelandii*(5%) | 70, 69, 66 | 73, 71, 76 | 85, 84, 81 | 6.5, 6.6, 6.5 | 6.5, 6.4, 6.5 | 6.3, 6.3, 6.2 | 25, 24, 26 | 28, 26, 28 | 33, 31, 32 | 7, 6, 3 | 4, 4, 3 | 2, 2, 1 | 0.90, 0.89, 0.90 | 0.84, 0.81, 0.82 | 0.76, 0.71, 0.75 |
| Coir pith + *C. indica* + *A. vinelandii*(10%) | 72, 71, 73 | 83, 78, 78 | 88, 86, 82 | 6.6, 6.6, 6.6 | 6.6, 6.5, 6.6 | 6.3, 6.3, 6.3 | 26, 25, 25 | 27,26, 24 | 33, 30, 34 | 6, 5, 4 | 4, 5, 4 | 3, 3, 1 | 0.90, 0.91, 0.92 | 0.87, 0.85, 0.81 | 0.66, 0.65, 0.66 |
| Coir pith + *C. indica* + *A. vinelandii*(15%) | 72, 71, 68 | 75, 69, 79 | 84, 84, 85 | 6.7, 6.5, 6.7 | 6.7, 6.7, 6.6 | 6.5, 6.3, 6.1 | 26, 24, 26 | 28, 27, 26 | 34, 30, 31 | 5, 4, 6 | 5, 6, 6 | 1, 3, 2 | 0.92, 0.90, 0.91 | 0.74, 0.73, 0.72 | 0.55, 0.54, 0.53 |
| Coir pith + *C. indica* + *A. vinelandii*(20%) | 72, 69, 65 | 86, 85, 82 | 87, 86, 86 | 6.8, 6.7, 6.8 | 6.5, 6.5, 6.4 | 6.4, 6.1, 6.2 | 25, 24, 24 | 30, 29, 28 | 33, 34, 34 | 6, 6, 6 | 6, 6, 6 | 2, 2, 3 | 0.90, 0.92, 0.91 | 0.58, 0.52, 0.53 | 0.37, 0.31, 0.33 |
| Coir pith + *C. indica* + *A. vinelandii*(25%) | 74, 74, 72 | 75, 74, 71 | 95, 89, 92 | 6.8, 6.6, 6.7 | 6.7, 6.8, 6.8 | 6.2, 6.2, 6.2 | 26, 25, 25 | 25, 24, 24 | 37, 36, 33 | 5, 5, 6 | 3, 2, 2 | 0, 3, 3 | 0.91, 0.89, 0.86 | 0.55, 0.54, 0.53 | 0.24, 0.24, 0.28 |
| Coir pith + *C. indica* + Urea (Control) | 62, 61, 68 | 73, 73, 71 | 65, 64, 61 | 6.8, 6.8, 6.7 | 6.7, 6.7, 6.6 | 6.6, 6.3, 6.4 | 26, 26, 27 | 30, 29, 26 | 28, 29, 30 | 6, 7, 6 | 5, 4, 3 | 3, 2, 1 | 0.91, 0.88, 0.88 | 0.88, 0.87, 0.86 | 0.78, 0.76, 0.73 |

**Table IV h.** Physical Properties of Biodegraded coir pith with *Calocybe indica* and *Azospirillum brasilense*.

| Composting Combinations | Moisture (%) | | | pH | | | Temperature (°C) | | | Salinity (ppt) | | | Electrical Conductivity (mmhos/cm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *C. indica* + *A. brasilense*(5%) | 69, 66, 62 | 73, 72, 71 | 85, 81, 80 | 6.5, 6.4, 6.6 | 6.5, 6.4, 6.5 | 6.3, 6.1, 6.1 | 25, 21, 24 | 29, 30, 31 | 33, 31, 34 | 7, 8, 6 | 4, 4, 3 | 2, 1, 2 | 0.90, 0.91, 0.90 | 0.84, 0.84, 0.85 | 0.76, 0.76, 0.78 |
| Coir pith + *C. indica* + *A. brasilense*(10%) | 72, 71, 72 | 84, 82, 80 | 88, 82, 84 | 6.7, 6.6, 6.8 | 6.6, 6.4, 6.4 | 6.3, 6.3, 6.2 | 26, 25, 27 | 27, 28, 30 | 35, 34, 31 | 6, 6, 2 | 5, 4, 6 | 3, 3, 3 | 0.91, 0.86, 0.85 | 0.87, 0.87, 0.86 | 0.64, 0.63, 0.62 |
| Coir pith + *C. indica* + *A. brasilense*(15%) | 69, 68, 68 | 75, 70, 72 | 84, 84, 80 | 6.7, 6.8, 6.6 | 6.6, 6.5, 6.6 | 6.5, 6.2, 6.5 | 26, 26, 22 | 28, 29, 27 | 34, 33, 32 | 5, 3, 4 | 5, 6, 3 | 1, 1, 1 | 0.92, 0.89, 0.88 | 0.74, 0.74, 0.72 | 0.55, 0.64, 0.61 |
| Coir pith + *C. indica* + *A. brasilense*(20%) | 72, 70, 71 | 85, 78, 80 | 87, 80, 86 | 6.8, 6.9, 6.4 | 6.5, 6.5, 6.4 | 6.4, 6.4, 6.2 | 25, 25, 24 | 34, 30, 33 | 33, 31, 33 | 7, 6, 6 | 6, 6, 6 | 2, 2, 3 | 0.90, 0.88, 0.88 | 0.56, 0.58, 0.56 | 0.37, 0.35, 0.36 |
| Coir pith + *C. indica* + *A. brasilense*(25%) | 71, 69, 66 | 78, 80, 80 | 95, 91, 87 | 6.8, 6.8, 6.8 | 6.6, 6.3, 6.2 | 6.2, 6.3, 6.3 | 26, 25, 24 | 25, 26, 31 | 39, 38, 37 | 5, 5, 4 | 3, 4, 3 | 0, 3, 0 | 0.91, 0.91, 0.92 | 0.55, 0.57, 0.50 | 0.27, 0.26, 0.25 |
| Coir pith + *C. indica* + Urea (Control) | 65, 65, 64 | 73, 71, 76 | 65, 62, 61 | 6.8, 6.7, 6.6 | 6.7, 6.6, 6.5 | 6.6, 6.6, 6.4 | 26, 24, 22 | 32, 30, 29 | 28, 28, 26 | 6, 4, 7 | 4, 2, 2 | 3, 0, 0 | 0.91, 0.92, 0.93 | 0.85, 0.84, 0.83 | 0.78, 0.76, 0.76 |

**Table IV i.** Chemical Properties of Biodegraded coir pith with *Pleurotus sajor caju* and *Azotobacter vinelandii.*

| Composting Combinations | Lignin (%) | | | Organic Carbon (%) | | | Nitrogen (%) | | | Phosphorous (%) | | | Potassium (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *P. sajor caju* + | 32, | 26, 26, | 24, | 6.17, 6.16, | 6.15, 6.14, | 6.14, 6.14, | 0.80, 0.81, | 0.80,0.80, | 0.80, 0.80, | 0.25, 0.25, | 0.23, 0.23, | 0.27, | 0.40, | 0.41, | 0.41, |
| *A. vinelandii*(5%) | 31, 32 | 24 | 23, 25 | 6.17 | 6.15 | 6.13 | 0.80 | 0.80 | 0.80 | 0.24 | 0.24 | 0.27, 0.25 | 0.40, 0.41 | 0.41, 0.41 | 0.40, 0.41 |
| Coir pith + *P. sajor caju* + | 32, | 26, 26, | 22, | 6.17, 6.17, | 6.15, 6.15, | 6.12, 6.13, | 0.80, 0.80, | 0.820.81, | 0.83, 0.82, | 0.25, 0.21, | 0.30, 0.30, | 0.31, | 0.40, | 0.41, | 0.42, |
| *A. vinelandii*(10%) | 31, 32 | 26 | 24, 23 | 6.17 | 6.14 | 6.11 | 0.80 | 0.82 | 0.84 | 0.25 | 0.26 | 0.31, 0.32 | 0.40, 0.40 | 0.40, 0.40 | 0.42, 0.40 |
| Coir pith + *P. sajor caju* + | 32, | 25, 24, | 22, | 6.17, 6.16, | 6.14, 6.13, | 6.11, 6.11, | 0.81, 0.81, | 0.81, 0.81, | 0.82, 0.84, | 0.25, 0.26, | 0.31, 0.30, | 0.32, | 0.41, | 0.42, | 0.42, |
| *A. vinelandii*(15%) | 32, 30 | 26 | 22, 22 | 6.16 | 6.13 | 6.11 | 0.81 | 0.81 | 0.81 | 0.25 | 0.29 | 0.32, 0.34 | 0.41, 0.41 | 0.41, 0.40 | 0.41, 0.42 |
| Coir pith + *P. sajor caju* + | 32, | 25, 25, | 20, | 6.17, 6.16, | 6.12, 6.13, | 6.10, 6.12, | 0.81, 0.81, | 0.86, 0.81, | 0.89, 0.86, | 0.25, 0.26, | 0.33, 0.32, | 0.36, | 0.41, | 0.44, | 0.48, |
| *A. vinelandii*(20%) | 29, 32 | 24 | 20, 21 | 6.16 | 6.13 | 6.10 | 0.82 | 0.85 | 0.85 | 0.26 | 0.33 | 0.36, 0.37 | 0.40, 0.41 | 0.40, 0.41 | 0.46, 0.46 |
| Coir pith + *P. sajor caju* + | 32, | 24, 25, | 18, | 6.17, 6.17, | 6.12, 6.12, | 6.08, 6.10, | 0.81, 0.81, | 0.89, 0.86, | 0.92, 0.89, | 0.25, 0.25, | 0.39, 0.36, | 0.45, | 0.41, | 0.45, | 0.50, |
| *A. vinelandii*(25%) | 30, 29 | 26 | 20, 18 | 6.17 | 6.13 | 6.08 | 0.81 | 0.85 | 0.89 | 0.26 | 0.38 | 0.46, 0.48 | 0.40, 0.42 | 0.420.43 | 0.51, 0.50 |
| Coir pith + *P. sajor caju* + | 32, | 30, 31, | 30, | 6.17, 6.17, | 6.16, 6.15, | 6.16, 6.16, | 0.80, 0.81, | 0.79, 0.76, | 0.80, 0.80, | 0.25, 0.26, | 0.21, 0.21, | 0.23, | 0.41, | 0.41, | 0.40, |
| Urea (Control) | 31, 31 | 30 | 31, 30 | 6.17 | 6.16 | 6.15 | 0.82 | 0.75 | 0.81 | 0.26 | 0.26 | 0.21, 0.21 | 0.41, 0.41 | 0.41, 0.40 | 0.40, 0.41 |

**Table IV j.** Chemical Properties of Biodegraded coir pith with *Pleurotus sajor caju* and *Azospirillum brasilense*.

| Composting Combinations | Lignin (%) | | | Organic Carbon (%) | | | Nitrogen (%) | | | Phosphorous (%) | | | Potassium (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *P. sajor caju* + *A.brasilense*(5%) | 32, 32, 32 | 32, 32, 31 | 30, 31, 30 | 6.17, 6.17, 6.16 | 6.14, 6.14, 6.13 | 6.13, 6.13, 6.12 | 0.80, 0.80, 0.81 | 0.80, 0.80, 0.81 | 0.81, 0.81, 0.81 | 0.25, 0.25, 0.25 | 0.26, 0.25, 0.25 | 0.28, 0.28, 0.27 | 0.39, 0.36, 0.38 | 0.39, 0.36, 0.38 | 0.39, 0.38, 0.39 |
| Coir pith + *P. sajor caju* + *A.brasilense*(10%) | 32, 31, 32 | 30, 32, 30 | 23, 26, 24 | 6.17, 6.16, 6.17 | 6.17, 6.16, 6.17 | 6.15, 6.15, 6.14 | 0.80, 0.81, 0.82 | 0.84, 0.83, 0.82 | 0.84, 0.83, 0.82 | 0.28, 0.26, 0.28 | 0.30, 0.26, 0.27 | 0.31, 0.31, 0.30 | 0.36, 0.36, 0.35 | 0.40, 0.38, 0.39 | 0.42, 0.41, 0.40 |
| Coir pith + *P. sajor caju* + *A.brasilense*(15%) | 32, 31, 30 | 31, 31, 30 | 22, 22, 21 | 6.17, 6.16, 6.15 | 6.14, 6.14, 6.13 | 6.12, 6.12, 6.11 | 0.81, 0.80, 0.81 | 0.83, 0.81, 0.80 | 0.85, 0.85, 0.86 | 0.24, 0.24, 0.21 | 0.25, 0.22, 0.23 | 0.38, 0.35, 0.34 | 0.41, 0.40, 0.39 | 0.42, 0.41, 0.42 | 0.44, 0.45, 0.43 |
| Coir pith + *P. sajor caju* + *A.brasilense*(20%) | 32, 30, 30 | 28, 29, 30 | 20, 20, 21 | 6.17, 6.17, 6.17 | 6.12, 6.12, 6.12 | 6.09, 6.08, 6.08 | 0.81, 0.82, 0.82 | 0.87, 0.84, 0.85 | 0.89, 0.86, 0.88 | 0.26, 0.24, 0.26 | 0.33, 0.31, 0.32 | 0.40, 0.41, 0.41 | 0.41, 0.41, 0.41 | 0.43, 0.43, 0.43 | 0.48, 0.49, 0.48 |
| Coir pith + *P. sajor caju* + *A.brasilense*(25%) | 32, 32, 31 | 24, 21, 24 | 18, 20, 18 | 6.17, 6.16, 6.16 | 6.10, 6.10, 6.11 | 6.08, 6.09, 6.07 | 0.81, 0.80, 0.81 | 0.89, 0.88, 0.88 | 0.94, 0.96, 0.99 | 0.26, 0.25, 0.25 | 0.34, 0.33, 0.34 | 0.45, 0.41, 0.45 | 0.41, 0.42, 0.41 | 0.45, 0.45, 0.44 | 0.54, 0.55, 0.52 |
| Coir pith + *P. sajor caju* + Urea (Control) | 32, 32, 32 | 30, 30, 31 | 30, 31, 31, | 6.17, 6.16, 6.17 | 6.16, 6.16, 6.15 | 6.17, 6.17, 6.16 | 0.80, 0.80, 0.80 | 0.79, 0.76, 0.78 | 0.80, 0.80, 0.81 | 0.25, 0.21, 0.23 | 0.22, 0.21, 0.22 | 0.20, 0.20, 0.21 | 0.41, 0.39, 0.41 | 0.40, 0.41, 0.41 | 0.40, 0.40, 0.41 |

**Table IV k.** Chemical Properties of Biodegraded coir pith with *Pleurotus florida* and *Azotobacter vinelandii.*

| Composting Combinations | Lignin (%) | | | Organic Carbon (%) | | | Nitrogen (%) | | | Phosphorous (%) | | | Potassium (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *P. florida* + *A. vinelandii*(5%) | 32, 32, 32 | 32, 32, 31 | 31, 31, 30 | 6.17, 6.16, 6.17 | 6.13, 6.13, 6.12 | 6.13, 6.12, 6.11 | 0.80, 0.80, 0.81 | 0.80, 0.80, 0.81 | 0.81, 0.81, 0.82 | 0.25, 0.25, 0.25 | 0.26, 0.25, 0.25 | 0.26, 0.28, 0.28 | 0.39, 0.38, 0.39 | 0.39, 0.38, 0.38 | 0.39, 0.38, 0.39 |
| Coir pith + *P. florida* + *A. vinelandii*(10%) | 32, 32, 31 | 30, 31, 30 | 26, 26, 24 | 6.17, 6.17, 6.15 | 6.15, 6.14, 6.15 | 6.15, 6.14, 6.13 | 0.80, 0.80, 0.80 | 0.83, 0.81, 0.82 | 0.83, 0.83, 0.82 | 0.28, 0.25, 0.28 | 0.28, 0.26, 0.27 | 0.31, 0.31, 0.30 | 0.36, 0.35, 0.36 | 0.40, 0.39, 0.40 | 0.41, 0.39, 0.40 |
| Coir pith + *P. sajor caju* + *A. vinelandii*(15%) | 32, 31, 31 | 31, 31, 31 | 30, 30, 30 | 6.17, 6.17, 6.18 | 6.14, 6.14, 6.13 | 6.12, 6.12, 6.12 | 0.81, 0.80, 0.81 | 0.83, 0.83, 0.83 | 0.84, 0.82, 0.83 | 0.24, 0.24, 0.26 | 0.25, 0.25, 0.26 | 0.30, 0.30, 0.32 | 0.41, 0.40, 0.40 | 0.42, 0.42, 0.41 | 0.43, 0.41, 0.41 |
| Coir pith + *P. florida* + *A. vinelandii*(20%) | 32, 30, 32 | 28, 29, 31 | 26, 24, 24 | 6.17, 6.18, 6.17 | 6.11, 6.11, 6.13 | 6.09, 6.08, 0.10 | 0.81, 0.81, 0.81 | 0.87, 0.86, 0.87 | 0.86, 0.87, 0.88 | 0.26, 0.21, 0.25 | 0.30, 0.28, 0.29 | 0.37, 0.36, 0.37 | 0.41, 0.41, 0.41 | 0.43, 0.42, 0.42 | 0.46, 0.43, 0.46 |
| Coir pith + *P. florida* + *A. vinelandii*(25%) | 32, 31, 32 | 24, 24, 23 | 22, 21, 22 | 6.17, 6.16, 6.16 | 6.11, 6.16, 6.14 | 6.08, 6.08, 6.07 | 0.81, 0.80, 0.81 | 0.89, 0.89, 0.89 | 0.89, 0.89, 0.89 | 0.26, 0.22, 0.26 | 0.34, 0.26, 0.28 | 0.35, 0.35, 0.34 | 0.41, 0.41, 0.40 | 0.45, 0.44, 0.45 | 0.48, 0.46, 0.48 |
| Coir pith + *P. florida* + Urea (Control) | 32, 32, 31 | 30, 30, 30 | 31, 31, 31 | 6.17, 6.17, 6.17 | 6.16, 6.16, 6.15 | 6.17, 6.16, 6.15 | 0.80, 0.80, 0.80 | 0.79, 0.79, 0.79 | 0.79, 0.79, 0.79 | 0.25, 0.24, 0.24 | 0.22, 0.21, 0.21 | 0.24, 0.23, 0.24 | 0.41, 0.40, 0.40 | 0.40, 0.40, 0.41 | 0.41, 0.41, 0.40 |

**Table IV I.** Chemical Properties of Biodegraded coir pith with *Pleurotus florida* and *Azospirillum brasilense*.

| Composting Combinations | Lignin (%) | | | Organic Carbon (%) | | | Nitrogen (%) | | | Phosphorous (%) | | | Potassium (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *P. florida* | 32, 31, | 32, 32, | 31, 32, | 6.17, 6.16, | 6.13, 6.13, | 6.13, 6.13, | 0.80, 0.80, | 0.80, 0.80, | 0.81, 0.80, | 0.25, 0.24, | 0.25, 0.25, | 0.26, 0.28, | 0.39, 0.36, | 0.39, 0.38, | 0.39, 0.38, |
| + *A.brasilense* (5%) | 32 | 31 | 31 | 6.17 | 6.14 | 6.12 | 0.81 | 0.80 | 0.81 | 0.25 | 0.26 | 0.29 | 0.38 | 0.38 | 0.39 |
| Coir pith + *P. florida* | 32, 30, | 28, 27, | 26, 26, | 6.17, 6.17, | 6.15, 6.14, | 6.14, 6.12, | 0.80, 0.80 | 0.83, 0.83, | 0.83, 0.82, | 0.26, 0.25, | 0.28, 0.27, | 0.30, 0.30, | 0.36, 0.36, | 0.40, 0.39, | 0.41, 0.40, |
| + *A.brasilense* (10%) | 30 | 27 | 25 | 6.17 | 6.13 | 6.13 | 0.80 | 0.82 | 0.83 | 0.27 | 0.28 | 0.31 | 0.36 | 0.38 | 0.41 |
| Coir pith + *P. florida* | 32, 31, | 32, 31, | 30, 30, | 6.17, 6.16, | 6.14, 6.14, | 6.14, 6.14, | 0.81, 0.81, | 0.83, 0.82, | 0.84, 0.85, | 0.24, 0.24, | 0.25, 0.25, | 0.30, 0.30, | 0.40, 0.37, | 0.42, 0.41, | 0.43, 0.42, |
| + *A.brasilense* (15%) | 32 | 27 | 27 | 6.16 | 6.14 | 6.12 | 0.80 | 0.82 | 0.85 | 0.24 | 0.26 | 0.32 | 0.38 | 0.39 | 0.43 |
| Coir pith + *P. florida* | 32, 30, | 27, 28, | 26, 26, | 6.17, 6.17, | 6.12, 6.12, | 6.10, 6.09, | 0.81, 0.81, | 0.86, 0.87, | 0.86, 0.86, | 0.26, 0.25, | 0.30, 0.31, | 0.35, 0.31, | 0.41, 0.40, | 0.43, 0.41, | 0.46, 0.46, |
| + *A.brasilense* (20%) | 31 | 25 | 25 | 6.17 | 6.11 | 6.10 | 0.80 | 0.85 | 0.86 | 0.25 | 0.31 | 0.34 | 0.40 | 0.39 | 0.47 |
| Coir pith + *P. florida* | 32, 31, | 24, 24, | 25, 21, | 6.17, 6.16, | 6.11, 6.12, | 6.09, 6.07, | 0.81, 0.81 | 0.89, 0.90, | 0.90, 0.91, | 0.26, 0.24, | 0.34, 0.33, | 0.35, 0.35, | 0.41, 0.41, | 0.45, 0.44, | 0.46, 0.47, |
| + *A.brasilense* (25%) | 32 | 24 | 25 | 6.17 | 6.12 | 6.09 | 0.80 | 0.89 | 0.92 | 0.26 | 0.33 | 0.34 | 0.40 | 0.45 | 0.48 |
| Coir pith + *P. florida* | 32, 32, | 32, 30, | 31, 32, | 6.17, 6.17, | 6.16, 6.17, | 6.18, 6.18, | 0.80, 0.80, | 0.79, 0.79, | 0.79, 0.79, | 0.25, 0.25, | 0.32, 0.21, | 0.24, 0.24, | 0.41, 0.40, | 0.40, 0.38, | 0.41, 0.41, |
| + Urea (Control) | 32 | 32 | 32 | 6.17 | 6.17 | 6.19 | 0.80 | 0.78 | 0.78 | 0.25 | 0.22 | 0.23 | 0.40 | 0.39 | 0.40 |

**Table IV. M.** Chemical Properties of Biodegraded coir pith with *Pleurotus eous* and *Azotobacter vinelandii.*

| Composting Combinations | Lignin (%) | | | Organic Carbon (%) | | | Nitrogen (%) | | | Phosphorous (%) | | | Potassium (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *P. eous* + | 32, 31, | 32, 32, | 31, 31, | 7.12, 7.12, | 7.12, 7.12, | 7.13, 7.14, | 0.80, 0.80, | 0.80, 0.81, | 0.81, 0.80, | 0.25, | 0.25, 0.24, | 0.26, 0.25, | 0.39, 0.36, | 0.39, 0.37, | 0.39, 0.36, |
| *A.vinelandii*(5%) | 32 | 31 | 30 | 7.12 | 7.11 | 7.15 | 0.81 | 0.81 | 0.81 | 0.25, 0.25 | 0.25 | 0.25 | 0.36 | 0.38 | 0.38 |
| Coir pith + *P. eous* + | 32, 32, | 27, 28, | 26, 26, | 7.12, 7.11, | 7.15, 7.14, | 7.13, 7.14, | 0.80, 0.80, | 0.83, 0.82, | 0.84, 0.82, | 0.28, | 0.27, 0.26, | 0.30, 0.31, | 0.36, 0.37, | 0.41, 0.40, | 0.41, 0.40, |
| *A.vinelandii*(10%) | 32 | 27 | 26 | 7.12 | 7.15 | 7.13 | 0.80 | 0.80 | 0.83 | 0.27, 0.26 | 0.26 | 0.30 | 0.36 | 0.40 | 0.41 |
| Coir pith + *P. eous* + | 32, 31, | 30, 30, | 30, 31, | 7.12, 7.11, | 7.13, 7.13, | 7.14, 7.14, | 0.81, 0.81, | 0.82, 0.81, | 0.84, 0.83, | 0.24, | 0.25, 0.24, | 0.30, 0.30, | 0.40, 0.40, | 0.40, 0.41, | 0.43, 0.46, |
| *A.vinelandii*(15%) | 31 | 30 | 28 | 7.11 | 7.12 | 7.13 | 0.80 | 0.81 | 0.83 | 0.24, 0.23 | 0.25 | 0.31 | 0.41 | 0.41 | 0.45 |
| Coir pith + *P. eous* + | 32, 32, | 30, 29, | 26, 26, | 7.12, 7.12, | 7.12, 7.12, | 7.11, 7.13, | 0.81, 0.81, | 0.86, 0.82, | 0.87, 0.84, | 0.25, | 0.30, 0.31, | 0.34, 0.33, | 0.41, 0.40, | 0.43, 0.42, | 0.46, 0.45, |
| *A.vinelandii*(20%) | 30 | 28 | 25 | 7.12 | 7.12 | 7.13 | 0.81 | 0.83 | 0.85 | 0.24, 0.25 | 0.30 | 0.34 | 0.41 | 0.41 | 0.45 |
| Coir pith + *P. eous* + | 32, 32, | 28, 29, | 25, 25, | 7.12, 7.12, | 7.10, 7.10, | 7.09, 7.09, | 0.81, 0.80, | 0.89, 0.83, | 0.91, 0.89, | 0.26, | 0.34, 0.30, | 0.35, 0.34, | 0.41, 0.40, | 0.45, 0.44, | 0.47, 0.46, |
| *A.vinelandii*(25%) | 31 | 28 | 25 | 7.12 | 7.10 | 7.11 | 0.81 | 0.86 | 0.89 | 0.25, 0.26 | 0.31 | 0.35 | 0.40 | 0.43 | 0.46 |
| Coir pith + *P. eous* + | 32, 31, | 32, 32, | 31. 30, | 7.12, 7.12, | 7.16, 7.16, | 7.18, 7.18, | 0.80, 0.80, | 0.79, 0.79, | 0.78, 0.78, | 0.25, | 0.20, 0.20, | 0.24, 0.23, | 0.41, 0.41, | 0.40, 0.41, | 0.41, 0.41, |
| Urea (Control) | 31 | 31 | 31 | 7.11 | 7.14 | 7.17 | 0.80 | 0.79 | 0.78 | 0.26, 0.26 | 0.21 | 0.23 | 0.41 | 0.41 | 0.40 |

**Table IV. M.** Chemical Properties of Biodegraded coir pith with *Pleurotus eous* and *Azotobacter vinelandii.*

| Composting Combinations | Lignin (%) | | | Organic Carbon (%) | | | Nitrogen (%) | | | Phosphorous (%) | | | Potassium (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *P. eous* + | 32, 32, | 30, 30, | 31, 31, | 7.12, 7.13, | 7.12, 7.12, | 7.11, 7.10, | 0.80, 0.80, | 0.80, 0.80, | 0.81, 0.80, | 0.25, 0.25, | 0.25, 0.25, | 0.26, 0.26, | 0.39, 0.37, | 0.39, 0.36, | 0.39, 0.39, |
| *A.brasilense*(5%) | 32 | 31 | 30 | 7.12 | 7.11 | 7.12 | 0.80 | 0.80 | 0.81 | 0.24 | 0.24 | 0.25 | 0.39 | 0.39 | 0.39 |
| Coir pith + *P. eous* + | 32, 31, | 29, 29, | 26, 26, | 7.12, 7.12, | 7.14, 7.14, | 7.13, 7.13, | 0.80, 0.81, | 0.83, | 0.83, 0.83, | 0.28, 0.28, | 0.28, 0.28, | 0.30, 0.31, | 0.36, 0.36, | 0.40, 0.41, | 0.41, 0.41, |
| *A.brasilense*(10%) | 32 | 31 | 26 | 7.12 | 7.13 | 7.12 | 0.81 | 0.82, 0.82 | 0.84 | 0.27 | 0.27 | 0.30 | 0.36 | 0.40 | 0.42 |
| Coir pith + *P. eous* + | 32, 32, | 32, 32, | 30, 30, | 7.13, 7.13, | 7.13, 7.13, | 7.14, 7.14, | 0.81, 0.81, | 0.83, | 0.84, 0.84, | 0.24, 0.24, | 0.25, 0.26, | 0.30, 0.30, | 0.40, 0.40, | 0.42, 0.42, | 0.43, 0.43, |
| *A.brasilense*(15%) | 32 | 32 | 29 | 7.11 | 7.13 | 7.12 | 0.80 | 0.83, 0.83 | 0.84 | 0.25 | 0.24 | 0.30 | 0.41 | 0.42 | 0.42 |
| Coir pith + *P. eous* + | 32, 32, | 30, 30, | 25, 25, | 7.12, 7.12, | 7.12, 7.12, | 7.11, 7.11, | 0.81, 0.80, | 0.86, | 0.86, 0.86, | 0.26, 0.25, | 0.30, 0.31, | 0.35, 0.34, | 0.41, 0.40, | 0.43, 0.42, | 0.46, 0.46, |
| *A.brasilense*(20%) | 32 | 32 | 24 | 7.13 | 7.12 | 7.10 | 0.81 | 0.86, 0.87 | 0.86 | 0.26 | 0.30 | 0.34 | 0.39 | 0.43 | 0.46 |
| Coir pith + *P. eous* + | 32, 31, | 25, 22, | 25, 25, | 7.12, 7.12, | 7.12, 7.11, | 7.09, 7.09, | 0.81, 0.81, | 0.89, | 0.90, 0.90, | 0.26, 0.25, | 0.34, 0.33, | 0.35, 0.35, | 0.41, 0.41, | 0.45, 0.44, | 0.46, 0.47, |
| *A.brasilense*(25%) | 32 | 21 | 25 | 7.11 | 7.12 | 7.10 | 0.80 | 0.87, 0.89 | 0.90 | 0.25 | 0.34 | 0.34 | 0.40 | 0.45 | 0.45 |
| Coir pith + *P. eous* + | 32, 31, | 32, 32, | 30, 30, | 7.12, 7.12, | 7.13, 7.13, | 7.12, 7.11, | 0.80, 0.80, | 0.79, | 0.79, 0.78, | 0.25, 0.26, | 0.22, 0.23, | 0.24, 0.23, | 0.41, 0.41, | 0.40, 0.40, | 0.41, 0.40, |
| Urea (Control) | 31 | 32 | 30 | 7.13 | 7.12 | 7.12 | 0.80 | 0.76, 0.76 | 0.79 | 0.26 | 0.24 | 0.24 | 0.41 | 0.40 | 0.41 |

**Table IV o.** Chemical Properties of Biodegraded coir pith with *Calocybe indica* and *Azotobacter vinelandii*.

| Composting Combinations | Lignin (%) | | | Organic Carbon (%) | | | Nitrogen (%) | | | Phosphorous (%) | | | Potassium (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *C. indica* | 32, 32, | 32, 32, | 31, 31, | 7.12, 7.12, | 7.12, 7.12, | 7.13, 7.13, | 0.80, 0.80, | 0.80, 0.80, | 0.82, 0.82, | 0.25, 0.25, | 0.25, 0.25, | 0.25, 0.25, | 0.39, 0.39, | 0.39, 0.39, | 0.39, 0.39, |
| + *A. vinelandii* (5%) | 31 | 31 | 30 | 7.11 | 7.12 | 7.14 | 0.80 | 0.81 | 0.83 | 0.24 | 0.26 | 0.26 | 0.38 | 0.39 | 0.40 |
| Coir pith + *C. indica* | 32, 31, | 27, 27, | 26, 26, | 7.12, 7.12, | 7.15, 7.15, | 7.13, 7.15, | 0.80, 0.80, | 0.82, 0.81, | 0.84, 0.84, | 0.28, 0.28, | 0.27, 0.27, | 0.31, 0.31, | 0.36, 0.35, | 0.41, 0.40, | 0.41, 0.41, |
| + *A. vinelandii* (10%) | 32 | 28 | 26 | 7.12 | 7.14 | 7.14 | 0.80 | 0.83 | 0.85 | 0.29 | 0.28 | 0.31 | 0.36 | 0.41 | 0.42 |
| Coir pith + *C. indica* | 32, 32, | 30, 30, | 30, 30, | 7.12, 7.12, | 7.13, 7.13, | 7.14, 7.15, | 0.81, 0.81, | 0.82, 0.81, | 0.86, 0.86, | 0.24, 0.24, | 0.25, 0.25, | 0.31, 0.30, | 0.40, 0.39, | 0.40, 0.40, | 0.43, 0.43, |
| + *A. vinelandii* (15%) | 32 | 31 | 29 | 7.11 | 7.14 | 7.14 | 0.81 | 0.82 | 0.86 | 0.25 | 0.25 | 0.30 | 0.40 | 0.41 | 0.44 |
| Coir pith + *C. indica* | 32, 32, | 30, 30, | 26, 26, | 7.12, 7.12, | 7.12, 7.12, | 7.11, 7.11, | 0.81, 0.80, | 0.86, 0.86, | 0.87, 0.87, | 0.25, 0.26, | 0.30, 0.30, | 0.33, 0.32, | 0.41, 0.41, | 0.44, 0.43, | 0.45, 0.45, |
| + *A. vinelandii* (20%) | 31 | 31 | 25 | 7.13 | 7.13 | 7.10 | 0.80 | 0.84 | 0.87 | 0.25 | 0.31 | 0.31 | 0.41 | 0.43 | 0.45 |
| Coir pith + *C. indica* | 32, 32, | 28, 29, | 25, 25, | 7.12, 7.12, | 7.10, 7.10, | 7.09, 7.10, | 0.81, 0.80, | 0.89, 0.89, | 0.90, 0.90, | 0.26, 0.25, | 0.34, 0.34, | 0.35, 0.34, | 0.41, 0.40, | 0.45, 0.44, | 0.48, 0.48, |
| + *A. vinelandii* (25%) | 32 | 28 | 24 | 7.12 | 7.11 | 7.09 | 0.81 | 0.89 | 0.89 | 0.26 | 0.31 | 0.31 | 0.41 | 0.46 | 0.47 |
| Coir pith + *C. indica* | 32, 31, | 32, 32, | 31, 31, | 7.12, 7.11, | 7.16, 7.15, | 7.18, 7.18, | 0.80, 0.80, | 0.79, 0.79, | 0.78, 0.78, | 0.25, 0.25, | 0.20, 0.20, | 0.24, 0.24, | 0.40, 0.40, | 0.40, 0.40, | 0.41, 0.40, |
| + Urea (Control) | 31 | 31 | 32 | 7.11 | 7.15 | 7.18 | 0.81 | 0.78 | 0.79 | 0.25 | 0.19 | 0.25 | 0.41 | 0.41 | 0.41 |

**Table IV p.** Chemical Properties of Biodegraded coir pith with *Calocybe indica* and *Azospirillum brasilense.*

| Composting Combinations | Lignin (%) | | | Organic Carbon (%) | | | Nitrogen (%) | | | Phosphorous (%) | | | Potassium (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 |
| Coir pith + *C. indica* + *A.brasilense*(5%) | 32, 32, 31 | 32, 32, 31 | 31, 32, 31 | 7.12, 7.12, 7.11 | 7.12, 7.12, 7.12 | 7.13, 7.13, 7.14 | 0.80, 0.80, 0.81 | 0.80, 0.80, 0.80 | 0.82, 0.82, 0.81 | 0.25, 0.25, 0.24 | 0.24, 0.25, 0.25 | 0.25, 0.25, 0.25 | 0.39, 0.39, 0.38 | 0.38, 0.39, 0.38 | 0.39, 0.39, 0.39 |
| Coir pith + *C. indica* + *A.brasilense*(10%) | 32, 32, 32 | 26, 26, 27 | 26, 26, 24 | 7.12, 7.13, 7.11 | 7.14, 7.14, 7.15 | 7.13, 7.14, 7.15 | 0.80, 0.80, 0.81 | 0.80, 0.81, 0.80 | 0.84, 0.84, 0.85 | 0.28, 0.29, 0.28 | 0.29, 0.30, 0.28 | 0.31, 0.30, 0.31 | 0.36, 0.36, 0.37 | 0.40, 0.38, 0.40 | 0.41, 0.40, 0.41 |
| Coir pith + *C. indica* + *A.brasilense*(15%) | 32, 32, 31 | 32, 32, 31 | 30, 30, 29 | 7.12, 7.13, 7.14 | 7.13, 7.12, 7.11 | 7.12, 7.11, 7.12 | 0.81, 0.80, 0.81 | 0.82, 0.83, 0.81 | 0.84, 0.86, 0.84 | 0.24, 0.24, 0.25 | 0.22, 0.22, 0.21 | 0.31, 0.31, 0.31 | 0.40, 0.41, 0.40 | 0.40, 0.41, 0.39 | 0.43, 0.44, 0.42 |
| Coir pith + *C. indica* + *A.brasilense*(20%) | 32, 33, 31 | 30, 30, 29 | 26, 26, 27 | 7.12, 7.12, 7.12 | 7.12, 7.12, 7.13 | 7.11, 7.10, 7.12 | 0.81, 0.81, 0.81 | 0.85, 0.84, 0.85 | 0.87, 0.87, 0.86 | 0.25, 0.23, 0.25 | 0.31, 0.31, 0.30 | 0.33, 0.32, 0.34 | 0.41, 0.40, 0.40 | 0.43, 0.43, 0.43 | 0.45, 0.45, 0.45 |
| Coir pith + *C. indica* + *A.brasilense*(25%) | 32, 32, 31 | 28, 29, 27 | 24, 24, 25 | 7.12, 7.11, 7.12 | 7.11, 7.10, 7.11 | 7.09, 7.09, 7.11 | 0.81, 0.81, 0.82 | 0.84, 0.84, 0.85 | 0.93, 0.91, 0.90 | 0.26, 0.27, 0.24 | 0.32, 0.32, 0.31 | 0.35, 0.35, 0.34 | 0.41, 0.40, 0.41 | 0.43, 0.43, 0.44 | 0.48, 0.48, 0.49 |
| Coir pith + *C. indica* + Urea (control) | 32, 32, 31 | 32, 32, 31 | 31, 31, 30 | 7.12, 7.11, 7.12 | 7.16, 7.16, 7.16 | 7.18, 7.18, 7.16 | 0.80, 0.80, 0.81 | 0.79, 0.78, 0.77 | 0.78, 0.79, 0.78 | 0.25, 0.25, 0.24 | 0.21, 0.21, 0.20 | 0.24, 0.24, 0.25 | 0.40, 0.40, 0.40 | 0.41, 0.41, 0.43 | 0.41, 0.41, 0.43 |

## Statistics Analysis of Variance

### Moisture

| Source | Sum of Squares | df | Mean Square | F - value | p - value |
|---|---|---|---|---|---|
| Type | 991.509 | 7 | 141.644 | 6.765 | <0.001 |
| Concentration | 6947.667 | 5 | 1389.533 | 66.361 | <0.001 |
| Day | 18046.542 | 2 | 9023.271 | 430.933 | <0.001 |
| Error | 8731.532 | 417 | 20.939 | | |
| Total | 34717.250 | 431 | | | |

$R^2$ ➔ 0.748

### pH

| Source | Sum of Squares | df | Mean Square | F - value | p - value |
|---|---|---|---|---|---|
| **Type** | 0.452 | 7 | 0.065 | 3.894 | <0.001 |
| **Concentration** | 2.159 | 5 | 0.432 | 26.026 | <0.001 |
| **Day** | 8.236 | 2 | 4.118 | 248.220 | <0.001 |
| **Error** | 6.918 | 417 | 0.0.17 | | |
| **Total** | 17.766 | 431 | | | |

$R^2$ ➔ 0.611

### Temperature

| Source | Sum of Squares | df | Mean Square | F - value | p - value |
|---|---|---|---|---|---|
| **Type** | 32.294 | 7 | 4.613 | 0.949 | 0.468 |
| **Concentration** | 184.150 | 5 | 36.830 | 7.576 | <0.001 |
| **Day** | 4391.866 | 2 | 2195.933 | 451.687 | <0.001 |
| **Error** | 2027.299 | 417 | 4.862 | | |
| **Total** | 6635.609 | 431 | | | |

$R^2$ ➔ 0.694

**Salinity**

| Source | Sum of Squares | df | Mean Square | F - value | p - value |
|---|---|---|---|---|---|
| **Type** | 12.887 | 7 | 1.841 | 2.087 | 0.044 |
| **Concentration** | 60.789 | 5 | 12.158 | 13.781 | <0.001 |
| **Day** | 1024.505 | 2 | 512.252 | 580.661 | <0.001 |
| **Error** | 367.873 | 417 | 0.882 | | |
| **Total** | 1466.053 | 431 | | | |

$R^2$ ➔ 0.749

**Electrical Conductivity**

| Source | Sum of Squares | df | Mean Square | F - value | p - value |
|---|---|---|---|---|---|
| **Type** | 0.021 | 7 | 0.003 | 0.361 | 0.924 |
| **Concentration** | 4.889 | 5 | 0.978 | 118.983 | <0.001 |
| **Day** | 9.153 | 2 | 4.577 | 556.918 | <0.001 |
| **Error** | 3.427 | 417 | 0.008 | | |
| **Total** | 17.490 | 431 | | | |

$R^2$ ➔ 0.804

**Lignin**

| Source | Sum of Squares | df | Mean Square | F - value | p - value |
|---|---|---|---|---|---|
| **Type** | 429.917 | 7 | 61.417 | 15.544 | <0.001 |
| **Concentration** | 1141.546 | 5 | 228.309 | 57.783 | <0.001 |
| **Day** | 1679.116 | 2 | 839.558 | 212.484 | <0.001 |
| **Error** | 1647.634 | 417 | 3.951 | | |
| **Total** | 4898.213 | 431 | | | |

$R^2$ ➔ 0.664

**Organic Carbon**

| Source | Sum of Squares | df | Mean Square | F - value | p - value |
|---|---|---|---|---|---|
| **Type** | 104.422 | 7 | 14.917 | 43894.028 | <0.001 |
| **Concentration** | 0.068 | 5 | 0.014 | 39.893 | <0.001 |
| **Day** | 0.027 | 2 | 0.013 | 39.456 | <0.001 |
| **Error** | 0.142 | 417 | 0.000 | | |
| **Total** | 104.658 | 431 | | | |

$R^2$ ➔ 0.999

**Nitrogen**

| Source | Sum of Squares | df | Mean Square | F - value | p - value |
|---|---|---|---|---|---|
| **Type** | 0.004 | 7 | 0.001 | 1.600 | 0.134 |
| **Concentration** | 0.247 | 5 | 0.049 | 132.123 | <0.001 |
| **Day** | 0.093 | 2 | 0.047 | 124.651 | <0.001 |
| **Error** | 0.156 | 417 | 0.000 | | |
| **Total** | 0.500 | 431 | | | |

$R^2$ ➔ 0.688

**Phosphorous**

| Source | Sum of Squares | df | Mean Square | F - value | p - value |
|---|---|---|---|---|---|
| **Type** | 0.017 | 7 | 0.002 | 3.155 | 0.003 |
| **Concentration** | 0.371 | 5 | 0.074 | 96.180 | <0.001 |
| **Day** | 0.213 | 2 | 0.107 | 138.052 | <0.001 |
| **Error** | 0.322 | 417 | 0.001 | | |
| **Total** | 0.923 | 431 | | | |

$R^2$ ➔ 0.651

**Potassium**

| Source | Sum of Squares | df | Mean Square | F - value | p - value |
|---|---|---|---|---|---|
| **Type** | 0.005 | 7 | 0.001 | 3.108 | 0.003 |
| **Concentration** | 0.183 | 5 | 0.037 | 146.381 | <0.001 |
| **Day** | 0.087 | 2 | 0.044 | 174.368 | <0.001 |
| **Error** | 0.104 | 417 | 0.000 | | |
| **Total** | 0.380 | 431 | | | |

$R^2$ ➔ 0.726

## 4. Discussion

Moisture, pH, temperature, salinity and electrical conductivity of raw and composted coir pith with combination or fungi and nitrogen fixing bacteria were estimated and the result were tabulated in table III a to III h. There was a definite variation in all the cases while composting. Moisture was observed to be fluctuate

in the sample treated with different combinations of the fungus. Temperature increased trend as composting proceeds. pH showed slightly decreasing values due to leaching out of phenolic acids during composting. Salinity and Electrical conductivity also show a decreasing trend during composting.

Table III i to III p presents the results of the treatments on the decomposition of lignin in coir pith. When coir pith was treated with *P.sajor caju, P. florida,* Pleurotus eous and Calocybe indica in addition to nitrogen fixing bacteria (*Azotobacter vinelandii* and *Azospirillum brasilense*), it was observed that *P.sajor caju* degrades lignin more effectively (18%) than other fungal species. When the chemical fertilizer, urea was added as an additional component along with four fungal species, the results revealed that the decomposition process was better in coir pith treated with *P.sajor caju*. As the decrease in lignin content observed in coir pith treated with *P.florida*, difference noticed was marginal i.e. from 32 to 31 %, indicated that it was not so effective in degrading the lignin. As an alternate to chemical fertilizer, when the bacterial strains were added, the results showed an encouraging trend. When *Azotobacter* was supplemented to *P.sajor caju* and added to coir pith, the bacterial strain was observed to degrade the lignin more effectively. In the case of *Azotobacter* supplemented with *P. florida*, the degradation was 24 % which was better than the degradation with Urea. As *Azotobacter* was added with *P. sajor caju*, the degradation of lignin was 18 % and it was 24% when *P. florida* and *Azotobacter* combination was tried. The *Azospirillum* + *P.sajor caju* + coir pith combination revealed that the lignin degradation was 18 % and the *Azospirillum* + *P. florida* + coir pith combination showed 25 % degradation. This confirms the fact that the bacterial strains are more effective in lignin degradation than chemical fertilizers, *viz.* urea. The other two fungal species *viz. Pleurotus eous* and *Calocybe indica* also show their efficiency in lignin degradation in combination with nitrogen fixing bacteria. The increase of organic carbon, enhancement of nitrogen, phosphorus and potassium as a function of different treatments (coir pith, mushroom species, urea and bacterial strains) are

detailed in Table III i to III p. The organic carbon content showed a decreasing trend, however the rate of decrease was more in bacterial strains treated samples 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 0.92 % and 0.94% when treated with *Pleurotus sajor caju* in combination with *Azotobacter vinelandii* and *Azospirillum brasilense* respectively and minimum (0.80%) when treated with both bacteria and urea. The phosphorus and potassium content was observed to be maximum in samples treated with a combination of organisms while it was minimum in urea.

Organic carbon and NPK content showed a declining trend in samples treated with a combination of the spawn of different fungal species, urea and bacterial strains, however treatment with the bacterial strains led to the degradation of the organic content faster. Among the two bacterial strains, *Azospirillum brasilense* was found to be more effective in enhancing nitrogen and phosphorus content in coir pith while *Azotobacter vinelandii* treatment led to enhancing the potassium content. The Nitrogen, Phosphorous and Potassium content in Azospirillum treated coir pith was greater than that of treated with urea. Maximum value of potassium was discernible in *Azotobacter* while it was minimum in urea. Coir pith has to be degraded so that it can be used as good base material for the growth of several crops and several trials have been worked out to degrade the coir pith lignin using a combination of mushroom and bacterial species which led to encouraging results. The white rot fungi belonging to the Basidiomycetes have been reported to be widely used to degrade lignin (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 which has direct application in lignocellulose bioconversion processes. The present study could confirm that when mushroom species have been used to decompose coir pith,

considerable quantity of lignin is degraded. The present study reports a reduction of lignin by about 30.1%. Among the four mushroom species tested, *Calocybe indica* was observed 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 meagre quantities. The reason for the increased activity of *C. indica* in lignin degradation may be attributed to the production of lignolyte. It has been reported that mushroom by enzymatic action degrade only the lignin content and leaving the other components intact (Arora *et al.,* 2002). However in the present study, it could be confirmed 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 findings in the present work confirms the findings that, the depolymerization of the lignin causes the reduction in their percentage from32% 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) which states that, 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 increased weight loss and reduction in the lignin content. 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 content. Venkitaswamy (2003) reported maximum yields of coconuts when coir pith compost was added with trace amounts of chemical fertilizers (NPK). But the

present study on composting using Nitrogen fixing bacteria, cause the enhancement of NPK content within the coir pith compost itself by their activity leading to the conversion into coir pith 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 along with mushroom species led to increased lignin degradation, revealing the fact that combination of bacterial and fungal strains have the capability to degrade lignin to a greater extent. Thus the present study confirms that individual mushroom species or mushroom species in combination with Nitrogen fixing bacteria not only influence lignin degradation but also enhance the NPK content in the biodegraded coir pith which forms an ideal organic manure.

## 5. Conclusion

Conventional composting of coir pith involves use of urea as a nitrogen source. Substituting urea with nitrogen fixing bacteria would lead to making the composting process more ecofriendly. IN present study, the addition of nitrogen fixing bacterial cultures like Azotobacter vinelandii and Azospirillum brasilense in the composting of coir pith by fungal species viz. *Pleurotus sajor caju, Pleurotus florida, Pleurotus eous* and *Calocybe indica* could enhance the biodegradation of lignin in coir pith and increase the nitrogen, phosphorous and potassium content. The nutrient status of the biodegraded coir pith was improved considerably. Among the lignin degrading fungi studied, it was observed that the potency of Pleurotus sajor caju to degrade the lignin in coir pith was higher in comparison to Pleurotus florida, Pleurotus eous and Calocybe indica. The biodegraded coir pith organic manure had the nutrient status required for plant growth and could be applied as a soil additive in agriculture/horticulture. This confirms the fact that the

biological waste coir pith could be converted into an ecofriendly value added product for economic benefit.

Chapter 5

# EFFICACY OF COMBINED ACTION OF *PLEUROTUS SAJOR CAJU* AND NITROGEN FIXING ORGANISMS (*AZOTOBACTER VINELANDII AND AZOSPIRILLUM BRASILENSE*) FOR THE BIODEGRADATION OF COIR PITH

Contents



## 1. Methodology

### 1.1 Sample Collection

Coir pith was collected from the coir industries in Kattukada, Alappuzha district. Fungal species were procured from Central Coir Research Institute (CCRI), Kalavoor, Alappuzha. Bacteria (*Azotobacter vinelandii*, MTCC No. 124 and *Azospirillum brasilense*, MTCC No. 125) were procured from Microbial Type Culture Collection (MTCC), Chandigarh. The composting process conducted in Rajiv Gandhi Chair in Contemporary Studies, School of Environmental Studies, Cochin University of Science and Technology (CUSAT). The biochemical estimations were carried out at Central Coir Research Institute, Alappuzha and Rajiv Gandhi Chair.

### 1.2 Composting.

The experiments consisted of 4 lots mounted in 5 kg coir pith heaps in triplicate. The first lot consisted of raw coir pith, the second was supplemented with *Pleurotus sajor caju* alone, and the third lot with both fungus (*Pleurotus sajor caju*) and bacteria (*Azotobacter vinelandii*). The fourth lot was supplemented

with *Pleurotus sajor caju* and *Azospirillum brasilense*. The coir pith heaps were moistened daily and monitored regularly for 30 day. A control composting experiment using the conventional method was also carried out simultaneously.

## 1.3 Estimation of Chemical Properties.

### 1.3.1 Lignin.

The lignin in coir pith was determined by Klason lignin method (Stephen and Carlton, 1992).

### 1.3.2 Nitrogen.

Nitrogen in the coir pith samples were estimate by Alkaline permanganate method using Kjeldahl distillation Unit (Vogel, 1961).

### 1.3.3 Phosphorus.

Phosphorous was estimated by Vanado Molybdo phosphoric yellow colour method.

### 1.3.4 Potassium.

Potassium was estimated by Flame photometry.

### 1.3.5 Organic Carbon.

Organic carbom was estimated by Walkey and Black method

### 1.3.6 Ammonia.

Estimation of Ammonia in coir pith sample is done by Nesslerization method.

## 1.4 Estimation of Enzyme Activity.

### 1.4.1 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.

### 1.4.2 Manganese Peroxidase.

Manganese peroxidase activity estimated by prepared 50 mM sodium tartarate buffer (pH-4.5), added MnSO4, H2O2 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.

## 1.5 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 (SEM JEOL JSM 6380 LV).

## 1.6 Estimation of Phenolic Compounds Formation by High Performance Liquid Chromatography (HPLC).

HPLC analysis was carried out with Shimadzu LC-8A liquid Chromatograph. The LC-8A has been designed to offer simple scale-up from nanogram to gram quantities. The flow rate set to 0.6 mL/ min. Photo Diode Array Detector (PDA) and Luna 5 micron C-18 column (Length 250×4.6 mm) were used for the analysis. Running time was for 30 minutes and the injection volume was 20 micro liters.

# 2 Result

## 2.1 Estimation of Chemical Properties.

The biodegraded coir pith using *Pleurotus sajor caju* in combination with *Azotobacter vinelandii* and *Azospirillum brasilense* causes reduction in Lignin,

Organic carbon and enhancement in the Nitrogen, Phosphorous and Potassium (NPK) content. The details of the chemical parameters given below.

**2.1.1 Lignin.**

The investigations reveal that definite variations were observed in lignin content in the coir pith under various treatments with ligninolytic mushroom *Pleurotus sajor caju* in combination with *Azotobacter vinelandii* and *Azospirillum brasilense.* 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 sajor caju* and *Azotobacter vinelandii* the maximum lignin reduction (17%) followed by the combination of *Pleurotus sajor caju* and *Azospirillum brasilense*. The details are given in table V c.

**2.1.2 Nitrogen.**

The investigations reveal that the treatment of coir pith with different mushroom species show compatible and novel changes in the case of 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% and 0.79% for *Pleurotus sajor caju & Azotobacter vinelandii and Pleurotus sajor caju & Azospirillum brasilense* respectively. From the results, it is clear that all the treatments lead to the enhancement of Nitrogen.

**2.1.3 Phosphorous.**

The Phosphorous content in coir pith also show enhancement with the treatment of all the four mushroom species. 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% & 2.78% respectively for *Pleurotus sajor caju & Azotobacter vinelandii and Pleurotus sajor caju & Azospirillum brasilense.* The details of the amount of Phosphorous given in table V a.

### 2.1.4 Potassium.

The values of Potassium in the biodegraded coir pith with the treatment with combination of mushroom and bacterial species were given in table V a. 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 all the mushrooms 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 0.38% in Biodegraded Coir pith Compost (BCC) using *Pleurotus sajor caju* & *Azospirillum brasilense* and 0.36% with *Pleurotus sajor caju* and *Azotobacter vinelandii*. Coir pith samples drawn out from the experimental heaps at regular intervals indicated increase in the potassium content.

### 2.1.5 Organic Carbon.

The Organic Content (OC) value for the raw and Biodegraded coir pith Compost (BCC) is given in table V b. The use of mushroom species with nitrogen fixing bacteria for the decomposition was found to be effective for the reduction of OC content from 7.34% (raw coir pith) to 6.11% and 4.36% respectively for *Pleurotus sajor caju* with *Azotobacter vinelandii* and *Azozpirillum brasilense*. The results indicated a decreasing trend in the carbon content in coir pith under the treatment with different combinations. The carbon content of the raw coir pith (untreated) kept as control and did not show any variation.

### 2.1.6 Ammonia.

Ammonia content of the compost was observed to be increased due to nitrogen fixation activity of Nitrogen fixing bacteria incorporated with *Pleurotus sajor caju*. 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 sajor caju* & *Azotobacter vinelandii* and 2991.12 mg/kg with a combination of *Pleurotus sajor caju* & *Azospirillum brasilense*. The details have been given in table V b.

## 2.2 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) (Hatakka, 1994; 2001). The enzymes produced by white rot fungi catalyze the initial polymerization of lignin are extracellular and unusually non specific (Cullen and Kersten, 2004). 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 my 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.

### 2.2.1 Lignin Peroxidase.

The enzyme assay was conducted for the enzyme produced by *Pleurotus sajor caju*. 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 Table Vd.

### 2.2.2 Manganese Peroxidase.

Manganese peroxidase assay is based on the oxidation of phenol red during coir pith degradation (Tien *et al*., 1984). The principle function of manganese peroxidase (MnP) is to oxidize Mn2+ to Mn3+, using H2O2 as oxidant (Pudelski, 1987). Activity of the enzyme is stimulated by simple organic acids which stabilize the Mn3+, thus producing diffusible oxidizing chelates. Table Ve 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.

## 2.3 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 Fig V l to V s. 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.

## 2.4 Phenolic Compound Analysis.

Study on the presence of phenolic compounds in the coir pith was carried out using HPLC studies on their phenolic compounds such as Catechol, Gallic acid, Picric acid and Pyrogallic acid. From the peaks obtained from the HPLC analysis, it is clear that all the phenolic compounds present in different treatment samples and their presence could be confirmed. Details have been furnished in Fig. Vd to Vk.

**Table V a.** Variation pattern of NPK (%) in Raw and Biodegraded coir pith

| Sl No | Composting Days | Nitrogen | | | | Phosphorous | | | | Potassium | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CP | CP+*PS* | CP+*PS*+*A.v* | CP+*PS*+*A.b* | CP | CP+*PS* | CP+*PS*+*A.v* | CP+*PS*+*A.b* | CP | CP+*PS* | CP+*PS*+*A.v* | CP+*PS*+*A.b* |
| 1 | 0 | 0.19 | 0.19 | 0.19 | 0.19 | 0.85 | 0.85 | 0.85 | 0.85 | 0.04 | 0.04 | 0.04 | 0.04 |
| 2 | 05 | 0.19 | 0.20 | 0.21 | 0.28 | 0.85 | 0.86 | 1.20 | 0.86 | 0.04 | 0.11 | 0.11 | 0.12 |
| 3 | 10 | 0.19 | 0.21 | 0.29 | 0.31 | 0.85 | 0.90 | 1.48 | 1.40 | 0.04 | 0.13 | 0.14 | 0.16 |
| 4 | 15 | 0.19 | 0.23 | 0.48 | 0.46 | 0.85 | 1.10 | 1.56 | 1.86 | 0.04 | 0.16 | 0.18 | 0.19 |
| 5 | 20 | 0.19 | 0.35 | 0.70 | 0.68 | 0.85 | 1.12 | 1.78 | 2.12 | 0.04 | 0.19 | 0.22 | 0.23 |
| 6 | 25 | 0.19 | 0.51 | 0.72 | 0.75 | 0.85 | 1.15 | 1.85 | 2.76 | 0.04 | 0.26 | 0.30 | 0.36 |
| 7 | 30 | 0.19 | 0.53 | 0.78 | 0.79 | 0.85 | 1.17 | 1.87 | 2.78 | 0.04 | 0.28 | 0.36 | 0.38 |

**CP**= Coir pith, ***PS***= *Pleurotus sajor caju*, ***A.v*** = *Azotobacter vinelandii*, ***A.b*** = *Azospirillum brasilense*

**Table V b.** Variation pattern of Organic carbon & Ammonia in Raw and Biodegraded coir pith

| Sl No | Composting Days | Organic Carbon (%) | | | | Ammonia (mg/kg) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CP | CP+*PS* | CP+*PS*+*A.v* | CP+*PS*+*A.b* | CP | CP+*PS* | CP+*PS*+*A.v* | CP+*PS*+*A.b* |
| 1 | 0 | 7.34 | 7.34 | 7.34 | 7.34 | 1261.16 | 1261.16 | 1261.16 | 1261.16 |
| 2 | 05 | 7.34 | 7.30 | 7.25 | 6.51 | 1261.16 | 1268.81 | 1321.18 | 1412.61 |
| 3 | 10 | 7.34 | 7.25 | 6.84 | 6.01 | 1261.16 | 1336.62 | 1516.12 | 1628.11 |
| 4 | 15 | 7.34 | 7.11 | 6.76 | 5.14 | 1261.16 | 1432.16 | 2022.72 | 2028.85 |
| 5 | 20 | 7.34 | 6.86 | 6.62 | 5.11 | 1261.16 | 1662.15 | 2416.19 | 2421.72 |
| 6 | 25 | 7.34 | 6.84 | 6.45 | 4.48 | 1261.16 | 2001.68 | 2810.10 | 2762.13 |
| 7 | 30 | 7.34 | 6.80 | 6.11 | 4.36 | 1261.16 | 2010.10 | 3016.27 | 2991.12 |

**CP**= Coir pith, ***PS***= *Pleurotus sajor caju*, ***A.v*** = *Azotobacter vinelandii*, ***A.b*** = *Azospirillum brasilense*

**Table V c.** Variation pattern of Lignin (%) in Raw and BCC.

| Sl No | Composting Days | Lignin | | | |
|---|---|---|---|---|---|
| | | CP | CP+*PS* | CP+*PS*+A.v | CP+*PS*+A.b |
| 1 | 0 | 32 | 32 | 32 | 32 |
| 2 | 5 | 32 | 30 | 30 | 30 |
| 3 | 10 | 32 | 30 | 26 | 26 |
| 4 | 15 | 32 | 28 | 22 | 21 |
| 5 | 20 | 32 | 26 | 21 | 20 |
| 6 | 25 | 32 | 25 | 18 | 18 |
| 7 | 30 | 32 | 23 | 17 | 18 |

**CP**= Coir pith, ***PS***= *Pleurotus sajor caju*, ***A.v*** = *Azotobacter vinelandii*, ***A.b*** = *Azospirillum brasilense*

**Table V d.** Activity of Lignin Peroxidase (U $ml^{-1}$) in BCC

| Sl No | Composting Days | CP+*PS* | CP+*PS*+*A.v* | CP+*PS*+*A.b* |
|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 |
| 2 | 05 | 0.4 | 0.8 | 0.7 |
| 3 | 10 | 2.8 | 3.1 | 2.8 |
| 4 | 15 | 5.1 | 7.2 | 5.1 |
| 5 | 20 | 8.6 | 11.8 | 10.7 |
| 6 | 25 | 10.2 | 12.5 | 13.1 |
| 7 | 30 | 15.9 | 18.6 | 16.8 |

**CP**= Coir pith, ***PS***= *Pleurotus sajor caju*, ***A.v*** = *Azotobacter vinelandii*, ***A.b*** = *Azospirillum brasilense.*

**Table V e.** Activity of Manganese Peroxidase (U $ml^{-1}$) in BCC

| Sl No | Composting Days | CP+*PS* | CP+*PS*+*A.v* | CP+*PS*+*A.b* |
|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 |
| 2 | 05 | 0.2 | 0.5 | 0.6 |
| 3 | 10 | 2.5 | 4.1 | 4.5 |
| 4 | 15 | 4.1 | 6.8 | 7.1 |
| 5 | 20 | 6.8 | 10.2 | 12.4 |
| 6 | 25 | 11.2 | 15.9 | 15.8 |
| 7 | 30 | 14.4 | 18.8 | 18.1 |

**CP**= Coir pith, ***PS***= *Pleurotus sajor caju*, ***A.v*** = *Azotobacter vinelandii*, ***A.b*** = *Azospirillum brasilense.*

## GRAPHS

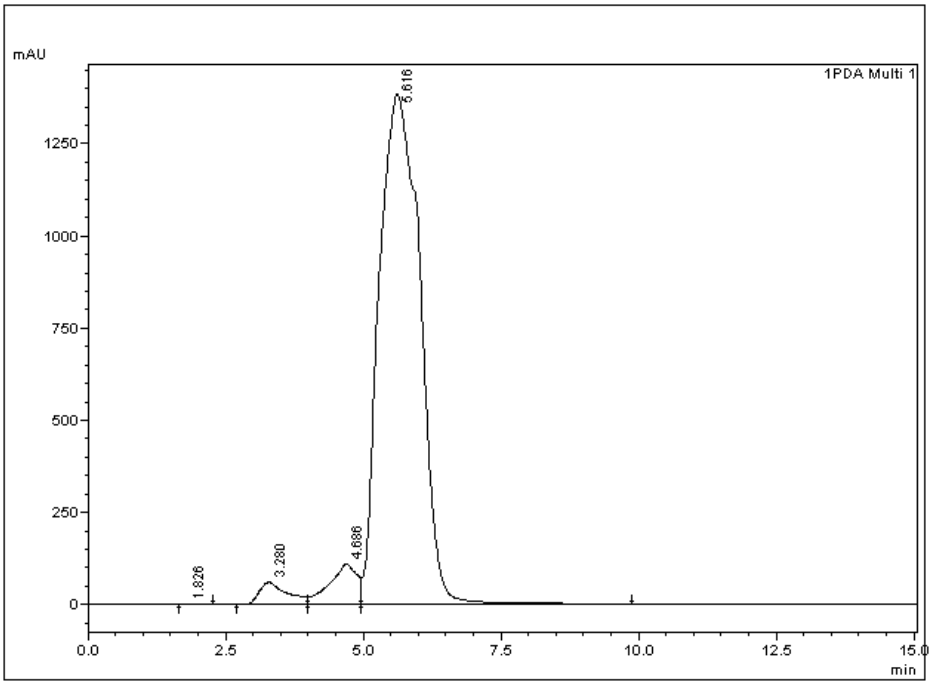



**Fig Vd.** HPLC Graph of Standards (Catechol).

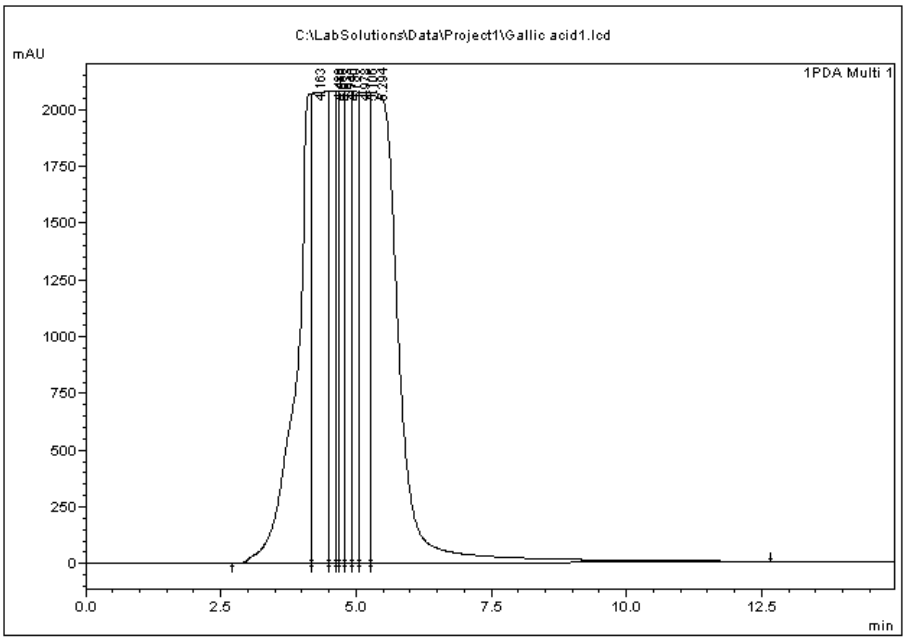



**Fig Ve.** HPLC Graph of Standards (Gallic acid).

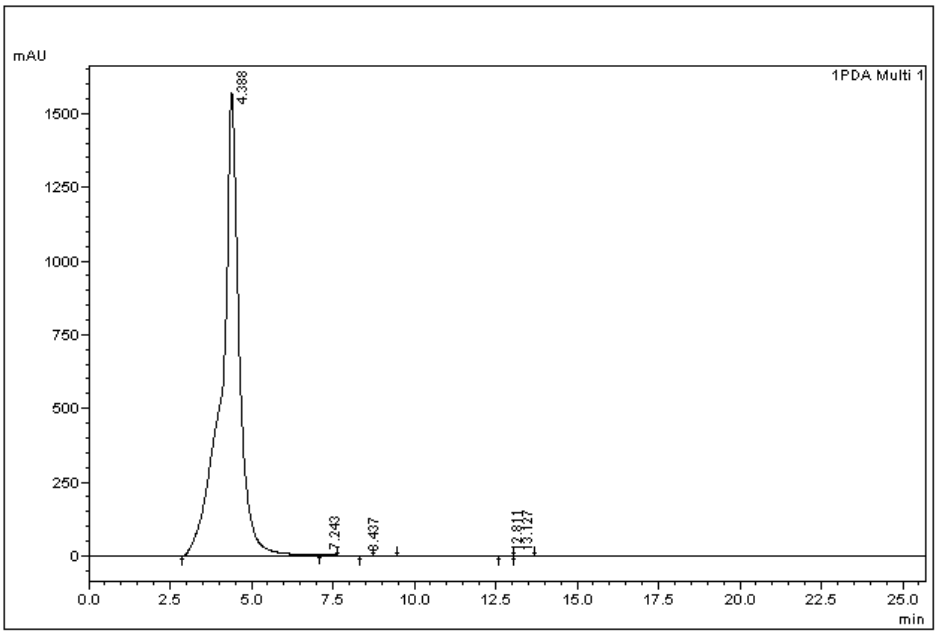



**Fig V f.** HPLC Graph of Standards (Picric acid).

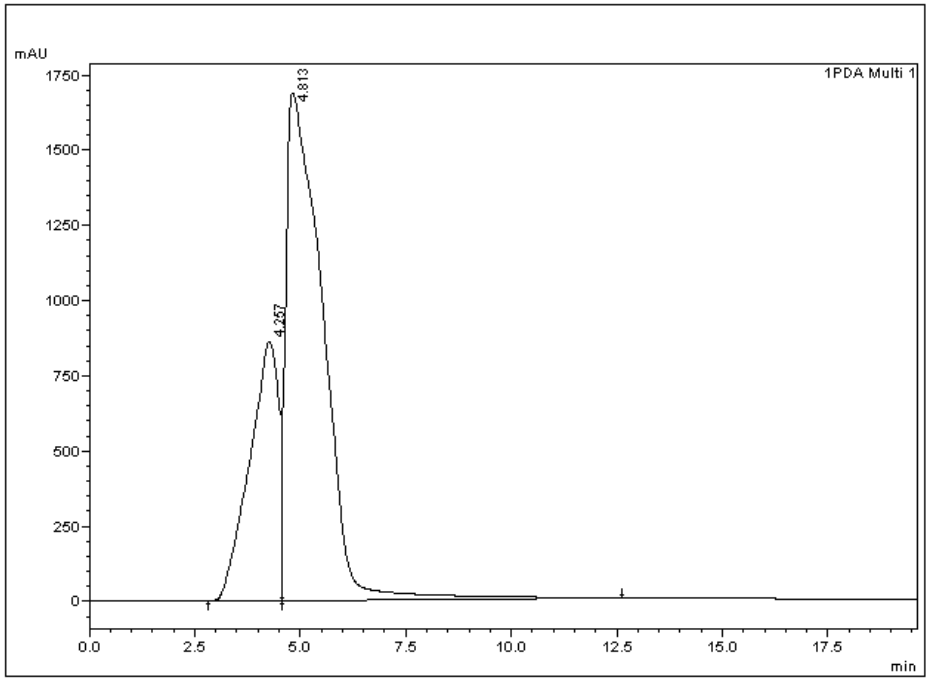



**Fig V g.** HPLC Graph of Standards (Pyrogallic acid).

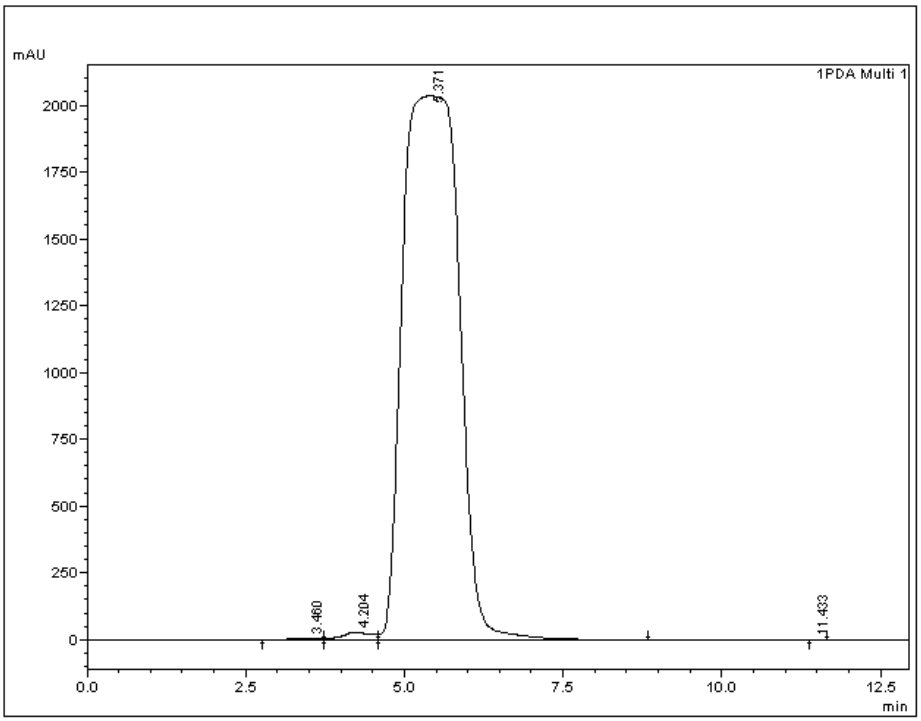



**Fig. V h.** HPLC Graph of Standards (Resorcinol).

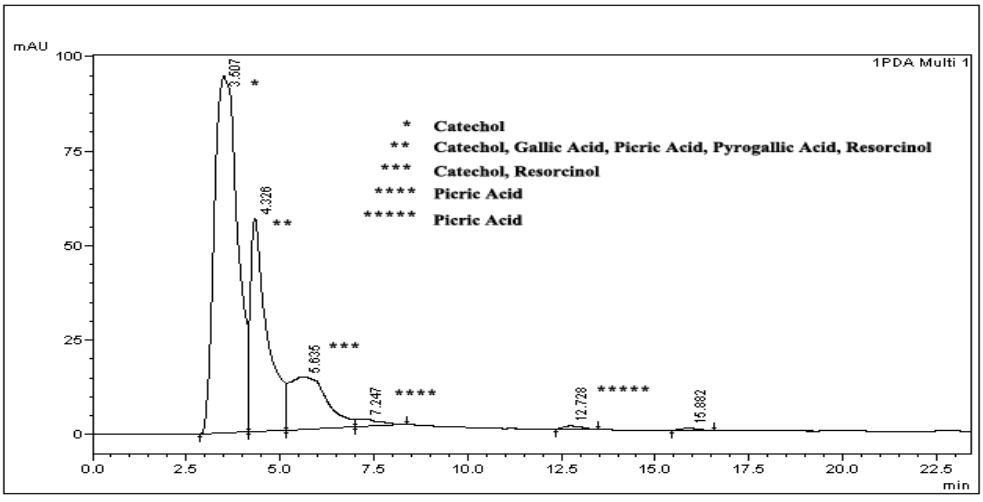



**Fig. V i.** HPLC Graph BCC with *Pleurotus sajor caju*

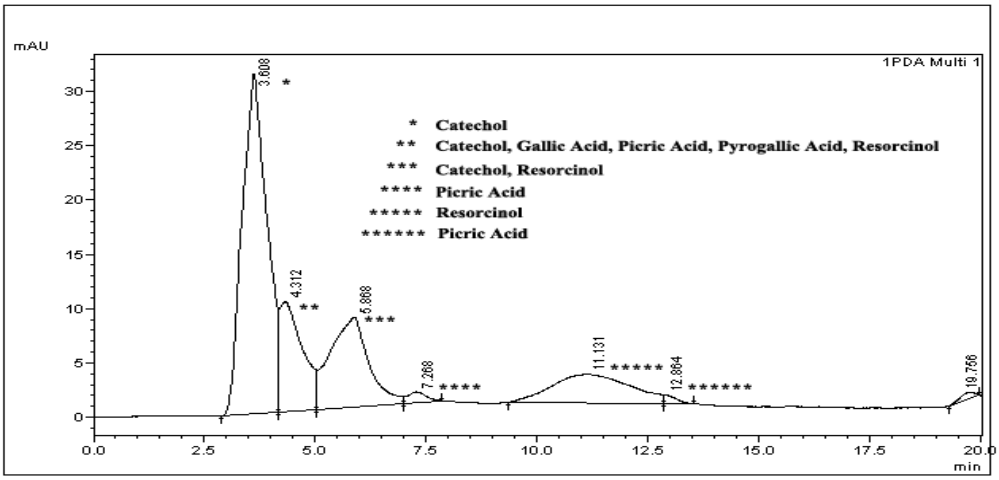



**Fig. V j.** HPLC Graph BCC with *Pleurotus sajor caju* and *A. vinelandii*

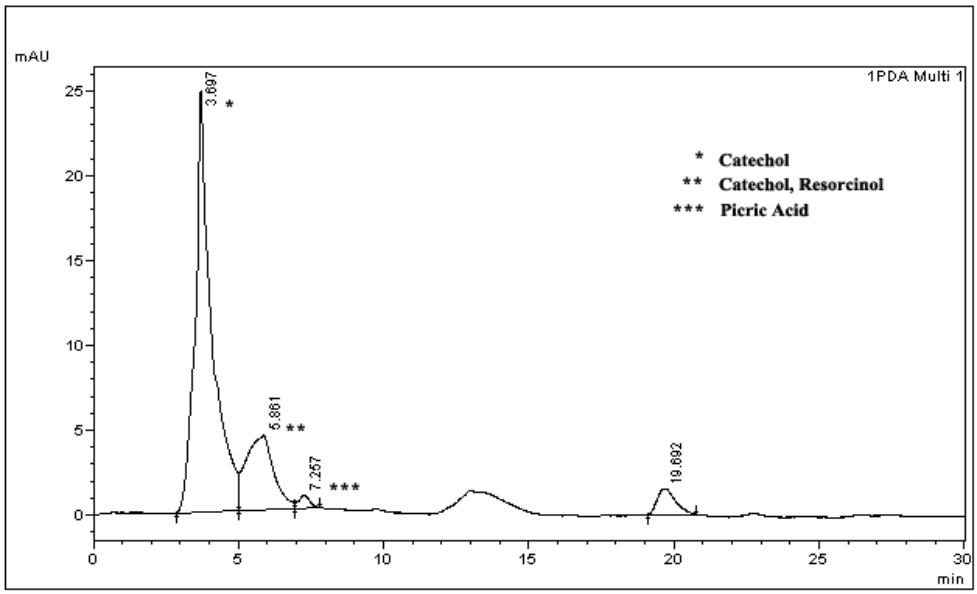



**Fig. V k.** HPLC Graph BCC with *Pleurotus sajor caju* and *A. brasilense*

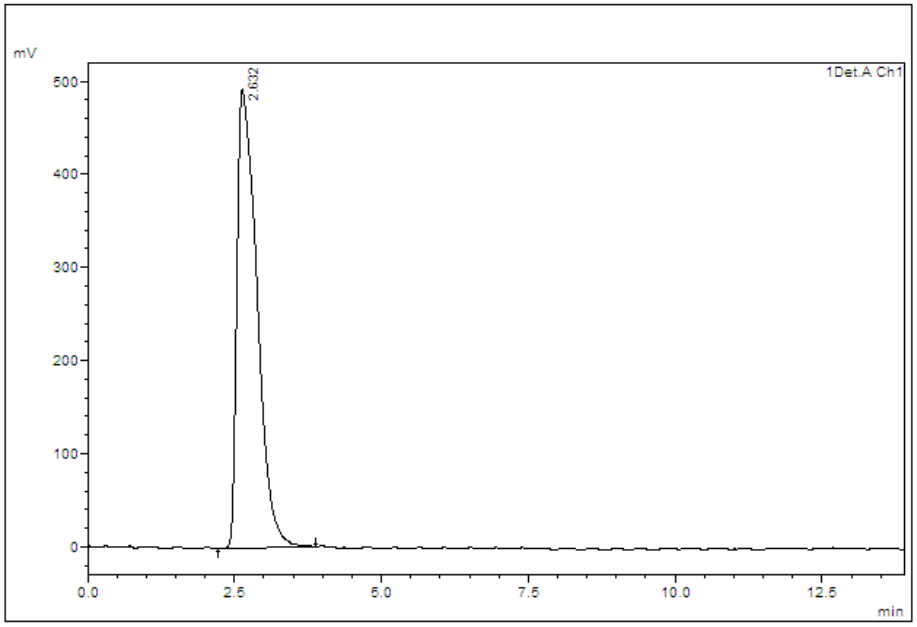



**Fig. V I.** HPLC Graph of Raw coir pith.

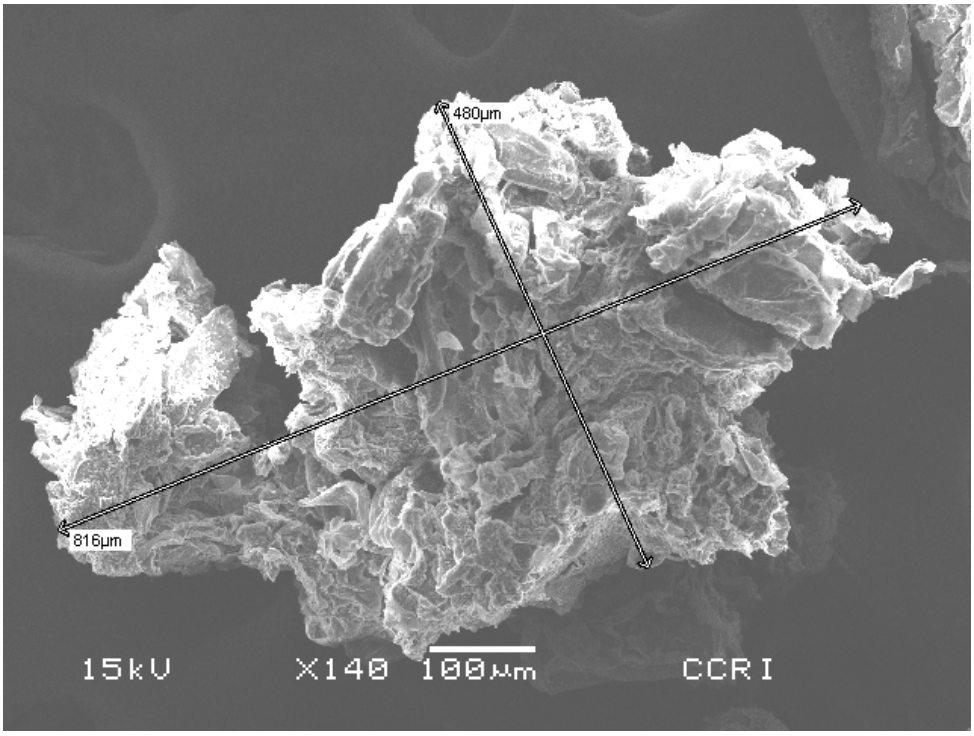



**Fig V I.** SEM images of Raw coir pith.

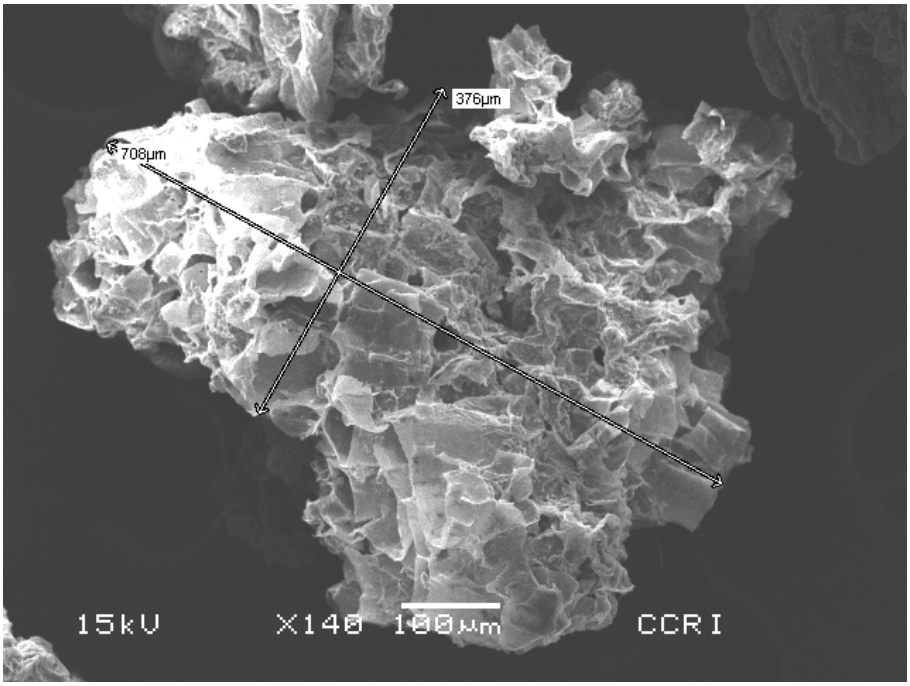



**Fig V m.** SEM image of *Pleurotus sojar caju* BCC.

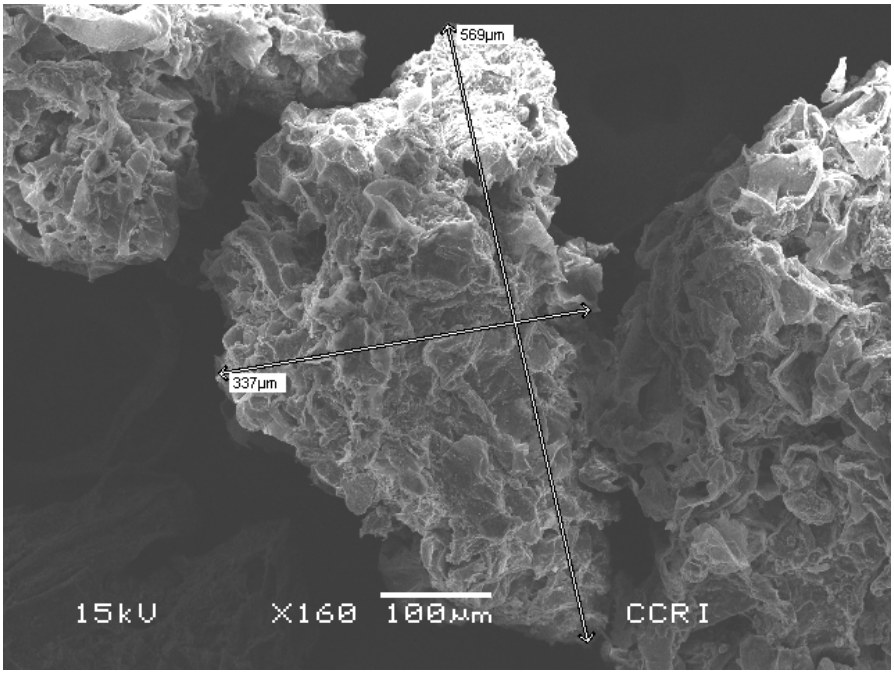



**Fig V n.** SEM image of with *Pleurotus sojar caju* + *A.v* BCC .

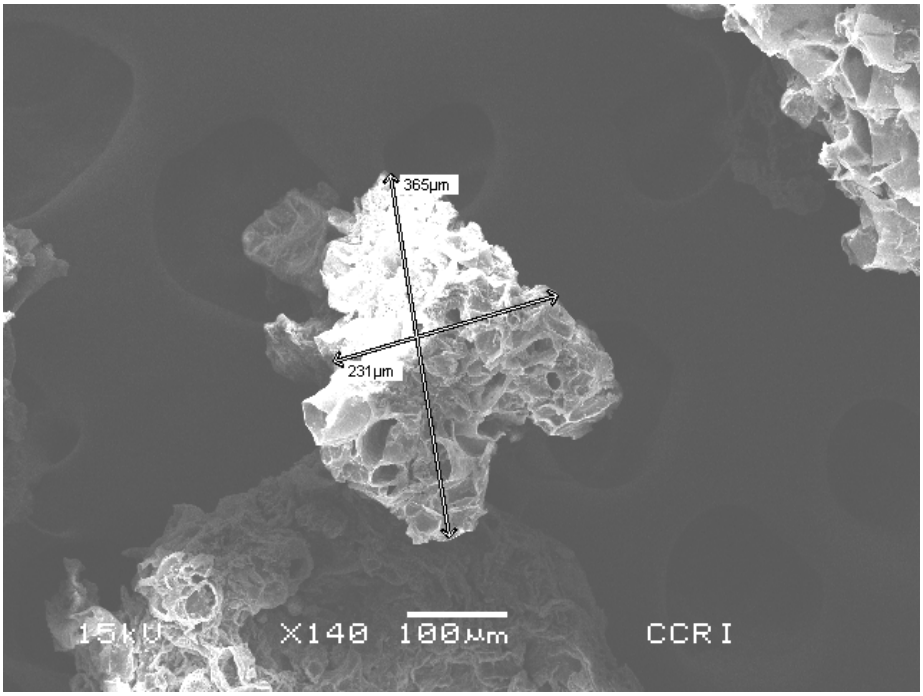



**Fig V o.** SEM image of *Pleurotus sojar caju*+ *A.b* BCC

## GRAPHS

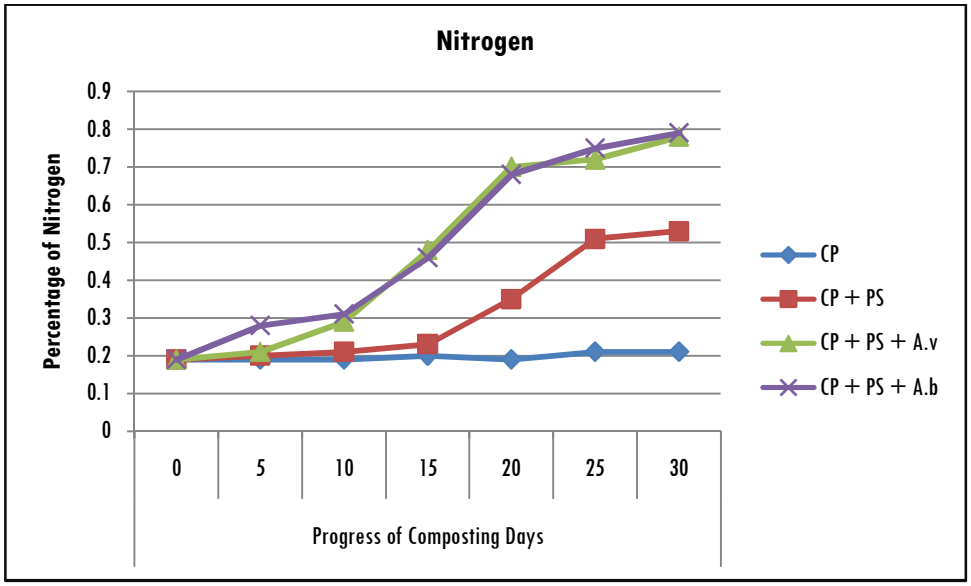



**Fig V t.** Enhancement of Nitrogen (%) during biodegradation of coir pith

**CP**- Coir Pith, ***PS***- *Pleurotus sajor caju*, ***A.v***- *Azotobacter vinelandii*, ***A.b***-*Azospirillum brasilense*

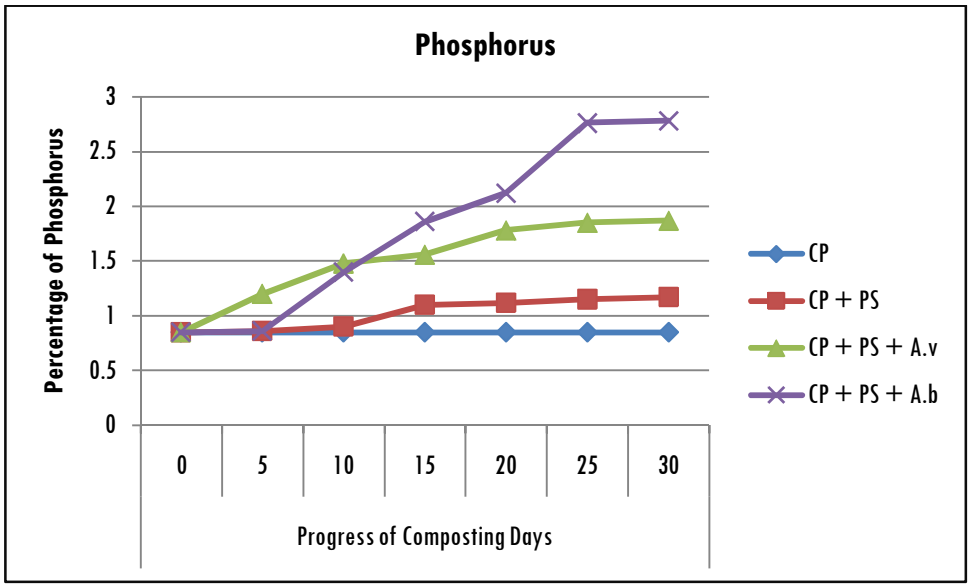



**Fig V u.** Enhancement of Phosphorus during biodegradation of coir pith.

**CP**- Coir Pith, ***PS***- *Pleurotus sajor caju*, ***A.v***- *Azotobacter vinelandii*, ***A.b***-*Azospirillum brasilense*

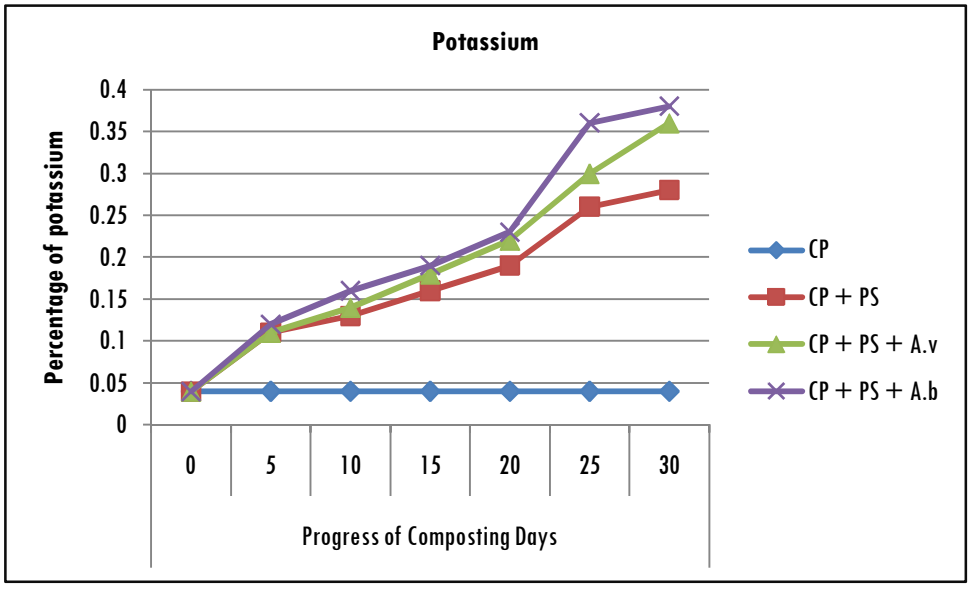



**Fig V v.** Enhancement of Potassium during biodegradation of coir pith.

**CP**- Coir Pith, ***PS***- *Pleurotus sajor caju*, ***A.v***- *Azotobacter vinelandii*, ***A.b***-*Azospirillum brasilense*

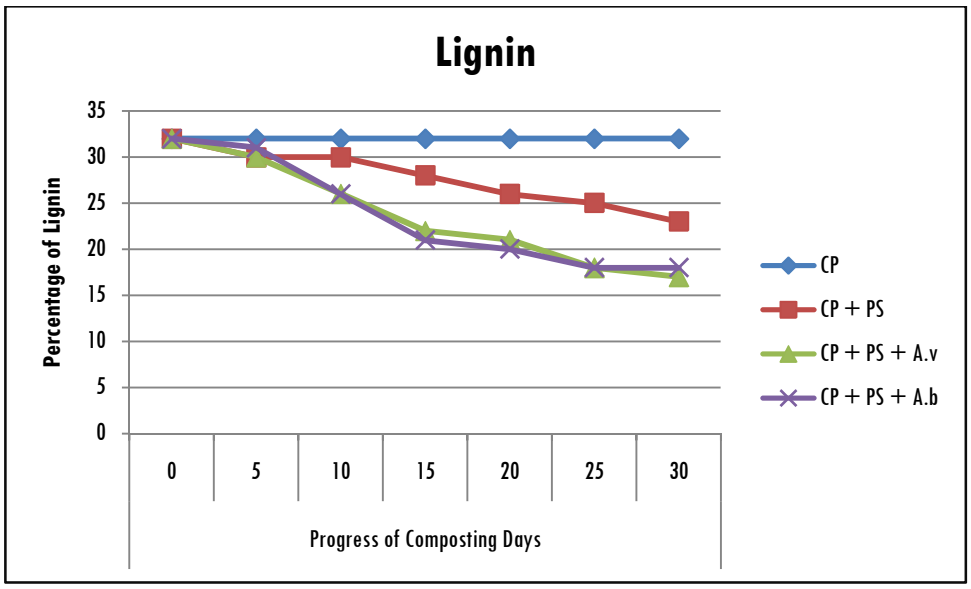
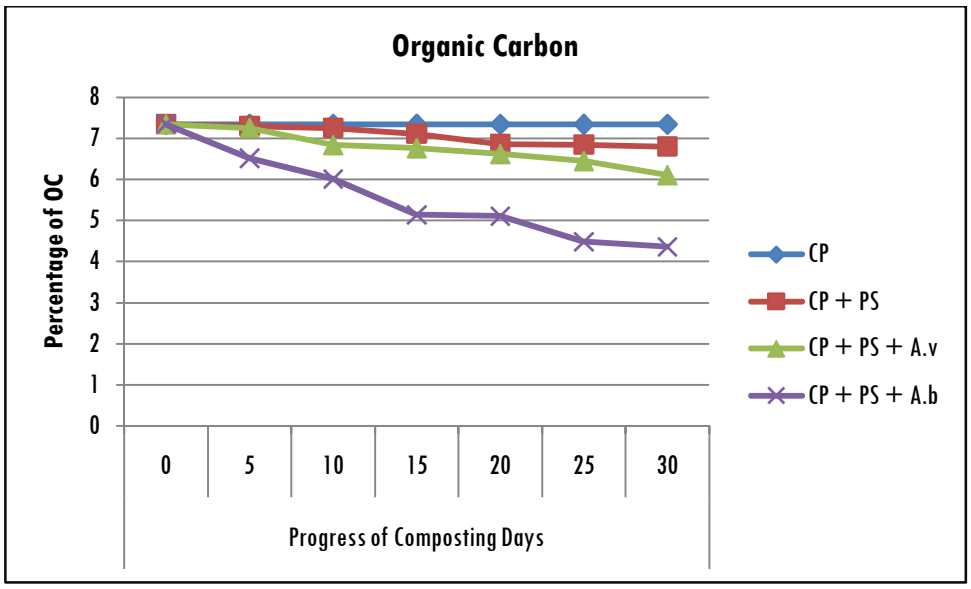


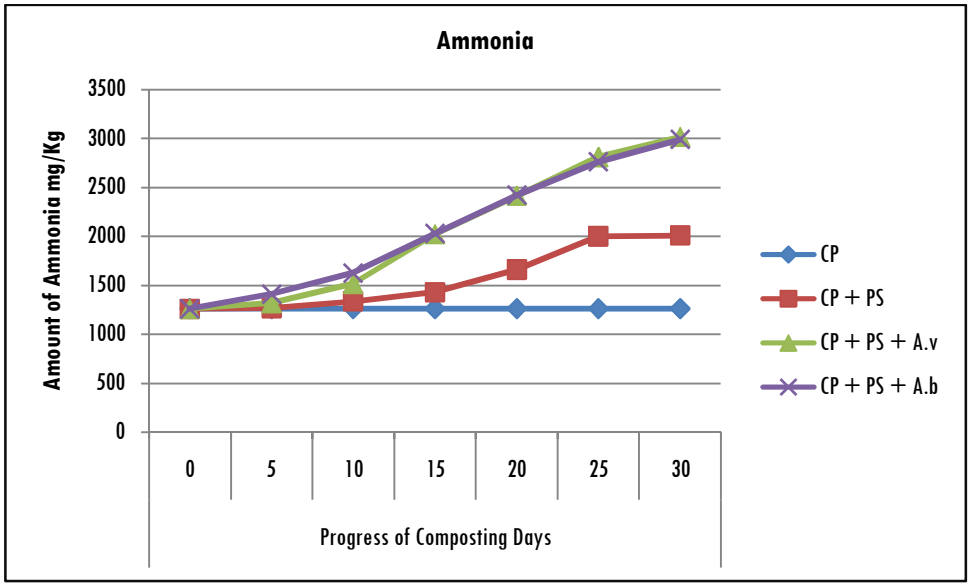

**Fig. V w.** Reduction of Lignin during biodegradation of coir pith

**CP**- Coir Pith, ***PS***- *Pleurotus sajor caju*, ***A.v***- *Azotobacter vinelandii*, ***A.b***-*Azospirillum brasilense*



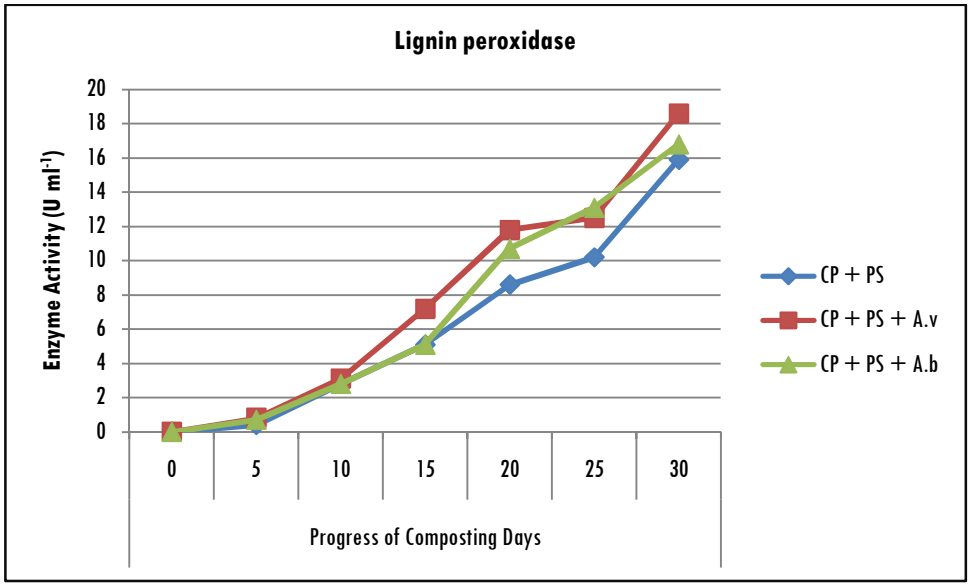

**Graph V x.** Reduction of Organic Carbon during biodegradation of coir pith.

**CP**- Coir Pith, ***PS***- *Pleurotus sajor caju*, ***A.v***- *Azotobacter vinelandii*, ***A.b***-*Azospirillum brasilense*



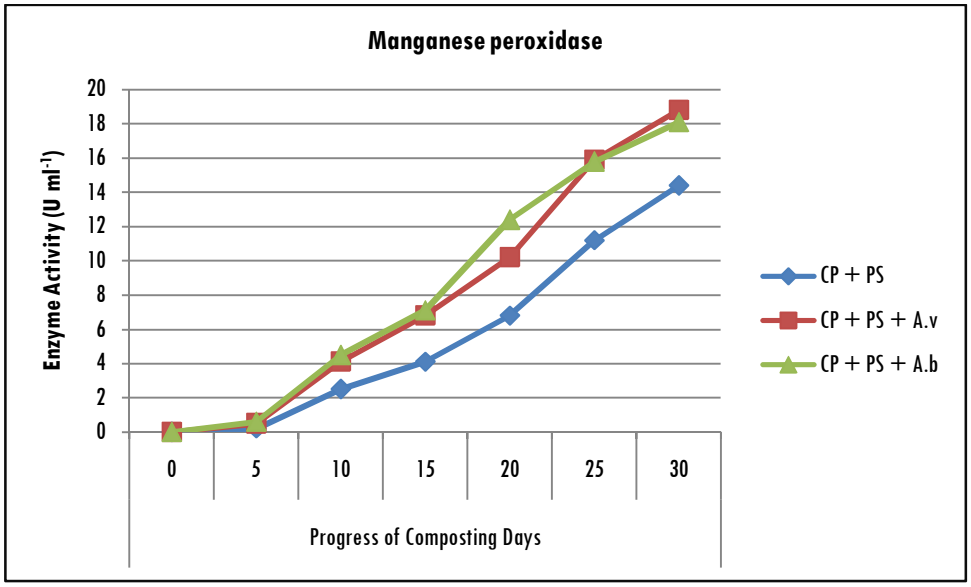

**Graph V y.** Enhancement of Ammonia during biodegradation of coir pith .

**CP**- Coir Pith, ***PS***- *Pleurotus sajor caju*, ***A.v***- *Azotobacter vinelandii*, ***A.b***-*Azospirillum brasilense*




**Graph V z.** Enzyme Activity (Lignin Peroxidase)

**CP**- Coir Pith, ***PS***- *Pleurotus sajor caju*, ***A.v***- *Azotobacter vinelandii*, ***A.b***-*Azospirillum brasilense*




**Graph V aa.** Enzyme Activity (Manganese Peroxidase)

**CP**- Coir Pith, ***PS***- *Pleurotus sajor caju*, ***A.v***- *Azotobacter vinelandii*, ***A.b***-*Azospirillum brasilense*

## 3. Discussion

Coir pith has been reported to have a C: N ratio of 112:1 (Nagarajan *et al*., 1985). White rot fungi which degrade cellulose and hemi cellulose as well as lignin are widely used to increase the digestibility of agro-residues (Kurtzman, 1981; Neelakantan, 1987; Zabrazil, 1987). The studies of coir pith degradation with the combination of *Pleurotus sajor caju* and nitrogen fixing bacteria (*Azotobacter vinelandii* and *Azospirillum brasilense*) shows a definite enhancement in nitrogen content after the coir pith biodegradation ( Table V a). Biodegraded Coir pith Compost (BCC) using *A.vinelandii* and *A.brasilense* has been observed to contain 0.78% and 0.79% nitrogen respectively. This is higher in comparison to that in raw coir pith and biodegradation with *Pleurotus sajor caju* alone. Barraquio *et al*., 2000; James *et al*., 2000 reported that, though a variety of nitrogen fixing bacteria like *Acetobaccter*, *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Derxia*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas* and

*Zoogloea* have been isolated from the rhizosphere of various crops, interest in the beneficial nitrogen fixing growth promoting rhizobacterial plant association has increased recently due to their potential use as biofertilizers (Vessey, 2003). As these bacterial species cannot act on the rigid chemical compounds present in coir pith, with the combination of fungal species *Pleurotus sajor caju*, the degradation has observed to be more effective. Phosphorous content has also been observed to increase significantly to an amount from 0.85% (raw coir pith) to 1.87% (*A. vinelandii*) and 2.78% (*A.brasilense*). A definite increase has also been observed in the potassium content in the different combinations from 0.04% in raw coir pith to 0.28% in BCC using *Pleurotus sajor caju* alone to 0.36% in combination with *A.vinelandii* and to 0.38% with *A.brasilense*. The use of these biological nitrogen fixing agents for the bio manure preparation is very important. It is reported that very significant reduction in the use of nitrogen fertilizer could be achieved if biological nitrogen fixation is made available to crop plants (Dawe, 2000).

Lignin is the most abundant aromatic polymer on earth, being produced by plants. The first step in lignin degradation is depolymerization, catalysed by lignolytic enzymes. Degradation of lignin is carried out by a group of basidiomycetes categorized as white rot fungi (Kamitsuji *et al*., 2004) Lignocellulosic wastes (LCW) refer to plant biomass wastes that are composed of cellulose, hemicelluloses and lignin (Mtui, 2009). Out of these three, lignin is the toughest materials which degrade very slowly in nature. In the present study, a reduction of lignin was observed by composting with combination of fungi and bacteria. Raw coir pith contains 32% lignin and which is reduced to 17% and 18% after biodegradation (Table V c). It should be noted that coir pith is resistant to biodegradation due to the presence of lignin. Normally coir pith is dumped as agricultural waste and accumulates as a waste product as heaps of course and fine dust (Ghosh *et al*, 2007). The coir pith 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 (Ramalingam *et al*, 2004). High content of

lignin in coir pith causes very slow decomposition following which it is used as raw organic manure for crops (Vinodhini *et al*, 2005). The degrading capacity of the combined consortium is more that by the individual organisms. Even, white rot fungi which are the most effective basidiomycetes for biological pretreatment of lignocellulosic materials (Sun and Cheng, 2002), when added with appropriate bacterial species, interesting results could be obtained.

Ammonia is the indicator of nitrogen fixation, and attempts have been made in the present study to show the enhancement of ammonia in coir pith after biodegradation. Table V b shows the value of ammonia in raw and biodegraded coir pith. Reghuvaran *et al*, 2011 reported a definite amount of increase in ammonia with the biodegradation of coir pith could be observed (Reghuvaran *et al*, 2011). Organic carbon was also observed to reduce considerably during the biodegradation (Table V b). There are reports stating that coir pith contains 87% organic matter and 13% ash content (Thampan, 1987).

Extracellular enzymes play an important role in the degradation of lignocelluloses. In our study the activity of Lignin peroxidase (LiP) and Manganese peroxidase (MnP) were estimated and the results have been tabulated in Table Vd & Ve. White rot fungi have been observed to secrete these lignin degrading enzymes. White rot fungi, including *P. ostreatus*, produce a wide range of enzymes (laccase, peroxidases, cellulases and xylanases) that degrade lignocelluloses (Kues and Liu., 2000). Several studies on *Pleurotus* species have been reported to grow on a wide spectrum of lignocellulosic waste materials due to their ability to secrete a range of degrading enzymes (cellulases, hemicellulases, xylanases, lignin peroxidase (LiP), manganese peroxidase (MnP) and laccases) and to produce protein rich biomass of fruiting bodies (Madan and Bisaria., 1983; Buswell and Chang., 1993; Rajarathnam *et al*., 1998). Among the ligninases produced from LCW (Lignocellulosic Waste), laccases have been studied the most (Nazareth and Sampy, 2003; Moldes *et al*., 2003, 2004; Couto *et al*., 2003; Couto and Sanroman,

2006; Mishra and Kumar, 2007; Alcantara *et al*., 2007; Minussi et al.,2007), followed by Manganese peroxidase and Lignin peroxidase (Couto et al., 2001, 2003; Wuyep *et al*., 2003; Velazquez- Cedeno *et al*., 2004; Couto and Sanroman, 2005; Alam *et al*., 2005; Asgher *et al*., 2006; Songulashvili *et al*., 2007; Elisashvili *et al*., 2008). Combined consortium of microorganisms produce more enzymes when compared to biodegradation by *Pleurotus sajor caju* alone and thereby action of the consortium accelerates the lignin degradation. As the combined action of these organisms yields more extracellular enzymes it can be concluded that, the presence of these nitrogen fixing bacteria could attribute to accelerating the enzyme production of *Pleurotus sajor caju* and thereby speed up the biodegradation of coir pith. These results are in concordance with many works, the main enzymes involved are lignin peroxidase, manganese peroxidase and laccase (Hao *et al*., 2006; Mtui and Nakamura, 2007, 2008; Mtui and Masalu, 2008).

The structure of coir pith has also been observed to vary during composting. Scanning Electron Microscope images of raw and biodegraded coir pith show variation in coir pith structures (Fig V l to V o). It has been observed that the average size of coir pith reduces after degradation which could be attributed to the breakdown of components. The raw coir pith particle size has been observed to be 816×480µm. *Pleurotus sajor caju* mediated BCC displayed size of 708×376 µm, *Pleurotus* and *A.vinelandii* BCC showed a size of 569×337 µm and the BCC using *Pleurotus* and *A. brasilense* BCC displayed size of 365×231 µm. The big sized particles show high aeration porosity and those containing small sized particle size show high water holding porosity *i.e.* smaller particles do not hold much aeration and bigger particles do not hold much water (Jeyeseeli and Poul Raj, 2010).

Large amounts of coir pith accumulate in the viscinity of coir processing units (approximately 7.5 million tons annually in India), causing severe disposal problems, fire hazards and ground water contamination due to release of phenolic compounds (Namasivayam *et al*., 2001). This also causes pollution of ground water

due to the percolation of leachates containing residual phenol from these dumps (Gopal and Gupta, 2001) especially during rainy season. The environment also serves as an ideal breeding ground for rodents and insect pests (Grimwood, 1975). Polyphenols are present /produced during coir pith biodegradation. Lignin, a main contributor of the total carbon of agro-industrial wastes, produces polycyclic aromatic hydrocarbon components such as benzopyrine, catechol, hydroquinone, phenanthrine and naphthalene when degraded by heat (Kjallstrand *et al*., 1998). Here the phenolic substances like catechol, gallic acid, picric acid, pyrogallic acid and resorcinol were taken as standards for HPLC analysis. The Rf (Retension Factor) values of the samples were almost similar for these standards. Gallic acid has the Rf value 4.358, which is almost similar to that observed in *PS+A.v BCC* (4.312) and *PS+A.b* BCC (4.326). The Rf values were studied for Picric acid (4.388), Pyrogallic acid (4.257) and Resorcinol (4.204). Catechol(3.280; 5.616). The similar Rf values were observed in the *CP+PS* (5.861), *PS+A.v* BCC (3.608; 5.868) and *PS+A.b* BCC (3.507; 5.635). Presence of resorcinol (3.460; 5.371) was also observed in the samples of biodegraded coir pith. Presence of phenolic compounds *viz*. Picric acid and Pyrogallic acid could also be confirmed (Fig V d to V k). In contrast, only one definite peak was observed in the HPLC graph of raw coir pith which shows the phenolics were absent in coir pith before biodegradation (Fig. V l). These results are in concordance with reports which state that resorcinol, pyrogallic acid and catechol were present in leachate samples drawn from coir retting areas.( Ravindranath and Sarma, 1998).

The potential of bioconversion of lignocellulosic wastes into value added products is emphasized in recent studies (Philippoussis & Zervakis 2000a; Poppe 2000). In our studies, we can conclude that coir pith can be converted to effective organic manure with biodegradation with *Pleurotus sajor caju* and Nitrogen fixing bacteria like *Azotobacter vinelandii* and *Azospirillum brasilense.* 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.

## 4. Conclusion

Bioremediation has been reported to be more effective through consortium action than single culture. In the present study, Pleurotus sajor caju has been experimented in combination with nitrogen fixing microorganisms viz. *Azotobacter vinelandii* and *Azospirillum brasilense*. This was aimed at replacing urea in the composting process. The study could yield results viz. increase in nitrogen content from 0.19% to 0.79%.The increase in the nitrogen content could be attributed to the inoculation of *Azotobacter vinelandii* and *Azospirillum brasilense*. Thus biodegradation of coir pith using a consortium of cultures consisting of lignin degrading fungi and nitrogen fixing bacteria could yield a value added fertilizer from biological waste.

Chapter 6

# Part I. USE OF BIODEGRADED COIR PITH FOR THE CULTIVATION OF MEDICINAL PLANTS

Contents



## 1. Introduction

Coir pith or coir dust is a major byproduct of coir fiber extraction industries (Reghuvaran *et al*., 2009). 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 (Ramalingam *et al*., 2004). Normally coir pith is dumped as agricultural waste and accumulates as a waste product as heaps of course and fine dust (Ghosh *et al*., 2007). High content of lignin (28.25%) in coir pith causes very slow decomposition following which it is used as raw organic manure for crops (Vinodhini *et al*., 2005). 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 (Akhmedova, 1992). Degradation of coir pith can be effectively carried out with suitable species of Basideomycetes fungus (*Pleurotus sajor caju*) 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 (Argypropoulose & Menachem, 1997). 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 (Ramaswamy, 1986). Coir pith (100%) used as potting medium showed a spectacular increase in water holding capacity of potting mixture when tomato plants were grown on coir pith based potting mixture (Baskaran & Saravanan, 1997). Among medicinal plants, brahmi (*Bacopa monnieri*), a well known prostrate herb, is distributed in damp, marshy areas throughout India. *Piper longum* can be cultivated successfully in organic matter rich fertile well drained forest soils. It is of South Asian origin and is found almost all over India. It is a component of Indian medicine reported as remedy for treating gonorrhea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infections, chronic gut related pain, arthritis and alleviation of anxiety (Guido & David, 1998). *Phyllanthus niruri* originated in India, usually occurs as a winter weed throughout and *P. niruri* is a herb of Euphorbiaceae family that grows upto 60 cm. In recent years, more and more people are complimenting their treatment with natural supplements (Dursum *et al*., 2004). Kalmegh (*Andrographis paniculata*) as ethanolic extract has an insulin sensitizing effect (Sadikun, 2008). Coconut coir dust is being marketed as a soilless medium substitute for sphagnum peat moss that inhibits fungus gnat (*Bradysia* sp.) development (Denise *et al*., 2002). Several other important studies that conducted on the antioxidant activity of some medicinal plants grown under organic farming conditions (Khalil *et al.,* 2007) have been reported. Growth of *A. paniculata* in vermicomposted coir pith has suggested that vermicomposted coir pith could be helpful for reclamation of soils from industrial sites for cultivation of *A. paniculata* in small scale nurseries (Vijaya *et al*., 2008). Aqueous extract of the leaves of *A. paniculata* has traditionally been used for treatment of various liver disorders and jaundice (Trivedi & Rawel, 2000).

The present study reports the findings on the growth trials carried out using biodegraded coir pith on medicinal plants (*Andrographis paniculata, Bacopa monneiri, Phyllanthus niruri,* and *Piper longum*).

## 2. Methodology.

### 2.1 Sample Collection.

Coir pith was collected from the coir industries in Kattukada, Alappuzha district was used for the study.

### 2.2 Collection of Medicinal Plants.

Experimental medicinal plants *viz. Phyllanthus niruri, Andrographis paniculata, Bacopa monnieri* and *Piper longum* were collected from Horticulture division, Kerala Agricultural University, Mannuthy, Trissur.

### 2.3Composting.

The efficacy of growth of medicinal plants in coir pith compost is studied by laying three sets of coir pith heaps in a shady place for composting. Each of the experiments were conducted by mounting 1 kg heaps of coir pith supplemented with 4 gms of *Pleurotus sajor caju* and 10 gms of urea as nitrogen supplement. Three sets were designed for 2 bacterial species and one mushroom species and a control by the standard composting method were maintained. All the heaps were moistened regularly to retain moisture.

### 2.4 Field Experiment.

Four experimental lots of coir pith composted using *P. sajor caju* in combination with *Acetobacter vinelandii, Azospirillum brasilense,* urea and fourth one kept as untreated as raw coir pith itself were compared with control (garden soil). Four sets of 13 pots were used for cultivation of medicinal plants. Each set of pot was maintained for each species of medicinal plant. Out of 13, pot A was filled with garden soil. B, C and D were filled with a mixture of garden soil and compost [composted with *P. sajor caju* and N-fixing bacteria (*Acetobactor vinelandii*) in proportion of 1:1, 1:3 and 3:1]. Pot E, F and G were filled with garden soil and compost (with mushroom and *Azospirillum brasilense*). Pot H, I and J were filled

with mixture of garden soil and compost (with mushroom and urea) and last three pots (Pot K, L and M) were filled with mixture of garden soil and raw coir pith. All treatments were carried out in duplicate. After 45 days, measurements were made of shoot and root length.

## 2.5 Estimation of Physical Properties.

### 2.5.1 Shoot Length.

The shoot length was estimated with a measurement tape.

### 2.5.2 Root Length.

The root length was estimated by a measurement tape.

## 2.6 Estimation of Chemical Properties.

### 2.6.1 Carbohydrate (Lee & Tournsean., 1958).

### 2.6.2 Protein (Lowry *et al*., 1951).

### 2.6.3 Chlorophyll (Arnon., 1949).

# 3. Results.

## 3.1 Physical Properties

### 3.1.1 Shoot Length

In result of all the 13 samples of different medicinal plants, the shoot length shows an interesting trend on pots containing biodegraded coir pith than garden soil. In garden soil (A), the length of shoot is 8 cm in the case of *Phyllanthus niruri,* 15 cm in *Andrographis paniculata*. 15 cm in *Bacopa monnierii* and 23 cm in case of *Piper longum.* In case of pots containing BCC with *Pleurotus sajor caju* and *Azotobacter vinelandii* with garden soil in different proportions (B, C and D) show increased shoot length (12 cm, 8 cm and 17 cm respectively) for *P. niruri*. The shoot length of 18 cm, 22 cm and 27 cm (Pot E, F and S) for BCC with *P.*

*sajor caju* and *Azospirillum brasilense*; 13 cm, 10 cm and 15 cm (Pot H, I and J) for BCC with *P. sajor caju* and urea and for raw coir pith, it was 8 cm, 3 cm and 10 cm (Pot K, L and M). In case of all medicinal plants, the increased shoot length is observed in plants grown in pots containing higher percentage of coir pith than garden soil (Table VI a to VI d).

### 3.1.2 Root Length

The results in root length of different medicinal plants cultivated in 13 pots containing different concentrations of raw and biodegraded coir pith with garden soil show variations. The pots of garden soil (A) show root length of 4 cm in *Phyllanthus niruri*. It is increased to 6 cm and 8 cm (B and D) in BCC with *P. sajor caju* and *A. vinelandii*. Root length show changes in pots E, F and G (4 cm, 3 cm and 6 cm) in case of BCC with *P. sajor caju* and *A. brasilense*. In case of pots having BCC with *P. sajor caju* and urea observed root length of 5 cm, 3 cm and 9 cm for pot H, I and J respectively. Almost similar results were observed in case of other three medicinal plants also. There is an increased root length were observed in plants grown in pots containing higher percentage of coir pith than garden soil (Table VI a to VI d).

## 3.2 Chemical Properties

### 3.2.1 Carbohydrate

Carbohydrate contents in the plant leaves is an important indicator of plant growth. The carbohydrate content in the leaf of plants which were grown in garden soil is observed to be 0.23 mg/g (Pot A). The contents were increased to 0.28 mg/g, 0.24 mg/g and 0.28 mg/g (Pot B, C and D resp.) for coir pith composted with *P. sajor caju* and *A. vinelandii*. Almost similar values were observed in BCC with *P. sajor caju* and *A. brasilense* (0.26 mg/g, 0.24 mg/g and 36 mg/g resp.). Pots H, I and J (BCC with *P. sajor caju* and urea) show reduced carbohydrate content in comparison with other two treatments (Fig. VI e to VI h).

### 3.2.2 Protein

Protein content in plants growing in biodegraded coir pith and garden soil show remarkable variations. Sample A (garden soil) show protein content of 0.40 mg/g, whereas the same in plants grown in BCC composted with *P. sajor caju* and *A. vinelandii* show increase in protein content to 0.65 mg/g, 0.53 mg/g and 0.78 mg/g respectively for Pot B, C and D. Comparing with other bacterial culture (*A. brasilense*) with P. sajor caju also show enhancement (0.62 mg/g, 0.50 mg/g and 0.70 mg/g for sample E, F and G resp). BCC with *P. sajor caju* and urea did not show much increase as much as the other two treatments shown (Fig. VI e to VI h).

### 3.2.3 Chlorophyll

Chlorophyll content also show enhancement in pots having BCC with combination of *Pleurotus sajor caju* and nitrogen fixing bacteria. The plants which grow in garden soil show chlorophyll content of 0.080 mg/g and which was increased to 0.186 mg/g, 0.180 mg/g and 0.191 mg/g (Pot B, C and D resp.) for BCC composted with *P. sajor caju* and *A. vinelandii*. Chlorophyll content is also show hike in plants growing in compost with *P. sajor caju* and *A. brasilense*. Similar observations were also observed in all four medicinal plants (Fig. VI e to VI h).

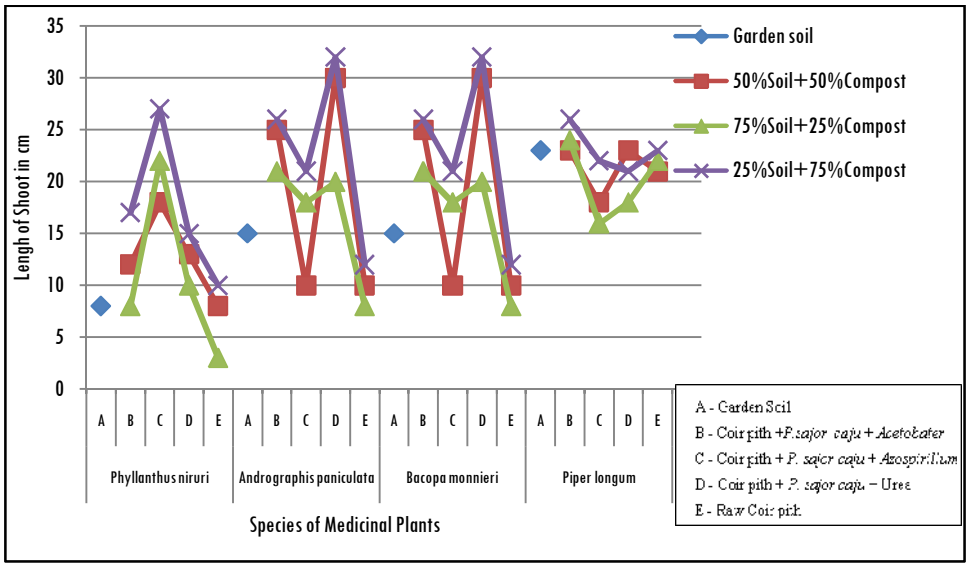



**Fig. VI a.** Effect of Biodegraded coir pith in Shoot length of Medicinal p

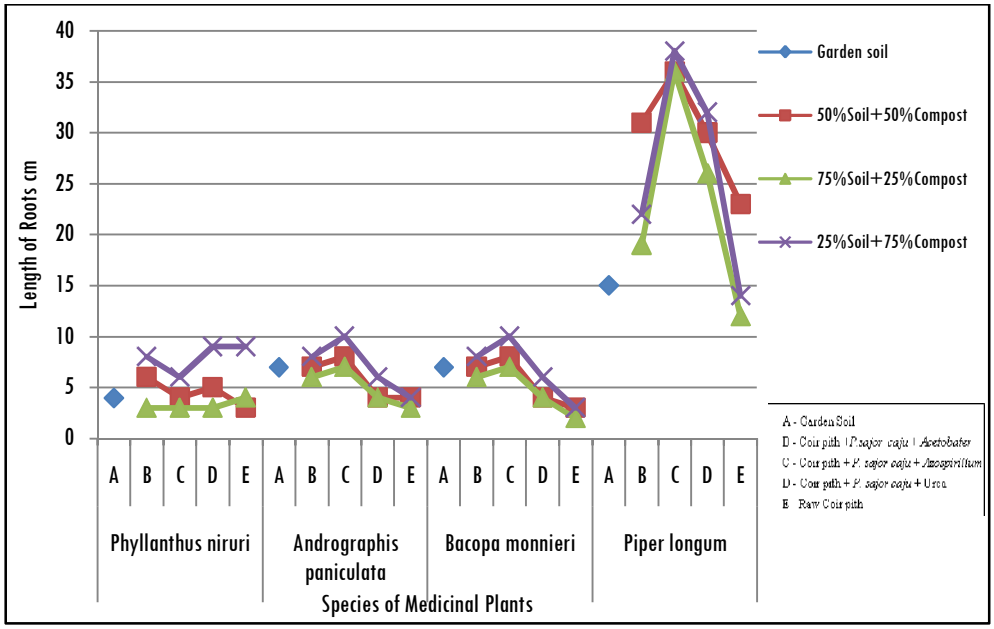



**Fig VI b.** Effect of Biodegraded coir pith in Root length of Medicinal plants

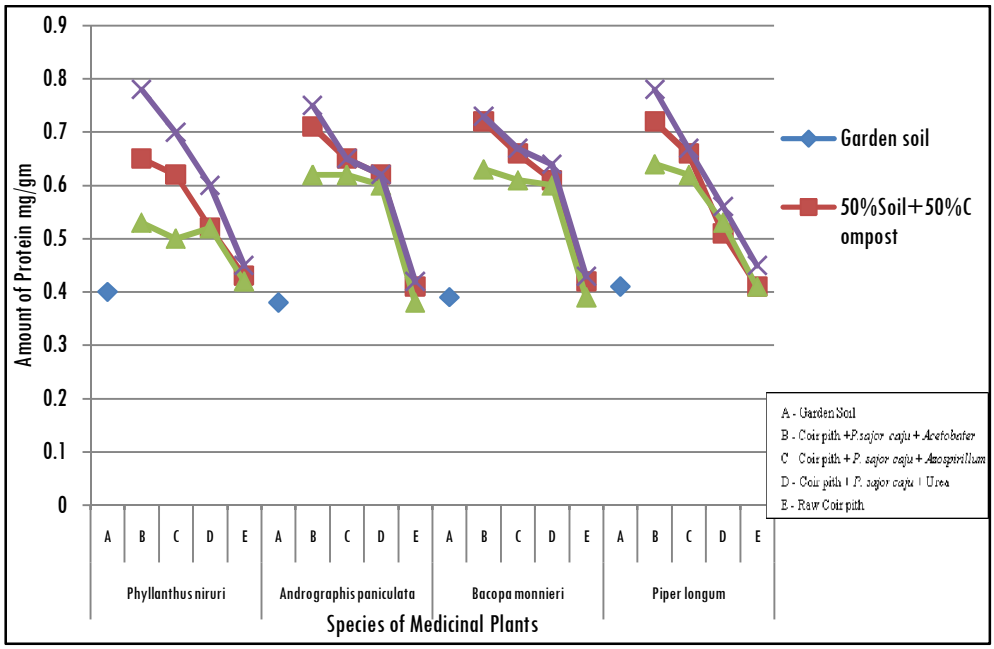



**Fig VI c.** Effect of Biodegraded coir pith in Protein content in Medicinal plants

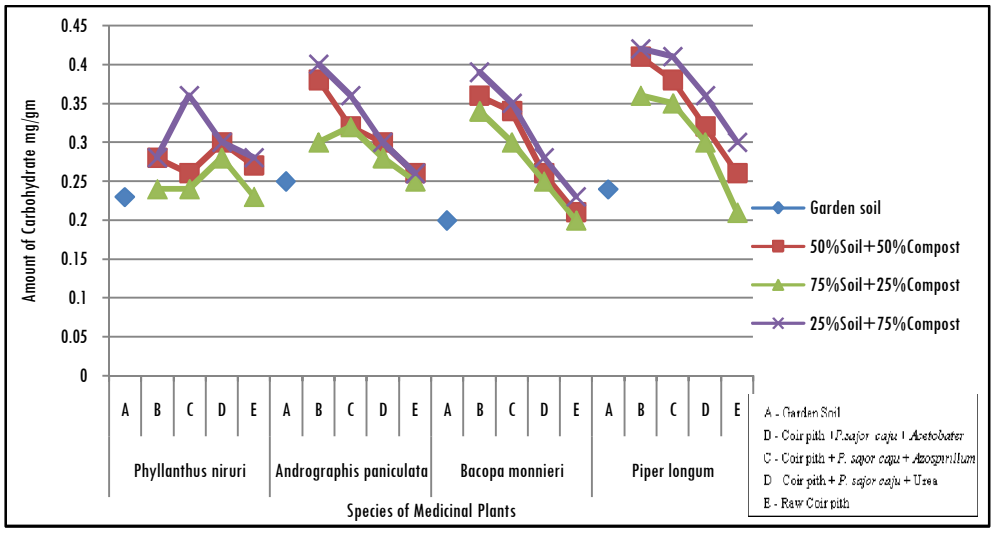



**Fig VI d.** Effect of Biodegraded coir pith in Carbohydrate content in Medicinal plants.

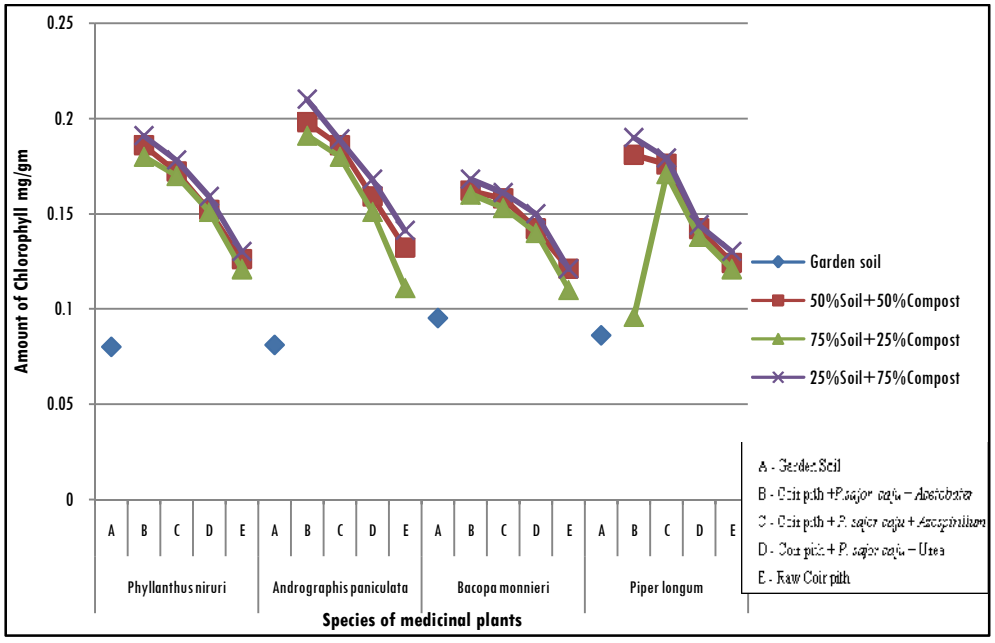



**Fig VI e.** Effect of Biodegraded coir pith in Chlorophyll content in Medicinal plants

VI. f *Andrographis paniculata*

VI g *Bacopa monnieri*

VI h *Phyllanthus niruri*

VI i *Piper longum*

## 4. Discussion

Medicinal plants are important natural resources and supplementation of biodegraded coir pith as manure could contribute to increasing the growth productivity of these plants, which could be cost effective compared to addition of ckemical fertilizers. The study has details on the effect of addition of biodegraded coir pith on the growth of medicinal plants *viz. Phyllanthus niruri, Andrographis paniculata, Bacopa monnieri* and *Piper longum.*

*Andrographis paniculata* is widely cultivated in Southern and Southeastern Asia, where it is used to treat infections and some diseases, often being used before antibiotics were created. Mostly the leaves and roots were used for medicinal purposes. *A. paniculata* is distributed in tropical Asian countries, often in isolated

patches. It can be found in a variety of habitats, such as plains, hillsides, coastlines, and disturbed and cultivated areas such as roadsides, farms, and wastelands. Native populations of *A. paniculata* are spread throughout south India and Sri Lanka which perhaps represent the center of origin and diversity of the species. *Bacopa monnieri* (Brahmi) is a perennial, creeping herb whose habitat includes wetlands and muddy shores. It commonly grows in marshy areas throughout India, Nepal, Sri Lanka, China, Taiwan, and Vietnam, and is also found in Florida, Hawaii and other southern states of the USA where it can be grown in damp conditions by the pond or bog garden. It has been used in traditional Ayurvedic treatment for epilepsy and asthma. It is also used in Ayurveda for ulcers, tumors, ascities, enlarged spleen, indigestion, inflammations, leprosy, anemia, and biliousness.

Pot culture experiments were carried out to study the effect of coir pith based potting mixture with garden soil on four different medicinal plants (*P. niruri, A. paniculata, B. monneiri* and *P. longum*). Plants grown in pots containing higher percentage of coir pith than soil, show increased shoot and root length (Fig. 1). *P. niruri* shows shoot length and root length 8 cm and 4 cm in 100% garden soil, 27 cm and 6 cm respectively in pot containing 25% soil and 75% composted coir pith, which is biodegraded with *P. sajor caju* and *Azospirillum*, secondly with 75% soil and 25% coir pith, same treatment also yielded 22 cm shoot and 3 cm root length respectively. Thus, composted coir pith with the combination of mushroom and bacteria provide NPK enrichment on pot culture. This medium provides easy movement of roots and thereby increased growth of plants. In case of *P. sajor caju* and *Acetobacter*, biodegraded coir pith compost (BCC) medium, values are 17 cm, 12 cm and 8 cm (shoot) in 25% soil+75% compost, 50% soil+50% compost and 75% soil+25% compost respectively. Root length was observed to be 8 cm, 6 cm and 3 cm for 25% soil+75% compost, 50% soil+50% compost and 75% soil+25% compost respectively. In case of BCC with *P. sajor caju* and urea also similar results were observed, but values were comparatively lower than that composted with N-fixing bacteria. *A. paniculata* also shows effective growth in pots

containing higher content of BCC; using *Acetobacter* shows improved growth in medium with compost and soil. In case of *B. monnieri* and *P. longum*, higher growth was observed in BCC using *Acetobacter*. Similar results were observed in studies using N-fixing bacteria. Medium containing coir pith: shoal leaf mould: sand (60:20:20 and 50:25:25 v/v) had higher cation exchange capacity (CEC) and water holding capacity (WHC) (Saravanan & Nanbisan, 1995). Coir pith is very efficient for tropical farming as soil conditioner and cyanobacteria are also capable of biodegrading coir pith with various kinds of pollutants (Malliga & Vishwajith, 2005).

*Piper longum* sometimes called Indian long pepper, is a flowering vine in the family *Piperaceae*, cultivated for its fruit, which is usually dried and used as a spice and seasoning. Long pepper has a similar, but hotter, taste to its close relative *Piper nigrum* - from which black, green and white pepper are obtained. Today, long pepper is a very rare ingredient in European cuisines, but it can still be found in Indian vegetable pickles, some North African spice mixtures, and in Indonesian and Malaysian cooking. It is readily available at Indian grocery stores, where it is usually labeled *pippali*. *Phyllanthus niruri* is a widespread tropical plant commonly found in coastal areas, best known by the common names stonebreaker or seed-under-leaf. A clinical study with *Phyllanthus niruri*, indicated that it may reduce the levels of urinary calcium (Nishiura *et al.*, 2004). A subsequent study of 150 patients over a 6 month period indicated that an extract of this herb reduces the incidence of stone formation, and concluded, "Regular self-administration of *P. niruri* after extracorporeal shock wave lithotripsy for renal stones results in an increased stone-free rate that appears statistically significant for lower caliceal location. Its efficacy and the absolute lack of side effects make this therapy suitable to improve overall outcomes after extracorporeal shock wave lithotripsy for lower pole stones." (Micali *et al.*, 2006).

A considerable increase in growth of rice plants is also observed using coir pith based cyanobacterial biofertilizer (Krishnaveni, 1999; Lavanya Priya, 1997). In present study, potting medium, composed of coir pith along with soil, is an efficient soil conditioner, and BCC using *P. sajor caju* and N-fixing bacteria show significant increase in growth due to its enriched NPK content. In case of *P. amaranthus*, protein content estimated was 0.40 mg/g in 100% soil (Fig. 2). But pots with combination of coir pith and soil shows variation in protein content. In case of coir pith composted with *P. sajor caju* and *Acetobacter*, protein content obtained was 0.78 mg/g, 0.65 mg/g and 0.53 mg/g in 25% soil+75% compost, 50% soil+50% compost and 75% soil+25% compost respectively. Carbohydrate (Fig. 3) and chlorophyll (Fig. 4) content also show similar variation. Garden soil alone shows 0.23 mg/g and 0.080 mg/g for carbohydrate and chlorophyll respectively. Combinations (*P. sajor caju* &*Acetobacter, P. sajor caju* & *Azospirillum, P. sajor caju* & urea) and raw coir pith have increase in carbohydrate and chlorophyll content than garden soil. In case of *A. paniculata*, parameters of protein, carbohydrate and chlorophyll are found to be 0.38mg/g, 0.25mg/g and 0.081mg/g respectively for garden soil. Composted and raw coir pith for *A. paniculata* shows variations. An increase in protein, chlorophyll and carbohydrate content has been observed in plants treated with BCC when compared with garden soil. Values for protein content were 0.75mg/g, 0.71mg/g & 0.62mg/g, carbohydrate content 0.40mg/g, 0.38mg/g & 0.30mg/g and chlorophyll 0.210mg/g, 0.198mg/g and 0.191mg/g for 25% soil+75% compost, 50% soil+50% compost and 75% soil+25% compost respectively. Similar results were observed in case of BCC with *Azospirillum*, urea and untreated raw coir pith, which show definite increase in protein, carbohydrate and chlorophyll content. In case of *B. monneiri* and *P. longum*, increased protein, carbohydrate and chlorophyll contents was observed in coir pith based potting mixtures. It is also reported that high percentage of rooting of acalypha and bougainvillea was observed in coir dust medium when compared to and or soil+organic manure (Lokesha *et al.*, 1988). Thus, present study confirms

that composted coir pith with *P. sajor caju* and N-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.

## 5. Conclusions

Nitrogen fixing bacteria are excellent and eco-friendly substitutes for urea for composting of coir pith with *P. sajor caju.* Composted coir pith based potting medium for cultivation of medicinal plants suggested that this compost can be used in reclamation of soils to enhance productivity of medicinal plants and also possibly other crops. This study could encourage the stake holders involved in cultivation of medicinal plants organically, economically to adopt environmental friendly ways of growing medicinal plants.

# Part II. USE OF BIODEGRADED COIR PITH FOR GARDEN PLANTS AS A SOIL LESS MEDIUM FOR ROOF GARDENING PRACTICES

Contents



## 1. Introduction

Roof gardens have received increased attention in recent years as an urban horticulture alternative (Boivin *et al*., 2001). Attention to roof garden relies on the fact that the plant material destroyed during the construction phase will be restored at the top of the building and will reduce the adverse effects of urbanization and deforestation (Osmundson, 1999).It has been established that roof gardens reduce temperature and solar irradiance, provide up to 50% reduction in the heat flux into buildings (Onmura *et al*., 2001). Despite their numerous advantages, roof gardens have not been yet considered a common practice (Panayiotis *et al*., 2003). The main obstacle for wide public acceptance is the high initial construction cost and the increased load that is exerted on the frame of the buildings. The last results mainly from the substrate weight (Scrivens, 1990). In extensive roof gardens, the minimal substrate depth in conjunction with the utilization of light weight materials (Fischer and Jauch, 1995) reduces substantially the weight of the construction. In intensive roof gardens with shrubs or trees, soil forms the basic constituent of the substrate, in order to support plant growth, in advent increases water-holding capacity and provide sufficient anchorage to the plants (FLL, 1995). Green roofs have certain advantages; first they reduce the quantity of runoff entering municipal

storm water management systems (Kolb, 2004; Liesecke, 1998, 1999; Rowe *et al*., 2003; Schade, 2000; U.S.EPA, 2003). Secondly they provide insulation for buildings, thus reducing energy consumption (Eumorfopoulou and Aravantinos, 1998; Lukenga and Wessels, 2001; Theodosiou, 2003). Third, they increase the life span of a typical roof by protecting the various roof components from damaging UV rays, extreme temperatures and rapid temperature fluctuations (Lukenga and Wessels, 2001; Stein, 1990). Fourth, they have the potential to reduce the Urban Heat Island Effect (Dimoudi and Nikolopoulou, 2003; Wong *et al*., 2003). Although these benefits have long been identified, research quantifying these benefits and sustainability of various plant texa for use on green roofs has been limited (Michael *et al*., 2005).

Higher plants effectively use only a small percentage of available water (Bres and Weston., 1993). Amending plant growth media with hydrophilic polymers (soil conditioners) has increased water retention (Johnson, 1984; Quinn, 1990) ; reduced watering frequency (Still, 1976; Taylor and Halfacre, 1986) ; improved soil texture (Wallace and Wallace., 1986b, 1986c) ; increased water infilteration (Mitchell, 1986; Wallace and Wallace 1986c) ; reduced erosion and water runoff (Wallace and Wallace, 1986d) and increased germination and improved early growth of plants (Cook and Nelson, 1986; Wallace and Wallace, 1986a, 1986b).

Coir pith is an excellent material as the soil conditioner, it is lignocellulosic in nature, brown coloured, light weight corky dust; particle size varies from 100-300 microns similar to peat moss. It has a porous structure and the pores are responsible for allowing good aeration around the roots of plants and retain water content in the pores for rewetting when dry. Chemical analysis reveals that coir pith contains three major constituents- cellulose, hemicelluloses and lignin. Cellulose is a polymeric chain of anhydrous glucose units and exists mainly in crystalline form. Hemicellulose is made up of mixed polymers of various pentose

and hexose sugars and is amorphous in nature. Lignin is an amorphous polymer of phenyl propane, which surrounds the cellulose in the cell wall. Lignin exists in situ as large molecular weight component but indefinite in size. The major properties of coir pith are high water holding capacity, *i.e*. 6-8 times than its weight, slow degradation (due to high lignocellulose content), excellent moisture retention (even after drying), high porosity, storing and releasing of nutrients over extended periods of time, acceptable electrical conductivity (EC), pH, cation exchange capacity (CEC), aeration and oxygenation. These properties provide enhanced root penetration, greater physical resiliency that withstand compression better, being a poor conductor of heat, helps keep soil temperature under control and thereby prove to be ideal medium for plant growth.

The present study aimed 1) to investigate the contribution of an effective light weight potting media for the roof garden practices, 2) To study the potency of this potting medium for the growth of garden plants, 3) to formulate and market the medium as an effective readymade potting medium for roof garden plants.

## 2 Materials and Methods

### 2.1 Collection of samples.

Coir pith was collected from the coir industries in Kattukada, Alappuzha district. White rot fungi, pith plus were procured from the Central Coir Research Institute (CCRI), Kalavoor, Alappuzha. The Bacteria (*Azotobacter vinelandii*, MTCC No. 124 and *Azospirillum brasilense*, MTCC No. 125) were procured from Microbial Type Culture Collection (MTCC), Chandigarh. The experimental studies were conducted in the Rajiv Gandhi Chair in Contemporary Studies, School of Environmental Studies, Cochin University of Science and Technology (CUSAT). The biochemical estimations were carried out at the Central Coir Research Institute, Alappuzha and Rajiv Gandhi Chair, CUSAT.

Plant saplings such as *Rosa rubiginosa, Jasmine sambae, Aralia elata, Gardenia jasminoides* and *Chrysalidocarpus lutescens* were procured from Horticulture division, Kerala Agricultural University (KAU), Mannuthy, Trissur, Kerala.

### 2.2 Composting.

The experiments consisted of 2 sets mounted in 5 kg quantities of coir pith heaps in triplicate. The first lot supplemented with both fungus (*Pleurotus sajor caju*) and bacteria (*Azotobacter vinelandii*). The second lot was supplemented with *Pleurotus sajor caju* and *Azospirillum brasilense*. The experimental coir pith heaps were moistened and monitored daily for 30 day. A control composting experiment using the conventional method was also carried out simultaneously.

### 2.3 Field Experiment

Fifteen pots were used for the study. Out of this, first five pots were filled with garden soil, next five with biodegraded coir pith with *Pleurotus sajor caju* and *Azotobacter vinelandii* and last five with biodegraded coir pith with *Pleurotus sajor caju* and *Azospirillum brasilense.* Plant the five different ornamental plant saplings to each set of pots. All the experiments conducted in duplicates. Keep the pots in place with sufficient sunlight. Pots were moistened regularly to enable good growth of the plants.

## 2.4 Estimation of Physical Properties.

### 2.4.1 Shoot Length.

The shoot length was estimated with a measurement tape.

### 2.4.2 Root Length.

The root length was estimated by a measurement tape.

## 2.5 Estimation of Chemical Properties.

### 2.5.1 Carbohydrate (Lee & Tournsean., 1958).

### 2.5.2 Protein (Lowry *et al*., 1951).

### 2.5.3 Chlorophyll (Arnon., 1949)

# 3 Results.

The increase in length of shoot and root shows the growth of plants. It is used as a measurement for the effective growth of plants in plant cultivation experiments.

## 3.1 Physical Properties

### 3.1.1 Shoot Length

In all the five experimental plants, shoot length is observed to be increased in plants grown in biodegraded coir pith with combination of *P. sajor caju* and nitrogen fixing bacteria (*A. vinelandii* and *A. brasilense*). *Rosa rubiginosa* show enhanced shoot length in pots containing coir pith composted with *P. sajor caju* and *A. vinelandii* (32.8 cm), the same in *P. sajor caju* and *A. brasilense* have a shoot length of 30.6 cm and the plant cultivated in garden soil alone show reduced shoot length (28.0 cm). In cases of all the other medicinal plants, the shoot length is enhanced with *P. sajor caju* and nitrogen fixing bacteria.

### 3.1.2 Root Length

Root lengths of five experimental ornamental plants were observed and the result shows an increase in the root lengths of plants grown in biodegraded coir pith with *P. sajor caju* and nitrogen fixing bacteria. Root length of 8.6 cm was observed on *Rosa rubiginosa* cultivated in garden soil alone. An increase of 9.4 cm and 9.8 cm were observed in plant cultivated in BCC with *P. sajor caju* and both nitrogen fixing bacteria, *A. vinelandii* and *A. brasilense* respectively. In other four

experimental plants also, a definite increase in root length were recorded in plants cultivated In Jasmine sambac the length of shoot observed as 18.4 cm and 16.5 cm for *A. vinelandii* and *A. brasilense* respectively. In *Aralia elata*, it was 10.8 cm and 10.4, in *Gardenia jasminoides* it was 22.2 and 20.5 and in *Chrysalidocarpus lutescens*, it was 36.4 cm and 37.5 respectively. Here the length of roots of plants grown in biodegraded coir pith show increased root length in comparison with garden soil.

## 3.2. Chemical Properties.

### 3.2.1. Carbohydrate.

Carbohydrate content of the plants grown in the more amount of biodegraded coir pith is enhanced (Fig III to V).

### 3.2.2. Protein.

Protein content of the plants grown in the more amount of biodegraded coir pith is enhanced (Fig III to V).

### 3.2.3. Chlorophyll.

Chlorophyll content of the plants grown in the more amount of biodegraded coir pith is enhanced (Fig III to V).

**Table VI e.** Physical properties of different Ornamental plants cultivated in biodegraded coir pith.

| Sl no | Potting media | *Rosa rubiginosa* | | *Jasmine sambae* | | *Aralia elata* | | *Gardenia jasminoides* | | *Chrysalidocarpus lutescens* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root |
| 1. | Garden soil | 28.0 | 8.6 | 16.1 | 6.7 | 8.9 | 6.5 | 18.1 | 8.2 | 36.2 | 10.1 |
| 2 | BCC (*P. sajor caju* + *A.vinelandii*) | 32.8 | 9.4 | 18.4 | 6.8 | 10.8 | 8.8 | 22.2 | 8.6 | 36.4 | 11.6 |
| 3 | BCC (*P. sajor caju* + *A. brasilense*) | 30.6 | 9.8 | 16.5 | 7.1 | 10.4 | 6.9 | 20.5 | 8.9 | 37.5 | 11.8 |

**Table VI e.** Chemical properties of different Ornamental plants cultivated in biodegraded coir pith.

| Sl no | Potting media | *Rosa rubiginosa* | | | *Jasmine sambae* | | | *Aralia elata* | | | *Gardenia jasminoides* | | | *Chrysalidocarpus lutescens* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pro | Car | Chl | Pro | Car | Chl | Pro | Car | Chl | Pro | Car | Chl | Pro | Car | Chl |
| 1. | Garden soil | 0.41 | 0.21 | 0.10 | 0.50 | 0.25 | 0.09 | 0.47 | 0.22 | 0.11 | 0.45 | 0.23 | 0.10 | 0.39 | 0.21 | 0.09 |
| 2 | BCC (*P. sajor caju* + *A.vinelandii*) | 0.77 | 0.31 | 0.18 | 0.81 | 0.29 | 0.18 | 0.78 | 0.30 | 0.18 | 0.68 | 0.35 | 0.19 | 0.75 | 0.29 | 0.19 |
| 3 | BCC (*P. sajor caju* + *A. brasilense*) | 0.78 | 0.30 | 0.20 | 0.75 | 0.31 | 0.18 | 0.69 | 0.32 | 0.21 | 0.68 | 0.32 | 0.20 | 0.71 | 0.29 | 0.20 |

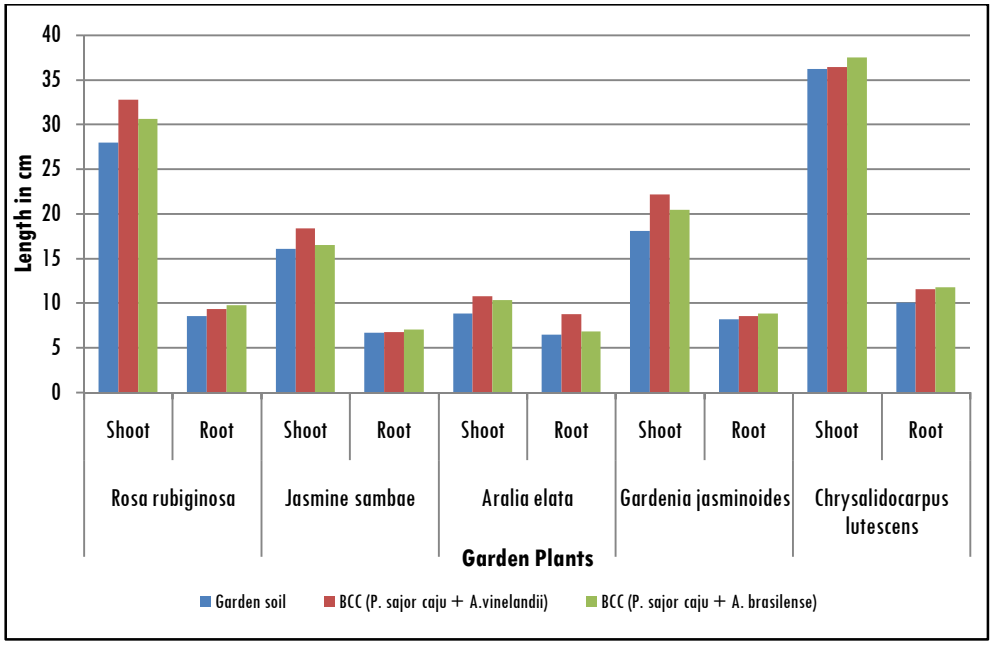



**Fig. VI j.** Effect of Biodegraded coir pith in Shoot and root length of Ornamental plants

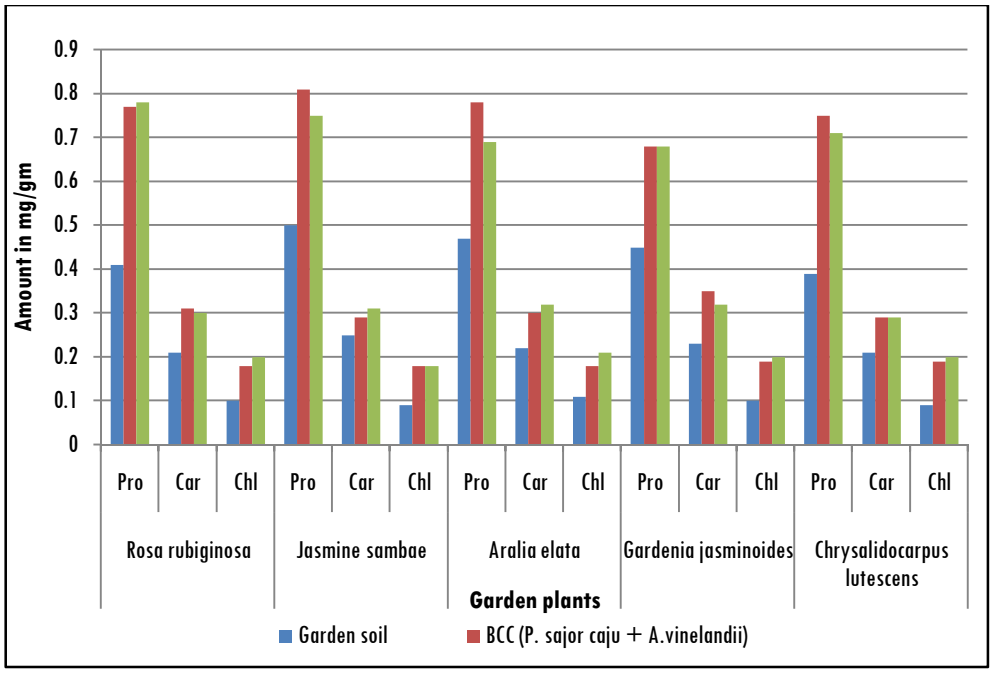



**Fig. VI k.** Effect of Biodegraded coir pith in Protein, Carbohydrate and Chlorophyll content of Ornamental plants

**Fig. VI l** *Rosa rubiginosa*

**Fig. VI m.** *Jasmine sambac*

**Fig VI n.** *Chrysalidocarpus lutescens*

**Fig VI o** *Aralia elata*

**Fig. VI p.** *Gardenia jasminoides*

**Fig. VI q.** Coir pith compost in different pots


**Fig VI r.** Packed Coir pith composted with *Pleurotus sajor caju* and *Azospirillum brasilense*


**Fig VI s.** Packed coir pith composted with *pleurotus sajor caju* and *azotobacter vinelandii.*

## 4. Discussion

This study on the biodegraded coir pith included analysis of Lignin, Organic carbon and NPK iin coir pith treated with *Pleurotus sajor caju* in combination with *Azotobacter vinelandii* and *Azospirillum brasilense* before and after the decomposition. The lignin content in the coir pith degraded by the combination of organisms is observed to vary with that in the raw untreated coir pith. The decomposition was observed to be 17% in samples treated with *P. sajor caju* and *A. vinelandii*. From the literature it is clear that the *Pleurotus* species are very efficient in decomposing various agricultural wastes (Mandhare *et al*., 2003).

The nitrogen, phosphorous and potassium contents show variation and enhance in content. The organic carbon is observed to decreases considerably. Treatments using both Azotobacter vinelandii & Azospirillum brasilense were given to the lignin content in the coir pith.

The pot culture experiment was carried out to study the effect of coir pith based potting media for garden plants *viz*. *Rosa rubiginosa, Jasmine sambae, Aralia elata, Gardenia jasminoides* and *Chrysalidocarpuslutescens*. The study was conducted over a period of 2 months as the plants grow to a standard size. Plants grown in the coir pith compost shows increased shoot and root length (Fig. II). *Rosa rubiginosa* show shoot length and root length 30.6 cm and 9.8 cm respectively in biodegraded coir pith (BCC) with *P. sajor caju* and *A. vinelandii*. In the treatment using *P. sajor caju* and *A. brasilense* the shoot and root length was observed to be 32.8 cm and 9.4 respectively. The shoot and root length of the same species grown in garden soil has been observed to be 28 cm and 8.6 cm respectively. Simultaneously in the case of *Jasminum sambae* the growth observed in garden soil was shoot and root length of 16.1 and 6.7 cm respectively. Effective growth in pots containing composted coir pith was also observed in *Aralia elata*. In all the species, longer shoot and root length was observed when the plants were cultivated in coir pith composted using combination of microorganisms. This composted coir pith provides NPK enrichment in pot culture. This could be attributed to the medium providing easy movement of roots leading to increased growth of plants. Biodegraded coir pith was observed to be efficient for the cultivation of medicinal plants such as *Phyllanthus amaranthus*, *Andrographis paniculata, Bacopa monneiri* and *Piper longum* (Reghuvaran and Ravindranath, 2010). A considerable increase in growth of rice plants has also been reported using coir pith based cyanobacterial biofertilizer (Krishna Veni., 1999 and Lavanyapriya., 1997). In the present study, the potting medium consisting of biodegraded coir pith has proved to be an efficient cultivating medium for garden plants.

Analysis of Protein, Carbohydrate and Chlorophyll content in the plants also display varying values for garden soil and biodegraded coir pith (Fig. VI e). In the case of *Rosa rubiginosa*, the protein content estimated was observed to be 0.41 mg/g in garden soil medium, the pots with coir pith compost medium show variation in protein content. In case of coir pith composted with *P. sajor caju* and *Azotobacter vinelandii* the protein content was observed 0.77 mg/g and the same with *P. sajor caju* and *Azospirillum brasilense* was 0.78 mg/g. The results of the other four species also show almost similar fluctuations in case of compost. The chlorophyll content and carbohydrate content also show similar variation. In the case of garden soil, the value for carbohydrate and chlorophyll is 0.10 mg/g and 0.21 mg/g respectively for *Rosa rubiginosa.* The biodegraded coir pith with *P. sajor caju* & *Azotobacter vinelandii* and *P. sajor caju* & *Azospirillum brasilense* have shown an increase in the carbohydrate and chlorophyll content when compared to garden soil. In the case of *Jasmine sambae*, the protein, carbohydrate and chlorophyll contents are observed to be 0.50 mg/g, 0.25 mg/g and 0.09 mg/g respectively. In all the plants, an increase in protein, carbohydrate and chlorophyll content has been observed in the plants treated with BCC when compared to garden soil. This confirms the findings of Lokesha *et al*., 1988 which reported that high percentage of rooting of acalypha and bougainvillea was observed in coir dust medium when compared to sand or soil+organic manure. Earlier, Baskaran and Saravanan (1997) have observed that 100% coir pith medium showed a spectacular increase of the water holding capacity of the potting mixture when tomato plants were grown on coir pith based potting mixture grown. Coir pith also has great potential for use as a source of many useful microorganisms including nitrogen fixing bacteria (Reghuvaran *et al*., 2012). The use of coir pith as a soil conditioner in tropical farming is well documented whereas that of utilization of coir pith as potting medium is very limited (Saravanan and Nambisan, 1995). The present study confirms that coir pith composted with *P. sajor caju*, *Azotobacter vinelandii*

and *Azospirillum brasilense* form an excellent potting media for roof gardening as soil less medium.

Coir pith composted with *Pleurotus sajor caju* and *Azotobacter vinelandii* has been marketed as AZOTO-V (Fig. VIII) and the same with *Pleurotus sajor caju* and *Azospirillum brasilense* can also be named AZOSPIRIL-B (Fig VII) and packed in 1 kg packets marketed for cultivation of ornamental plants mainly for roof gardening avoid soil for the cultivation.

## 5. Conclusions

Nitrogen fixing bacteria are excellent and eco-friendly substitute for urea during composting of coir pith using mushroom spawn of *P. sajor caju.* Composted coir pith based potting medium for cultivation of ornamental plants suggests that this compost can be used as substrate of soils due to its enhanced productivity. Coir pith composted with a combination of fungus and nitrogen fixing bacteria could be excellent potting media for cultivation of garden plants. Growth is observed to be better than garden soil for the cultivation of plants, therefore can be used as a soilless media for cultivation of ornamental plants. Use of this compost can replace garden soil in roof gardening practices, thereby reduce the weight exerted on terrace for roof gardening. Being efficient for cultivation of plants, the biodegraded coir pith can be marketed by the brand names AZOTO-V and AZOSPIRIL-B and could be cost effective medium for ornamental plants for the use of farmers. This study will encourage those involved in the cultivation of ornamental plants to adopt organic, economical and environmental friendly ways for roof gardening practices.

Chapter 7

# SUMMARY AND CONCLUSIONS

## Summary and Conclusions

Environmental pollution can be avoided by the development of clean technologies using microbial consortia and is one of the main areas of environmental biotechnology. Composting is a natural process that turns organic material into a dark rich substance. This substance, called compost or humus, is a wonderful conditioner for the soil. It also termed the process of bioconversion of waste into hygienic soil conditioner and fertilizer. Coir industry provides livelihood to a large work force in southern states of India viz. Kerala, Tamil Nadu, Karnataka and Andhra Pradesh. However accumulation of coir pith hillocks and coconut husk leachates causes pollution of both land and water in the environment.

White rot fungi are the group of fungi, which can degrade the lignin present in the coir pith. More often the presence of lignin is responsible for the difficulty of biodegradation of coir pith. The biodegradative ability of different fungal species Pleurotus *sajor caju, Pleurotus florida, Pleurotus eous* and *Calocybe indica* has been studied and the results revealed that all the tested white rot fungi degrade coir pith and reduce the percentage of lignin present in it together with enhancing the NPK and ammonia content.

Although coir pith is a problematic waste, it has been found to harbor useful micro-organisms with potential use as plant nutrient. Nitrogen fixing bacteria from the raw coir pith accumulated in coir fiber extraction units could be isolated and confirmed through molecular techniques. Therefore it can be

concluded that coir pith can be a source of many useful micro-organisms including nitrogen fixing bacteria.

In this study, a novel improved technology could be developed to convert the recalcitrant coir pith into environmental friendly organic manure. The standard method of composting involves the substitution of urea with nitrogen fixing bacteria *viz*. *Azotobacter vinelandii* and *Azospirillum brasilense* leading to the development of an improved method of coir pith. The combined action of the microorganisms could enhance the biodegradation of coir pith. In the present study, *Pleurotus sajor caju*, an edible mushroom which has the ability to degrade coir pith, and the addition of nitrogen fixing bacteria like *Azotobacter vinelandii* and *Azospirillum brasilense* could accelerate the action of the fungi on coir pith. The use of these microorganisms brings about definite changes in the NPK, Ammonia, Organic Carbon and Lignin contents in coir pith. This study will encourage the use of biodegraded coir pith as organic manure for agri/horti purpose to get better yields and can serve as a better technology to solve the problem of accumulated coir pith in coir based industries.

The efficiency of the biodegraded coir pith by this improved method was estimated by growing the plants in the compost. A number of medicinal and ornamental plants were cultivated in coir pith compost successfully. Four medicinal plants were used for the study and it was grown in pots filled with garden soil and compost. An increase in the growth of the plants was observed in the pots containing higher percentage of coir pith compost. Coir pith facilitates easy growth of plant roots and provides sufficient nutrients for its growth. In the case of ornamental plants, coir pith can be used exclusively without mixing garden soil. Therefore the composted coir pith by this improved method can be used as a soil less media for roof gardening practices.

Coir pith composted with *Pleurotus sajor caju* and *Azotobacter vinelandii* can be developed on a brand name AZOTO-V and that with *Pleurotus sajor caju*

and *Azospirillum brasilense* can be named AZOSPIRIL-B, packed in 1 kg packets and marketed for cultivation of ornamental plants mainly for roof gardening replacing garden soil.

**COSTING FOR COIR PITH COMPOST**

**BASIS. 5 MT**

**RECURRING EXPENDITURE**

| | |
|---|---|
| 1. PITHPLUS @ Rs.50 / KG | 500.00 |
| 2. *Azotobacter/Azospirillum* @ Rs.50 / KG | 100.00 |
| 3. LABOUR CHARGE @ Rs.300 / HEAD | 600.00 |
| 4. OVERHEAD | 100.00 |
| TOTAL | Rs.1300.00 |

i.e. 0.26 /= per Kg.

say, 26 paise per Kg.

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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 15$^{th}$ day of composting and 30$^{th}$ 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 30$^{th}$ day, it was evident that the values showed a marginal increase in all the treatments when compared to 15$^{th}$ day of treatment; however the increase noticed was not significant.

The organic carbon content of coir pith on 15$^{th}$ and 30$^{th}$ 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

**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 |

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.

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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# Efficacy of biodegraded coir pith for cultivation of medicinal plants

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This study presents coir pith biodegradation with white rot fungus and nitrogen fixing bacteria for cultivation of medicinal plants (*Phyllanthus amaranthus. Andrographis paniculata. Bacopa monneiri. Piper longum*). Proportion (25% garden soil and 75% compost) yielded an effective growth of all medicinal plants. Thus, composted coir pith with nitrogen fixing bacteria is as an effective potting medium for cultivation of medicinal plants.

**Keywords**: Coir pith, Lignin, Medicinal plants, Potting mixture, Soil reclamation

## Introduction

Coir pith or coir dust is major byproduct 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 lignocelluloses in plant biomass[5]. Degradation of coir pith can be effectively done with suitable species of Bacideomycetes fungus (*Pleurotus sajor caju*) and in combination with nitrogen fixing bacteria[1]. Lignin[6] ($3\times10^{11}$ metric tons on planet with an annual biosynthetic rate of $2\times10^{10}$ tons) constitutes second most abundant group of biopolymers in biosphere. 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]. Coir pith (100%) used as potting medium showed a spectacular increase of water holding capacity of potting mixture when tomato plants were grown on coir pith based potting mixture[8].

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Among medicinal plants, brahmi (*Bacopa monnieri*), a well known prostrate herb, is distributed in damp, marshy areas throughout India. *Piper longum* can be cultivated successfully in organic matter rich fertile well drained forest soils. It is of South Asian origin and is found almost all over India. It is a component of Indian medicine reported as remedy for treating gonorrhea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infections, chronic gut related pain, arthritis and alleviation of anxiety[9]. *Phyllanthus niruri* originated in India, usually occur as a winter weed throughout the hotter parts. *P. niruri* is a herb of Euphorbiaceae family that grows upto 60 cm. In recent years, more and more people are complimenting their treatment with natural supplements[10]. Kalmegh (*Andrographis paniculata*) as ethanolic extract has an insulin sensitizing effect[11]. Coconut coir dust is being marketed as a soilless medium substitute for sphagnum peat moss that inhibits fungus gnat (*Bradysia* sp.) development[12]. Several other important studies also conducted on antioxidant activity of some medicinal plants grown under organic farming conditions[13]. Growth of *A. paniculata* in vermicomposted coir pith has suggested that vermicomposted coir pith could be helpful for reclamation of soils from industrial sites for cultivation of *A. paniculata* in a small scale nursery[14]. Aqueous extract of leaves of *A. paniculata* has traditionally been used for treatment of various liver disorders and jaundice[15].

Table 1—Lignin, organic carbon, nitrogen, phosphorous and potassium content of raw and biodegraded coir pith with mushroom species

| Sl No. | Treatment | Lignin | Organic carbon | Nitrogen | Phosphorous | Potassium |
|---|---|---|---|---|---|---|
| 1 | Garden soil, kg/ha | - | 0.63 | 66.92 | 15.32 | 268.8 |
| 2 | Raw coir pith, % | 32 | 14.93 | 1.293 | 27.5 | 0.046 |
| 3 | Coir pith+*P sajor caju*+*Acetobacter*, % | 20 | 7.76 | 0.672 | 19.8 | 0.046 |
| 4 | Coir pith+ *P sajor caju*+*Azospirillum*, % | 19 | 6.27 | 0.543 | 15.4 | 0.038 |
| 5 | Coir pith+ *P sajor caju*+Urea, % | 20 | 5.37 | 0.465 | 13.2 | 0.050 |

This study presents coir pith biodegradation with white rot fungus and nitrogen fixing bacteria for cultivation of medicinal plants (*A. paniculata, B. monneiri, P. amaranthus,* and *P. longum*).

## Experimental Section

Coir pith was collected from Cherthala, Alapuzha district, India. Microbial species procured from Microbiology division of CCRI, Alappuzha. Experimental protocol, field experiment and biochemical analysis of coir pith was carried out at Rajiv Gandhi Chair in Contemporary Studies, CUSAT.

### Composting Process

Four lots of coir pith (1 kg each) in duplicate were laid as separate heaps. *P. sajor caju* (12 g) spawn and N-fixing bacteria (*Acetobactor*) were thoroughly mixed with first lot of coir pith. Same amount of fungus was supplemented with 5 g of N-fixing bacteria (*Azospirillum*) and added to second sample. N-fixing bacteria was immobilized in lignite as carrier. Third sample of coir pith was treated with mushroom (12 g) and Urea (5 g). Fourth sample was kept as untreated. Moisture content was maintained and study was monitored regularly for 45 days, by drawing out samples at periodic intervals.

### Field Experiment

Four treatments (coir pith sample composted with *P. sajor caju* in combination with *Acetobacter, Azospirillum*, urea and fourth one kept as untreated as raw coir pith itself) were compared with control (garden soil). Four sets of 13 pots were used for cultivation of medicinal plants. Each set of pot was maintained for each species of medicinal plant. Out of 13, first one was filled with garden soil. Second, third and fourth were filled with a mixture of garden soil and compost [composted with *P. sajor caju* and N-fixing bacteria (*Acetobactor*) in proportion of 1:1, 1:3 and 3:1]. Fifth, sixth and seventh pots were filled with garden soil and compost (with mushroom and *Azospirillum*). Eighth, ninth and tenth pots were filled with mixture of garden soil and compost (with mushroom and urea) and last three pots were filled with mixture of garden soil and raw coir pith. All treatments were carried out in duplicate. After 45 days, measurements were made of shoot and root length. Aqueous extract of leaf was analyzed for carbohydrate[16], protein[17], and chlorophyll[18].

### Constituent Analysis of Coir pith and Soil

Analysis of lignin was carried out by Modefied Klason Lignin assay, N by Kjel dahl method[19], organic carbon by Walkey and Black method[20], P by Spectrophotometer and K by Flame photometer.

## Results and Discussion

Lignin content, organic carbon and NPK of garden soil sample and coir pith before and after decomposition with *P. sajor caju* and N-fixing bacteria were estimated (Table 1).Lignin content of raw coir pith (32%) varied after biodegradation with a combination of organisms [*P. sajor caju* and N- fixing bacteria (*Acetobacter* and *Azospirillum*) and urea]. Decomposition was up to 19% with *P.sajor caju* and *Azospirillum. Pleurotus spp.* is known to decompose and utilize various agricultural wastes[21]. From current results, an increase in nitrogen component of substrate, and a proportionate decrease in carbon were also observed as also reported in earlier works[22-24]. A salient finding observed is that concentration of nitrogen in spent substrates increased with reduction in C: N ratio[25]. In all treatments, a reduction was observed of lignin content, which confirms action of microbial

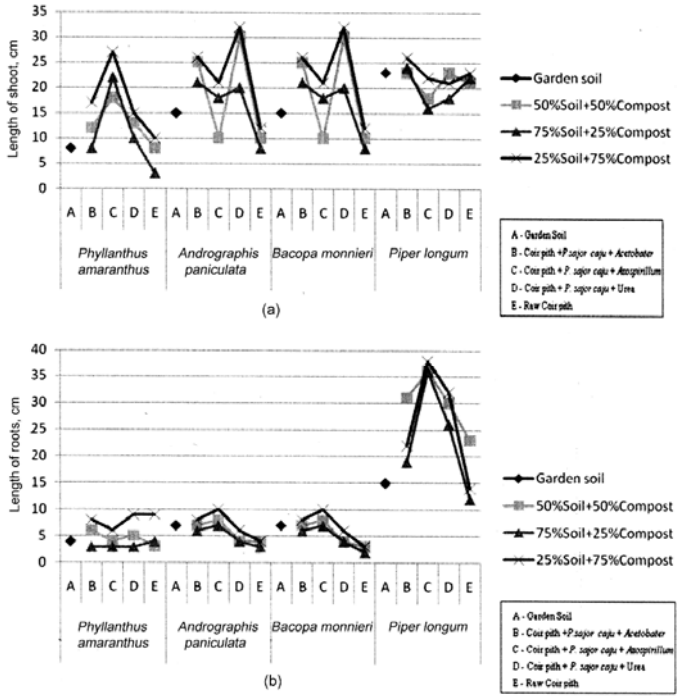


Fig. 1—Effect of biodegraded coir pith on: a) Shoot length of medicinal plants; b) Root length of medicinal plants

agents effectively. There was a definite reduction in organic carbon content of raw coir pith when treated with mushroom and bacteria.

A pot culture experiment was carried out to study effect of coir pith based potting mixture with garden soil on four different medicinal plants (P. amaranthus, *A. paniculata, B. monneiri* and *P. longum*). Plants grown in pots containing higher percentage of coir pith than soil, show increased shoot and root length (Fig. 1). *P. amaranthus* shows shoot length and root length 8 cm and 4 cm in 100% garden soil, 27 cm and 6 cm respectively in pot containing 25% soil and 75% composted coir pith, which is biodegraded with *P. sajor caju* and *Azospirillum,* secondly with 75% soil and 25% coir pith, same treatment also yielded 22 cm shoot and 3 cm root length respectively. Thus, composted coir pith with the combination of mushroom and bacteria provide NPK enrichment on pot culture. This medium provides easy movement of roots and thereby increased growth of plants. In case of *P. sajor caju* and *Acetobacter*, biodegraded coir pith compost (BCC) medium, values are 17 cm, 12 cm and 8 cm (shoot) in 25% soil+75% compost, 50% soil+50% compost and 75% soil+25% compost respectively. Root length was observed to be 8 cm, 6 cm and 3 cm for 25% soil+75% compost, 50% soil+50% compost and 75% soil+25% compost respectively. In case of BCC with *P. sajor caju* and urea also similar results were observed, but values were comparatively lower than that composted with N-fixing bacteria.

*A. paniculata* also shows effective growth in pots containing higher content of BCC; using *Acetobacter* shows improved growth in medium with compost and soil. In case of *B. monnieri* and *P. longum*, higher growth

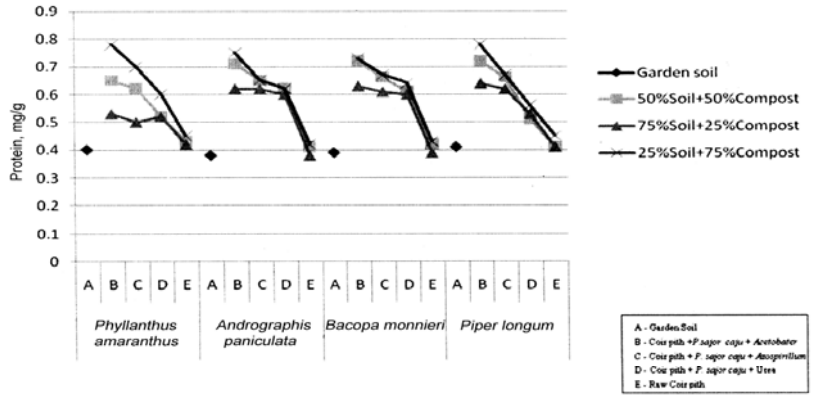


Fig. 2—Effect of biodegraded coir pith on protein content in medicinal plants

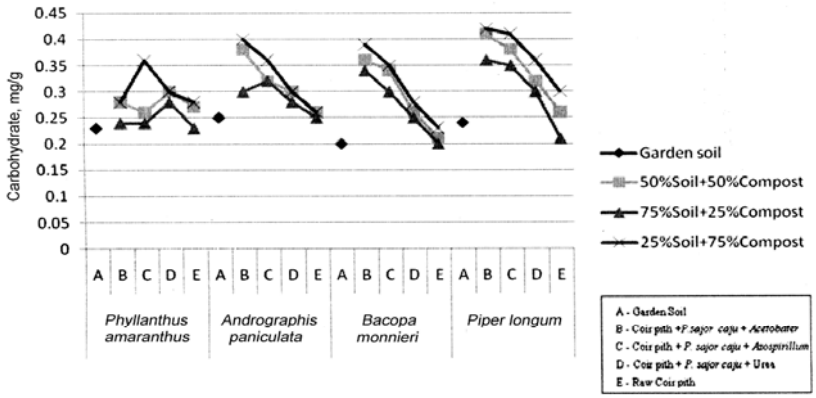


Fig. 3—Effect of biodegraded coir pith on carbohydrate content in medicinal plants

was observed in BCC using *Acetobacter*. Similar results were observed in studies using N-fixing bacteria. Medium containing coir pith: shoal leaf mould: sand (60:20:20 and 50:25:25 v/v) had higher cation exchange capacity (CEC) and water holding capacity (WHC)[26]. Coir pith is very efficient for tropical farming as soil conditioner and cyanobacteria are also capable of biodegrading coir pith with various kinds of pollutants[27]. A considerable increase in growth of rice plants is also observed using coir pith based cyanobacterial biofertilizer[28,29]. In present study, potting medium, composed of coir pith along with soil, is an efficient soil conditioner, and BCC using *P. sajor caju* and N-fixing bacteria show significant increase in growth due to its enriched NPK content.

In case of *P. amaranthus*, protein content estimated was 0.40 mg/g in 100% soil (Fig. 2). But pots with combination of coir pith and soil shows variation in protein content. In case of coir pith composted with *P. sajor caju* and *Acetobacter*, protein content obtained was 0.78 mg/g, 0.65 mg/g and 0.53 mg/g in 25% soil+75% compost, 50% soil+50% compost and 75% soil+25% compost respectively.

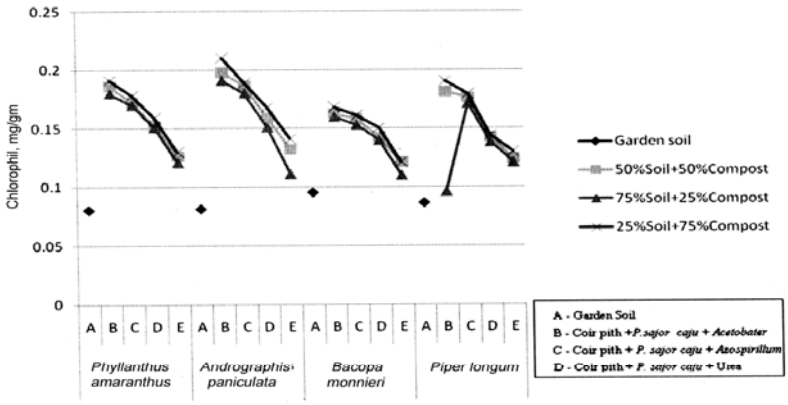


Fig. 4—Effect of biodegraded coir pith on chlorophyll content in medicinal plants

Carbohydrate (Fig. 3) and chlorophyll (Fig. 4) content also show similar variation. Garden soil alone shows 0.23 mg/g and 0.080 mg/g for carbohydrate and chlorophyll respectively. Combinations (*P. sajor caju* &*Acetobacter, P. sajor caju* & *Azospirillum, P. sajor caju* & urea) and raw coir pith have increase in carbohydrate and chlorophyll content than garden soil. In case of *A. paniculata,* parameters of protein, carbohydrate and chlorophyll are found to be 0.38mg/g, 0.25mg/g and 0.081mg/g respectively for garden soil. Composted and raw coir pith for *A. paniculata* shows variations. An increase in protein, chlorophyll and carbohydrate content has been observed in plants treated with BCC when compared with garden soil. Values for protein content were 0.75mg/g, 0.71mg/g & 0.62mg/g, carbohydrate content 0.40mg/g, 0.38mg/g & 0.30mg/g and chlorophyll 0.210mg/g, 0.198mg/g and 0.191mg/g for 25% soil+75% compost, 50% soil+50% compost and 75% soil+25% compost respectively. Similar results were observed in case of BCC with *Azospirillum*, urea and untreated raw coir pith, which show definite increase in protein, carbohydrate and chlorophyll content. In case of *B. monneiri* and *P. longum,* increased protein, carbohydrate and chlorophyll contents was observed in coir pith based potting mixtures. It is also reported that high percentage of rooting of acalypha and bougainvillea was observed in coir dust medium when compared to sand or soil+organic manure[30]. Thus, present study confirms that composted coir pith with *P. sajor caju* and N-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.

## Conclusions

N-fixing bacteria are excellent and eco-friendly substitute for urea for composting of coir pith with *P. sajor caju.* Composted coir pith based potting medium for cultivation of medicinal plants suggested that this compost can be used in reclamation of soils for its enhanced production, and also possibly other crops. This study will encourage those involved in cultivation of medicinal plants organically, economically and using environmental friendly ways.

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First author thanks Prof N Chandramohanakumar, Rajiv Gandhi Chair in Contemporary Studies (RGCCS) for permission to carry out this investigation. Authors also thank Mr Arun Augustine, K Pillai Raji, and Mrs Kala K Jacob, RGCCS, for continuous encouragement and support.

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*Full Length Research Paper*

# Biochemical aspects and formation of phenolic compounds by coir pith degraded by *Pleurotus sajor caju*

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**Coir pith is a byproduct of fibre extraction from coconut husk. Coir pith has been produced in large quantities and is dumped as waste as its bulk at the production site itself and disposal efforts are in vain owing to the quantity. Several biological, biochemical and microbial methods have been tried and are underway to degrade the coir pith into useful product. A basidiomycete fungus *viz. Pleurotus sajor caju*, has the ability to slowly degrade the coir pith and is capable of detoxifying phenolic compounds by producing biopolymerizing enzymes. The present work is targeted to degrade the biochemical constituent present in coir pith includes lignin which is considered as recalcitrant under normal conditions and the production of phenolic compounds which are the break down products of lignin. The enhancement of Nitrogen, phosphorous and potassium (NPK) also shows the enrichment of the compost. Biodegraded product can be used as efficient organic manure and as hydroponic systems for growing roses and vegetables.**

**Key words:** Coir pith, lignin, biodegradation, basidiomycete.

## INTRODUCTION

Coconut (*Cocos nucifera*) is cultivated in tropical countries. The fibrous mesocarp of coir is used to make ropes. The waste of coir yarn industry (coir pith) gets accumulated in large quantities making their disposal difficult, though it is used as soil conditioner (Christopher et al., 2007). India is the leading producers of coconut. It is an important oil seed and cash crop grown in south Indian states especially in Kerala, Tamil Nadu and Karnataka. About 7.5× $10^5$ tons of coir pith is produced annually in India (Pillai et al., 1952). It is available either from retted or unretted processing industries of coir fiber, where for every ton of fiber extracted, the coir dust is produced to the extent of 2 tons. Coir pith constitutes as much as 70% of the coconut husk. It is estimated that, at present there is an accumulated stock of 10×$10^6$ metric tons of coir pith in the southern states of India (Ghosh et al., 2007). Fiber extracted from husk is used in production of mats, matting, rubberized coir mattresses, yarn, ropes etc. After extraction of coir fiber from husk, the, coir pith is unutilized (Photograph 1 and 2).

Coir pith is known as coir dust and is the major by-product of the coir fiber extraction industries. It is a ligocellulosic waste material consists of lignin 20 to 40%, cellulose 40 to 50%, hemicelulose 15 to 35% and protein 2.04% (Sjostrom, 1993). It has a high water holding capacity of 8 times its weight. 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. It is essentially 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). It should be noted that coir pith is resistant to

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**Photograph 1.** Accumulation of coir pith on coir fiber extraction industries.

**Photograph 2.** Collection of sample by the author.

biodegradation due to the presence of lignin (33 to 35%). Lignins constitute the second most abundant group of biopolymers in the biosphere. It is estimated that the planet currently contains $3\times10^{11}$ metric tons of lignin with an annual biosynthetic rate of approximately $2\times10^{10}$ tons (Argyropoulos and Menachem, 1997). It is an aromatic polymer composed of phenyl propane subunits including coumaryl, guaicyl and syringyl moeities that are covalently linked together by a variety of bonds, mainly β-aryl ether bonds. It is also present in the fiber and is responsible for the stiffness of coir. Oyster mushroom belonging to *Pleurotus* species has the ability to degrade lignin slowly under favorable conditions. This is reason for the selection of a suitable species of basidiomycetes

fungus called *Pleurotus sajor caju*, which has the ability to slowly degrade. The cellulosic compounds present in the coir waste support the initial growth of this fungus and acts as co-substrate for lignin degradation.

The lignin degradation in nature has been considered to occur by the action of wood rot fungi mostly of the basidiomycete class (Odier et al., 1981). Most of the mushroom species have bee recognized for their property of degradation of the natural lignocellulosic wastes, in their original form or preformed (composted) form (Somasundaram Rajarathnam et al., 1998). White rot fungi, which have lignocelluloses degrading enzymes, play important roles in carbon recycling in nature, because lignin, next to cellulose is the second most abundant organic compound on earth (Kanmani et al., 2009). It is the most efficient lignin degraders, due to their ligninolytic system which is comprised of manganese peroxidase (MnP), lignin peroxidase (LiP) and laccase (Hatakka, 1994; Higuchi, 2004). It is due to their powerful degrading capabilities towards varius recalcitrant chemicals, white rot fungi and their lignin degrading enzymes have long been studied for biotechnical applications such as bioleaching (Takano et al., 2001). 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 lycine, threonine and tryptophan which are present in appreciable amount. It degrades the phenolic group, as observed by the decrease 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 has drawn considerable attention as an appropriate host for the production of lignin degrading enzymes or direct application in lignocelluloses bioconversion processes (Ruggeri and Sassi, 2003; Bosco et al., 1999). White rot fungi in partilular, having great importances due to their potential use of their enzymes for bioremediation, industrial and biotechnological applications (Jordaan et al., 2004; Novotny et al., 2004).

Studies of Lignin biodegradation are also of great importance for possible biotechnological applications, since lignin polymers are a major obstacle to the efficient utilization of lignocellulosic material in a wide range of industrial processes (Eggert et al., 1996). Lignin in favorable conditions degraded to form phenolic components such as resorcinol, guaicol and catechol. Resorcinol otherwise called resorcinol, which is a chemical compound from the dihydroxy phenols. It is the 1, 3- isomer of benzenediol with the formula C6H4 $(OH)_2$. Resorcinol crystallizes from benzene as colorless needles which are readily soluble in water, alcohol and ether, but insoluble in chloroform and carbon disulfide. IUPAC name of Guaicol is 2-methoxy phenol having the molecular formula $C_7H_8O_2$, which is a naturally occurring organic compound with the formula $C_6H_4$ $(OH)(OCH_3)$. This colourless aromatic oil is derived from guaiacum or wood creosote. Samples darken upon exposure to air and light. Guaiacol is present in wood smoke, resulting from the pyrolysis of lignin. Catechol, formerly known as pyrocatechol, or 1, 2-dihydroxybenzene, is an organic compound with the semi-empirical formula $C_6H_4$ $(OH)_2$. It is the *ortho* isomer, one of three isomeric benzenediols. This colourless compound occurs naturally in trace amounts. About 20 M kg are produced annually, mainly as a precursor to pesticides, flavors and fragrances.

## MATERIALS AND METHODS

Coir pith collected from the fibre extracted units in areas of Cherthala, Alappuzha district in Kerala was used in the study. The *Pleurotus sajor caju* procured from the microbiology division of 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 2010 to November 2010.

### Composting

One 1 Kg of washed coir pith in duplicates was laid on a shady area. Added 12 g of *Pleurotus sajor caju* spawn was thoroughly mixed with the sample of coir pith. Five grams of urea (M.W 60.06, SRL chemicals, Pvt Ltd) were added to the foregoing substrate. The heap was moistened by sprinkling water to maintain moisture to 200% and monitored for 30 days.

### Chemicals

All chemicals (urea (extra pure), catechol, resorcinol and guaicol) and reagents were purchased from sisco research laboratories, Bombay, India.

### Analysis

The composted coir pith was subjected to analysis for lignin content, organic cabon content, NPK, formation of phenolic compounds such as resorcinol, guaicol and catechol. Lignin was estimated by modefied Klason lignin assay method, Nitrogen was estimated by Kjel dahl method (Vogel, 1961) organic carbon by Walkey (1934) method, the estimation of phosphorous was done with spectrophotometry (CARY 50 Probe, UV-Visible spectrophotometer), potassium by flame photometry (ELICO flamephotometer CL 378, range 50-1000 ppm) and the estimation of phenolics by Thin Layer Chromatography (TLC). Thin layer chromatography was done with a flat plate of ordinary glass 20×20 $cm^2$ was washed with hot water and dried in oven. Prepare slurry of silica gel G in distilled water by mixing 30 g of silica gel G in 75 ml of distilled water. Stir the slurry evenly to the glass plate and allowed to dry. Apply the samples and the standards to one side of the plate with a micropipette or capillary tube as small spots. Pour the developing solvent system (benzene: ethyl acetate: acetic acid, 85: 15: 1) into a glass tank. Place the thin layer plates to this tank so that it stands in the solvents with the spotted end dipping in solvent. Once the solvent reaches three-fourth of the plate, remove It from the tank, dry and were exposed to iodine vapour. The

**Table 1.** Lignin Content,pH and organic carbon content of raw and treated Coir pith mushroom species.

| S/ No | Treatment sample | Lignin content | | pH | | Organic carbon | |
|---|---|---|---|---|---|---|---|
| | | 30th day (%) | 30th day (%) | 15th day (%) | 30th day (%) | 15th day (%) | 30th day (%) |
| 1 | Raw coir pith | 32 | 32 | 6.3 | 6.3 | 6.28 | 6.28 |
| 2 | Composting with *Pleurotus sajor caju* | 32 | 20 | 6.7 | 6.8 | 6.17 | 6.16 |

**Table 2.** Nitrogen, phosphorous and potassium contents of raw and treated coir pith mushroom species.

| S/No | Treatment sample | Nitrogen | | Phosphorous | | Potassium | |
|---|---|---|---|---|---|---|---|
| | | 15th day (%) | 30th day (%) | 15th day (%) | 30th day (%) | 15th day (%) | 30th day (%) |
| 1 | Raw coir pith | 0.73 | 0.73 | 0.24 | 0.24 | 0.28 | 0.28 |
| 2 | Composting with *Pleurotus sajor caju* | 0.84 | 0.86 | 0.29 | 0.48 | 0.42 | 0.41 |

samples then identified by comparing the Rf value of the standards.

## RESULTS AND DISCUSSION

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

Composting is the most suitable technique for transforming organic waste into usable agricultural amendments (Vargas et al., 2007). Although the waste composition is very diverse, lignocelluloses is the most abundant component which is responsible for limiting degradation (Dixon and Langer, 2006). The lignin content of raw coir pith has observed as 32% lignin. Where as the coir pith when treated with *Pleurotus sajor caju*, the lignin content showed varying levels. The decomposition is to 30.4%. It is observed that the amount of lignin in the sample first increased from 32 to 35% in the first five days. Then the amount of lignin started decreasing and in a span of thirty days the lignin content decreased to about 20%. The decrease in lignin content can be explained by the hypothesis that may be the fungi first extracts all the lignin from the cells and then acts on it. Our experiment proved that by the action of *Pleurotus sajor caju* the amount of lignin could be reduced from 32 to 20%. It is also seen that the action of *Pleurotus sajor caju* on the washed sample of coir pith is more than that on the unwashed sample. This can be explained by the fact that washing exposes the cell walls thus increasing the surface area for decomposition.

The pH of the raw coir pith on 15th day and 30th day of composting did not show any variation and it remained at 6.3. The pH was showed acidic and at no point of time it was either neutral or towards alkaline, however without the difference, it 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 *Pleurotus sajor caju* showed that a decrease in the carbon content from 6.28 to 6.17 (15 days) and the 30th day sample shows the reduction of carbon 6.16%. This shows the organic carbon in the pith is utilized by the organism and break down to form its products.

Nitrogen, phosphorous and potassium (NPK) content of raw and treated coir pith with mushroom species (*Pleurotus sajor caju*) on 15th and 30th day of composting are presented in Table 2 and Figure 1. The results of the study revealed some interesting information on different variables on account of decomposition. The nitrogen content of the raw coir pith was 0.73%, but after the treatment of 15 days of treatment, the amount of the same is increased to 0.84% and which is again increased to 0.86%. In the case of Phosphorous, the raw coir pith holds 0.24% and which in reach to 0.29% in 15th day and which again increased to 0.48% after the time of

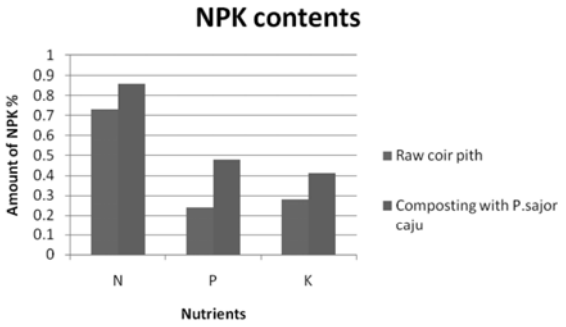


**Figure 1.** Nitrogen, phosphorous and potassium content of raw and biodegraded coir pith.

composting (30th day). The same enrichment of the nutrient amount is also observed in the case of Potassium also the, it accounts 0.28% potassium in the case of raw coir pith. After the degradation of 15 days it was increased to 0.42%.But there was a bit reduced amount of Potassium was observed when it reached to the 30th day (0.41%) (Figure 1).

It is observed from the available reports, the mushroom species with its enzymatic action degrade only the lignin content and leaving the other components intact (Arora et al., 2002). But the present study confirms that when the coir pith was treated with the mushroom *Pleurotus sajor caju*, the nutrient content of coir pith such as Nitrogen, Phosphorous and Potassium showed the variation and it get increased. In addition to the fungi, several reports brought strong evidence of some bacteria can degrade lignin effectively (Crawford, 1978; Haider et al., 1978; Kawakami., 1976; Trojanowski et al., 1977).

Cullen and Kersten (2004) reported that the enzymes from white rot fungi that catalyse the initial depolymerization of lignin are extra cellular and unusually non specific. The lignin degradation of white rot fungi is extensively studied, and their results revealed that the three kinds of extra cellular phenol oxidases, namely lignin peroxidases (LiP) Manganese peroxidase (MnP) and laccase (Lac) are responsible for initiating the depolymerization of lignin(Moriya 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 20% with the treatment of coir pith with the mushroom. From this it may be described that these enzymes also produced by Mushroom and are involved in the lignin polymerization.

This work is in harmony with the findings of Reghuvaran et al. (2009) that they indicate the degradation of coir pith different mushroom species in combination with the bacterial species. Instead of nitrogen fixing bacteria, here urea was used as the main nitrogen supplier and the action of the mushroom itself showed a definite capability to degrade the lignin and thereby exhibited a drastic reduction in lignin percentage. It is reported that the maximum yields of coconuts when coir pith compost was added with trace amounts of chemical fertilizers (Venkitaswamy, 2003). But the present work of composting with the mushroom species causes the enhancement of MPK content with in the coir pith compost itself by the action, and can cause the improved activity of organic manure.

The thin chromatographic results of the phenolic standards and the coir pith samples are given in Table 3. Spotted samples of coir pith along with phenolic standards such as resorcinol, guaicol and catechol. On comparing the values retention factor (Rf) of raw and coir pith samples, with these standards we can interpret the presence of phenolic compounds. It is very evident from the results that up to the fifteenth day sample, only onespot was obtained whose value corresponded to the value of catechol and guaicol. On the twenty-fifth and thirtieth day sample, three spots were obtained corresponding to catechol, guaicol and resorcinol. Thus it can be suggested that these phenolic compounds are formed during the degradation of coir pith. It can also state that these compounds are the breakdown products of lignin thus decreasing the total lignin content (Figure 2 to 4).

Thus the present study confirms that the mushroom species, *Pleurotus sajor caju* influences lignin degradation effectively. Coir pith is highly non-degradable mainly due to the presence of high percentage of lignin. It is one of the major pollutants of land and water in south Indian states. Our works find application in such places. From our work we can conclude that by the action of

**Table 3.** Thin layer chromatographic results of phenolic standards and coir pith samples.

| No of days | Solvant front(cm) | Solute front(cm) | | Rf value | Colour |
|---|---|---|---|---|---|
| 0th Day | | Resorcinol | 3.5 | 0.2554 | Yellow |
| | | Guaicol | 10.0 | 0.7299 | Brown |
| | | Catechol | 4.9 | 0.3576 | Brown |
| | | Raw coir pith | 5.5 | 0.4014 | White |
| | | Sample i | ---- | -------- | ------- |
| 5th Day | | Resorcinol | 6.0 | 0.4545 | Yellow |
| | | Guaicol | 11.4 | 0.8636 | Brown |
| | | Catechol | 6.8 | 0.5151 | Brown |
| | | Sample i | 5.4 | 0.4091 | White |
| 10th Day | | Resorcinol | 6.2 | 0.4460 | Yellow |
| | | Guaicol | 12.1 | 0.8705 | Brown |
| | | Catechol | 7.5 | 0.5395 | Brown |
| | | Sample i | 7.4 | 0.5323 | Brown |
| | | Sample ii | 7.7 | 0.5539 | Brown |

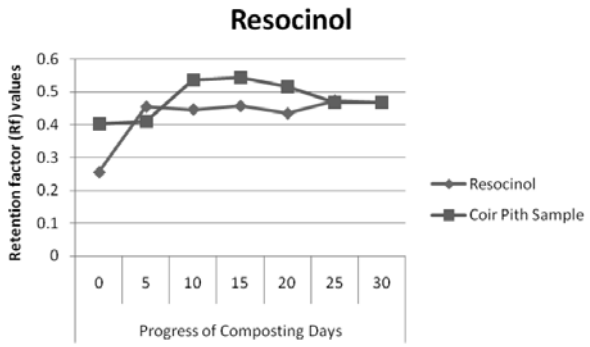


**Figure 2.** Enhancement of resorcinol on biodegraded coir pith sample.

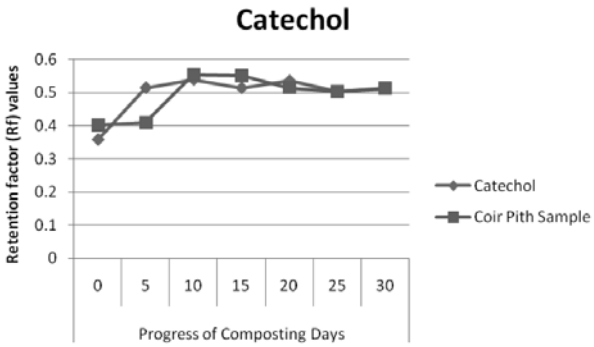


**Figure 3.** Enhancement of catechol on biodegraded coir pith sample.

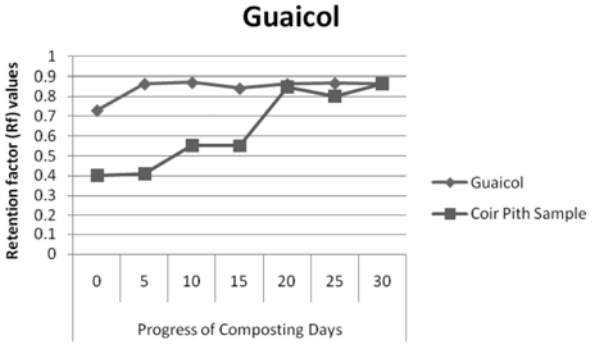


**Figure 4.** Enhancement of catechol on biodegraded coir pith sample.

*Pleurotus sajor caju*, the amount of lignin in the coir pith can be reduced considerably thus converting the waste pith into a useful product in an eco-friendly manner. The product obtained after bio-degradation can be used as manure and as hydr Coir pith oponic systems for growing roses and vegetables.

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*Full Length Research Paper*

# Isolation and characterization of nitrogen fixing bacteria from raw coir pith

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**Coir fibre is the hard fibre extracted from the coconut husk and coir pith is a lignocellulosic byproduct released during the extraction of coir fibre. The pith is not degraded under normal environmental conditions and accumulates in the fibre extraction units occupying sprawling space in the units. The inherent properties of coir pith make it useful as a plant nutrient. Nitrogen fixing bacteria could be isolated from the coir pith and four strains were amplified with *nifH* gene *viz.* NF-4 (*Lysinibacillus* sp.), NF-7 (*Ochrobactrum* sp.), NF-12 (*Paenibacillus* sp.) and an uncultured bacterial clone which shows only 50% similarity to NF-18 (*Clostridium* sp). The present study targeted the isolation and characterization of natural flora of nitrogen fixing organisms in the coir pith.**

**Key words:** Coir pith, *nif*H gene, nitrogen fixation, organic manure.

## INTRODUCTION

Coir pith or coir dust is a major byproduct of coir fiber extraction industries (Reghuvaran and Ravindranath, 2010). Normally, the pith is dumped as an agricultural waste and accumulates in the form of heaps of coarse and fine dust. Coir pith thus produced decomposes very slowly in the soil as its pentosan-lignin ratio is below 0.5 (Ghosh et al., 2007), and because of the chemical and structural complexity of its lignin-cellulose complex (Ramalingam et al., 2005). Large amounts of coir pith (approximately 7.5 million tons annually in India) accumulate nearby coir processing units, causing severe disposal problems, fire hazards and ground water contamination due to the release of phenolic compounds (Namasivayam et al., 2001). Coir pith contains 87% of organic matter, 6.28% organic carbon, 0.73% nitrogen (Reghuvaran and Ravindranath, 2010) and 13% of ash content (Thampan, 1987). Lignin is generally synthesized by polymerization of coniferyl, sinapyl and p-coumaryl alcohol to produce large molecules of indefinite size in which aromatic monomers are linked by a variety of chemical bonds. The structural feature has important implications for effective bio-degradation by microorganisms (Crawford and Crawford, 1976; McCarthy et al., 1984). It is estimated that 15,840 million coconuts are produced annually in India. In agro-industrial wastes; lignin is a main contributor of the total carbon producing polycyclic aromatic hydrocarbon components such as benzopyrine, catechol, hydroquinone, phenanthrene and naphthalene when degraded by heat (Kjallstrand et al., 1998). Coir pith is low in nitrogen content, C: N ratio mounting to 112:1 (Nagarajan et al., 1985). Microbial degradation of this waste is generally considered to be safe, effective and an environmental friendly process and certain mushrooms have showed good potentials for degrading coir pith (Vijaya et al., 2008).

Nitrogen fixation can be considered as one of the most interesting microbial activity as it makes the recycling of nitrogen on earth possible and gives a fundamental contribution to nitrogen homeostasis in the biosphere (Aquilantia et al., 2004). It is the reduction of $N_2$ (atmospheric nitrogen) to $NH_3$ (ammonia). Free living prokaryotes with the ability to fix atmospheric dinitrogen (diazotrophs) are ubiquitous in soil. In natural ecosystems, biological nitrogen fixation is the most important source of nitrogen. The capacity for nitrogen fixation is

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widespread among bacteria. The estimated contribution of free living N-fixing prokaryotes to the nitrogen input of soil ranges from 0 to 60 kg/ha/year (Burgmann et al., 2003). In recent years, many studies have addressed the importance and contribution of biological nitrogen fixation in ecologically unique terrestrial and aquatic habitats by focusing on the diversity of *nif*H sequences (Zehr et al., 2003). Such studies have provided a rapidly expanding database of *nif*H sequences and revealed a wide diversity of uncultured diazotrophs (Tan et al., 2003).

Plant-associated nitrogen fixing bacteria have been considered as one of the possible alternatives for inorganic nitrogen fertilizer for promoting plant growth and yield (Ladha and Reddy, 2000). A variety of nitrogen fixing bacteria like *Acetobacter, Arthrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Beijerinckia, Derxia, Enterobacter, Herbaspirillum, Klebsiella, Pseudomonas* and *zoogloea* have been isolated from the rhizosphere of various crops (Barraquio et al., 2000). A significant reduction in the use of nitrogen-fertilizer could be achieved if biological nitrogen fixation is made available to crop plants (Dawe, 2000). Nitrogenous fertilizers are one of the most widely used chemical fertilizers, as deficiency of nitrogen in the soil often limits crop yields. Consumption of nitrogen fertilizer in Asia has increased from 1.5 to 47 million tones (mt) during the last 35 years (Dawe, 2000). Only less than 50% of the added nitrogen is available to the plants. The enzymatic reduction of nitrogen to ammonia replenishes the loss of nitrogen from soil-plant ecosystems and is achieved through biological nitrogen fixation. Diazotrophs in the soil are the main source of nitrogen input in primary production ecosystems (Cleveland, 1999).

Nitrogen fixers in the environment are diverse. Bacteria of the genus *Azospirillum* are well-known examples of so-called associative nitrogen fixers, which are widespread in the soils of tropical, subtropical and temperate regions. These bacteria develop close relationships with the roots of various wild and agricultural plants (Tyler et al., 1979; Steenhoubt and Vanderleyden, 2000). The studies of these microorganisms carried out over the last few decades have primarily been aimed at gaining insight into the molecular nature of plant-microbial interactions in order to develop efficient modern genetic and agricultural biotechnologies (Burdman et al., 2001; Fedonenko et al., 2001). Associative nitrogen fixing bacteria such as *Azospirillum brasilense, Herbaspirillum seropedicae* and *Acetobacter diazotrophicus* may benefit their host plants as nitrogen biofertilizers and plant growth promoters. The latter two organisms were the first nitrogen-fixing bacteria suggested to be endophytes (Baldani et al., 1997; James and Oliveres, 1997). *A. lipoferum* and *A. brasilense* were for long the only known members of the genus *Azospirillum (*Tarrand et al., 1978).

A large number of *nif*H primers have been designed to study the diversity of diazotrophs (Poly et al, 2001; Rosch et al., 2002; Widmer et al., 1999; Shaffer et al., 2000). All nitrogen fixers carry a *nif*H gene that encodes the Fe protein of the nitrogenase. In this study, the structure of the *nif*H gene pool was investigated by RFLP analysis of the *nif*H gene, which has been amplified from DNA directly extracted from soil samples (Poly et al., 2001) and other techniques, such as PCR cloning (Zehr et al., 1995, 1998). The *nif*H genes are very diverse, some of them are characteristic of an ecological niche (Chelius and Lepo, 1999; Shaffer et al., 2000), which shows the habitats of soil nitrogen fixing bacteria and the structure of *nif*H gene pools relationships.

## MATERIALS AND METHODS

### Sampling location

Samples of coir pith were collected from the accumulated heap in the Alappuzha district of Kerala in India. The samples were randomly collected in sterile plastic bags and stored at 4°C in the laboratory for the further experiments.

### Isolation of nitrogen fixing bacteria

General plating techniques were followed for screening and isolation. Individual colonies were picked, purified and assayed as pure cultures for nitrogenase activity using N-deficient medium. Pure cultures of nitrogen fixing isolates were readily obtained by repeated sub-culturing and confirmed through Gram staining technique.

### Extraction and analysis of DNA

Genomic DNA was obtained by using standard bacterial procedure (Sambrook et al., 1989).

The DNA stock samples were quantified by UV spectrophotometer at 260 and 280 nm using the convention that one absorbance unit at 260 nm wavelength equals 50 µg DNA per ml. The absorbance in the UV range of 260 and 280 nm were studied for determination of DNA concentration and purity. Purity of DNA was confirmed on the basis of optical density ratio at 260:280 nm. The quality of DNA was further confirmed using agarose gel electrophoresis (Naniatis et al., 1982). 16S rDNA fragment was amplified by PCR from the bacterial genomic DNA using 16s rDNA universal primers (10 to 30 F: 5' –GAG TTT GAT CCT GGC TCA G-3' and 530 R: 5'-G(AT)A TTA CCG CGG CGG CTG-3').

### PCR amplification of the *nif*H gene fragment

One hundred nanogram of DNA were used as template in PCR. Selected primers *Nif*H for-5' TAYGGNAARGGNGGHATYGGYATC and *Nif*H rev -5' ATRTTRTTNGCNGCRTAVABBGCCATCAT were used to amplify (Poly et al., 2001). PCR was carried out in a final reaction volume of 25 µL in 200 µL capacity thin wall PCR tubes. The PCR tubes with all the components were transferred to thermal cycler.

The thermo cycling conditions consisted of an initial denaturation step at 94°C for 3 min, 30 amplification cycles of 45 s at 94°C, 30 s at 55°C, 60 s at 72°C and a final extension step at 72°C for 5 min with Gene Amp PCR system (Perkin-Elmer Co., Norwalk, Conn.).

**Analysis of DNA amplification by AGE**

Commercially available 100 bp ladder was used as standard molecular weight DNA. Analysis of the PCR products was carried out by electrophoresis. Electrophoresis was done by 5 µL of PCR product with 4 µL bromophenol blue (loading dye) in agarose gels (1.5%). The voltage of 100 V and current of 45 A for a period of 1 h 20 min till the bromophenol blue travelled 6 cm from the wells was applied. We viewed the gels on UV transilluminator and photographed the gel for documentation.

**Purification and DNA sequencing of samples**

Amplified PCR product was purified using column purification as per manufacturer's guidelines (Thermo Scientific, Fermentas Molecular Biology Tools). The isolated DNA having ratio between 1.8 to 2.0 can be considered to be of good purity and further used for sequencing reaction.

**Sequencing of purified 16SrDNA gene segment**

The concentration of the purified DNA was determined and was subjected to automated DNA sequencing on ABI3730xl genetic analyzer (Applied Biosystems, USA).

**16S rRNA sequence analysis**

Each nucleic acid sequence was edited manually to correct falsely identified bases and trimmed to remove unreadable sequences at the 3′ and 5′ ends (considering peak and quality values for each base) using the sequence analysis tools. The edited sequences (16S rDNA) were then used for similarity searches using Basic Local Alignment Search Tool (BLAST) programme in the NCBI GenBank (www.ncbi.nlm.nih.gov) DNA database for identifying the bacterial strains. The phylogenetic tree was constructed by the methods implemented in the TREECONW software package.

**Sequence deposition**

The 16s rRNA, nifH gene fragments of the strain NF4, NF7, NF12 and NF18 have been deposited in the GenBank under the accession numbers JN230510, JN230511, JN230512 and JN230513, respectively.

## RESULTS AND DISCUSSION

Coir pith is a rigid fluffy material and in the present study, an attempt was made to isolate microorganisms in the coir pith samples drawn from accumulated heaps of coir pith on coir fiber extraction units stored for the past 3 years without any type of treatment (Figure 6). Four nitrogen fixing bacteria composed of different physiological and biochemical characters were isolated and sequenced. Out of the 19 isolated colonies, four strains were amplified with *nifH* gene *viz.* NF-4 (*Lysinibacillus sp.*), NF-7 (*Ochrobactrum sp*), NF-12 (*Paenibacillus sp*) and an uncultured bacterial clone was isolated which shows only 50% similarity to NF-18 (*Clostridium sp.*). By repeated plating on to nitrogen deficient agar media, pure cultures of the four bacterial colonies were obtained. High molecular weight DNA was observed in agarose gel evaluation.16s rDNA fragment was amplified by PCR from genomic DNA using 16S rDNA universal primer. Column purification yielded contaminant free PCR product.

A large number of *nifH* primers were designed to study the diversity of diazotrophs (Poly et al., 2001; Rosch et al., 2002; Widmer et al., 1999; Shaffer et al., 2000). Here, the design of the appropriate primers was done with utmost priority for the novel primers, otherwise the use of highly generated primers in combination with low stringency amplification conditions could result in biased conclusions. In the present study, predesigned degenerated primers were used for the coir pith based micro flora analysis viz. *Nif*H for-5' TAYGGNAARGGN-GGHATYGGYATC and NifH rev -5' ATRTTRTTN-GCNGCRTAVABBGCCATCAT (Edwards et al., 1989). The *nif*H primers were designed from the available *nif*H sequences of different organisms from NCBI GenBank (www.ncbi.nlm.nih.gov). After the amplification of microbial DNA from coir pith with *nif* H primers (Figure 1), the edited sequences (16s rDNA) were then subjected to the similarity searches using BLAST programme (Table 1). The BLAST results show that the four cultures have greater similarity with *Lysinibacillus* sp., *Ochrobactrum* sp., *Paenibacillus* sp. and *Clostridium* sp. The results have been furnished in Figures 2 to 5.

Coir pith is very slow in microbial decomposition due to the presence of lignin and accumulates in coir fiber extraction units. The microflora inhabiting coir pith is therefore limited, as the lignocellulose complex resists biodegradation. Herein, an effort is made here to isolate the microorganisms in coir pith which possess the nitrogen fixing ability. The DNA isolated from the natural microflora in coir pith showed similarities with *Lysinibacillus* sp., *Ochrobactrum* sp., *Paenibacillus* sp. and *Clostridium* sp.

These bacteria are important nitrogen fixing species and most of them have other applications too. It is observed that activities that have been found to be associated with *P. polymyxa* treatment on plants in field experiments include nitrogen fixation, soil phosphorous solubilization, production of antibiotics, auxins, cytokinins, chitinase and hydrolytic enzymes, as well as the promotion of increased soil porosity (Timmusk and Wagner, 1999; Timmusk et al., 1999).

All these activities might be of importance for plant growth promotion. Timmusk et al. (2009) reported *P. polymyxa* B2, B5 and B6 antagonistic mechanisms against the well characterized model of oomycetic pathogens *Phytophthora palmivora* and *Pythium aphanidermatum*. *P. polymyxa* (previously *Bacillus polymyxa*; Ash et al., 1994) is a common soil bacterium belonging to plant growth promoting rhizobacteria

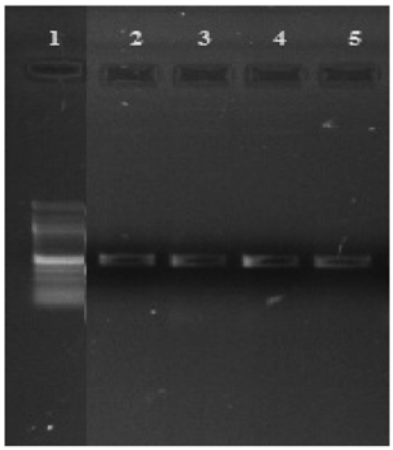
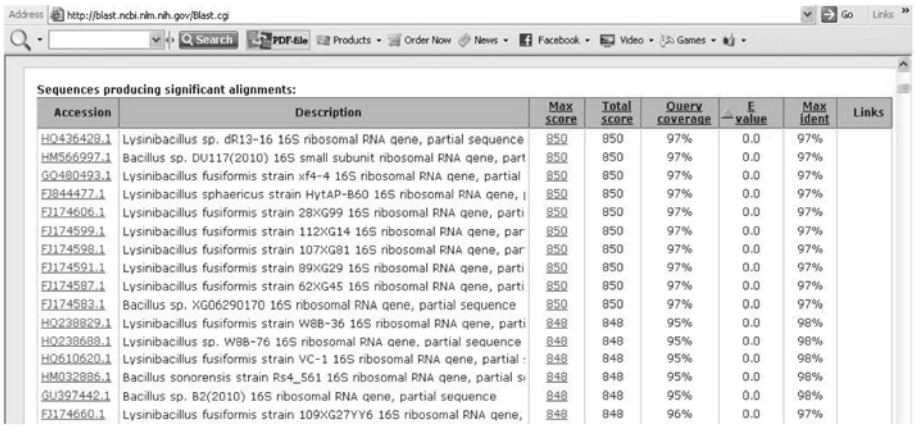



**Figure 1.** Amplification of coir pith DNA with *nifH* primers. Lane 1, 100 bp molecular weight marker, lane 2, NF-4; lane 3, NF-7; lane 4, NF-12 and lane 5, NF-18.

**Table 1.** Blast results of top two genes showed maximum similarity.

| Organisms | Accession No | Description | Maximum identity (%) |
|---|---|---|---|
| *Lysinibacillus sp.* | FJ 174660.1 | *Lysinibacillus fusiformis* strain 109XG27YY6 16S ribosomal RNA gene, Partial sequence. | 97 |
| | HM 032886.1 | *Bacillus sonorensis* strain 16S ribosomal RNA gene, Partial sequence. | 98 |
| *Ochrobactrum sp.* | HM 629806.1 | *Ochrobactrum sp.* BE3. 16S ribosomal RNA gene, Partial sequence. | 96 |
| | EU 301689.1 | *Ochrobactrum tritici.* 16S ribosomal RNA gene, Partial sequence. | 96 |
| *Paenibacillus sp.* | EU 912456.1 | *Paenibacillus sp.* BL18-3-2. 16S ribosomal RNA gene, Partial sequence. | 98 |
| | FJ 468006.1 | *Paenibacillus polymyxa* strain. MS 0102 16S ribosomal RNA gene, Partial sequence. | 98 |
| *Clostridium sordellii* sp. | HP 259293.1 | *Clostridium sordellii* strain MA2 16S ribosomal RNA gene, Partial sequence. | 85 |
| | HQ 259292.1 | *Clostridium sordellii* MA1 16S ribosomal RNA gene, Partial sequence. | 85 |

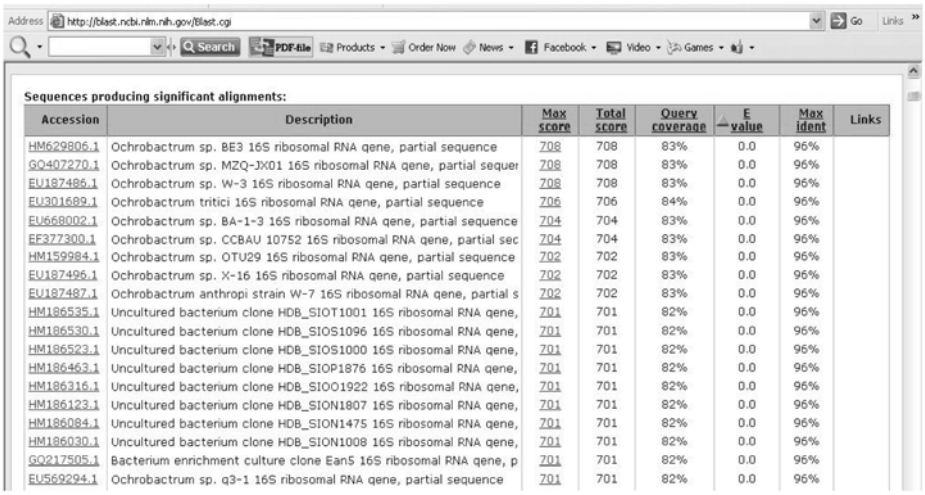
(PGPR) also present in the coir pith sample. The activities associated with *P. polymyxa* include nitrogen fixation (Heulin et al., 1994), soil phosphorous solubilization (Jisha and Alagawadi, 1996), as well as promotion of increased soil porosity (Gouzou et al., 1993; Timmusk and Wagner, 1999; Timmusk et al., 1999). Besides, it produces antimicrobial substances active against fungi and bacteria (Rosado and Seldin, 1993; Picard et al., 1995; Kajimura and Kaneda, 1996). *P. polymyxa* also has been used for the control of plant



Sequences producing significant alignments:

| Accession | Description | Max score | Total score | Query coverage | E value | Max ident | Links |
|---|---|---|---|---|---|---|---|
| HQ436428.1 | Lysinibacillus sp. dR13-16 16S ribosomal RNA gene, partial sequence | 850 | 850 | 97% | 0.0 | 97% | |
| HM566997.1 | Bacillus sp. DU117(2010) 16S small subunit ribosomal RNA gene, part | 850 | 850 | 97% | 0.0 | 97% | |
| GQ480493.1 | Lysinibacillus fusiformis strain xf4-4 16S ribosomal RNA gene, partial | 850 | 850 | 97% | 0.0 | 97% | |
| FJ844477.1 | Lysinibacillus sphaericus strain HytAP-B60 16S ribosomal RNA gene, | 850 | 850 | 97% | 0.0 | 97% | |
| FJ174606.1 | Lysinibacillus fusiformis strain 28XG99 16S ribosomal RNA gene, parti | 850 | 850 | 97% | 0.0 | 97% | |
| FJ174599.1 | Lysinibacillus fusiformis strain 112XG14 16S ribosomal RNA gene, par | 850 | 850 | 97% | 0.0 | 97% | |
| FJ174598.1 | Lysinibacillus fusiformis strain 107XG81 16S ribosomal RNA gene, par | 850 | 850 | 97% | 0.0 | 97% | |
| FJ174591.1 | Lysinibacillus fusiformis strain 89XG29 16S ribosomal RNA gene, parti | 850 | 850 | 97% | 0.0 | 97% | |
| FJ174587.1 | Lysinibacillus fusiformis strain 62XG45 16S ribosomal RNA gene, parti | 850 | 850 | 97% | 0.0 | 97% | |
| FJ174583.1 | Bacillus sp. XG06290170 16S ribosomal RNA gene, partial sequence | 850 | 850 | 97% | 0.0 | 97% | |
| HQ238829.1 | Lysinibacillus fusiformis strain W8B-36 16S ribosomal RNA gene, parti | 848 | 848 | 95% | 0.0 | 98% | |
| HQ238688.1 | Lysinibacillus sp. W8B-76 16S ribosomal RNA gene, partial sequence | 848 | 848 | 95% | 0.0 | 98% | |
| HQ610620.1 | Lysinibacillus fusiformis strain VC-1 16S ribosomal RNA gene, partial : | 848 | 848 | 95% | 0.0 | 98% | |
| HM032886.1 | Bacillus sonorensis strain Rs4_561 16S ribosomal RNA gene, partial s | 848 | 848 | 95% | 0.0 | 98% | |
| GU397442.1 | Bacillus sp. B2(2010) 16S ribosomal RNA gene, partial sequence | 848 | 848 | 95% | 0.0 | 98% | |
| FJ174660.1 | Lysinibacillus fusiformis strain 109XG27YY6 16S ribosomal RNA gene, | 848 | 848 | 96% | 0.0 | 97% | |

**Figure 2.** Blast result of Culture 4. Based on the 16s rDNA analysis, the culture 4 showed 97% similarity with *Lysinibacillus* sp. (accession no: HQ436428.1).



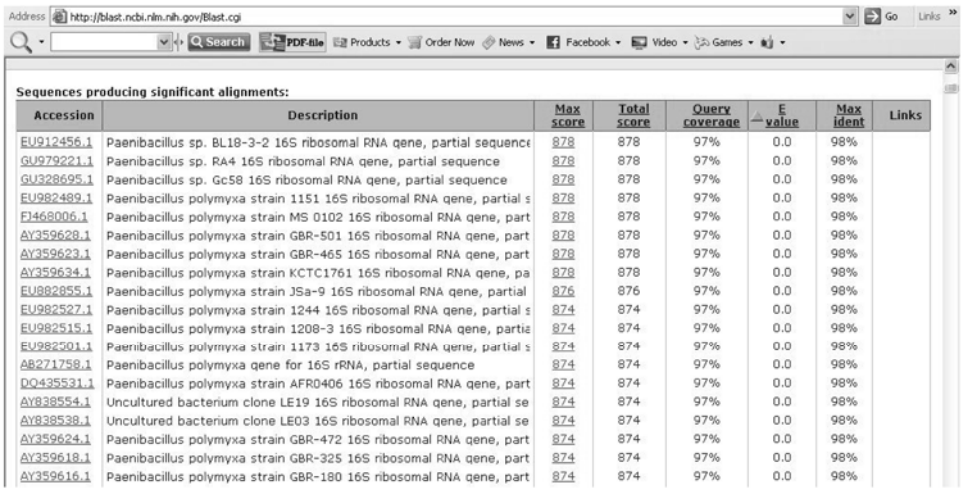
Sequences producing significant alignments:

| Accession | Description | Max score | Total score | Query coverage | E value | Max ident | Links |
|---|---|---|---|---|---|---|---|
| HM629806.1 | Ochrobactrum sp. BE3 16S ribosomal RNA gene, partial sequence | 708 | 708 | 83% | 0.0 | 96% | |
| GQ407270.1 | Ochrobactrum sp. MZQ-JX01 16S ribosomal RNA gene, partial sequer | 708 | 708 | 83% | 0.0 | 96% | |
| EU187486.1 | Ochrobactrum sp. W-3 16S ribosomal RNA gene, partial sequence | 708 | 708 | 83% | 0.0 | 96% | |
| EU301689.1 | Ochrobactrum tritici 16S ribosomal RNA gene, partial sequence | 706 | 706 | 84% | 0.0 | 96% | |
| EU668002.1 | Ochrobactrum sp. BA-1-3 16S ribosomal RNA gene, partial sequence | 704 | 704 | 83% | 0.0 | 96% | |
| EF377300.1 | Ochrobactrum sp. CCBAU 10752 16S ribosomal RNA gene, partial sec | 704 | 704 | 83% | 0.0 | 96% | |
| HM159984.1 | Ochrobactrum sp. OTU29 16S ribosomal RNA gene, partial sequence | 702 | 702 | 83% | 0.0 | 96% | |
| EU187496.1 | Ochrobactrum sp. X-16 16S ribosomal RNA gene, partial sequence | 702 | 702 | 83% | 0.0 | 96% | |
| EU187487.1 | Ochrobactrum anthropi strain W-7 16S ribosomal RNA gene, partial s | 702 | 702 | 83% | 0.0 | 96% | |
| HM186535.1 | Uncultured bacterium clone HDB_SIOT1001 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| HM186530.1 | Uncultured bacterium clone HDB_SIOS1096 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| HM186523.1 | Uncultured bacterium clone HDB_SIOS1000 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| HM186463.1 | Uncultured bacterium clone HDB_SIOP1876 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| HM186316.1 | Uncultured bacterium clone HDB_SIOO1922 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| HM186123.1 | Uncultured bacterium clone HDB_SION1807 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| HM186084.1 | Uncultured bacterium clone HDB_SION1475 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| HM186030.1 | Uncultured bacterium clone HDB_SION1008 16S ribosomal RNA gene, | 701 | 701 | 82% | 0.0 | 96% | |
| GQ217505.1 | Bacterium enrichment culture clone Ean5 16S ribosomal RNA gene, p | 701 | 701 | 82% | 0.0 | 96% | |
| EU569294.1 | Ochrobactrum sp. q3-1 16S ribosomal RNA gene, partial sequence | 701 | 701 | 82% | 0.0 | 96% | |

**Figure 3.** Blast result of Culture 7. Based on the 16s rDNA analysis, the culture 7 showed 96% similarity with *Ochrobactrum* sp. (accession no: HM629806.1).

disease (Mavingui and Heulin, 1994; Kim, 1995; Shishido et al., 1996; Dijksterhuis et al., 1999; Kharbanda et al., 1999). It displayed potent antimicrobial properties against both Gram negative and Gram positive pathogenic bacteria. The antimicrobials produced by this strain were isolated from the fermentation broth and subsequently analyzed by liquid chromatography-mass spectrometry.

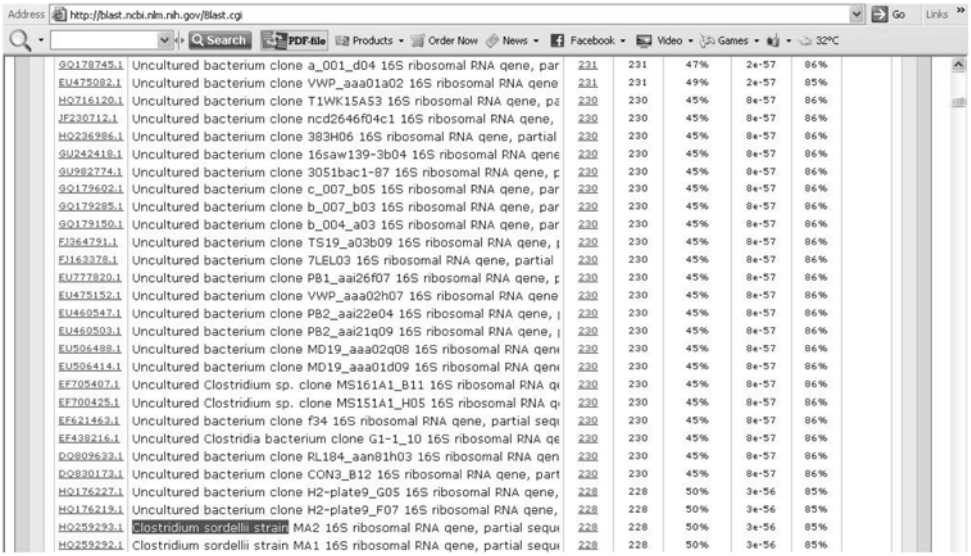
Another important bacteria isolated from the coir pith



Sequences producing significant alignments:

| Accession | Description | Max score | Total score | Query coverage | E value | Max ident | Links |
|---|---|---|---|---|---|---|---|
| EU912456.1 | Paenibacillus sp. BL18-3-2 16S ribosomal RNA gene, partial sequence | 878 | 878 | 97% | 0.0 | 98% | |
| GU979221.1 | Paenibacillus sp. RA4 16S ribosomal RNA gene, partial sequence | 878 | 878 | 97% | 0.0 | 98% | |
| GU328695.1 | Paenibacillus sp. Gc58 16S ribosomal RNA gene, partial sequence | 878 | 878 | 97% | 0.0 | 98% | |
| EU982489.1 | Paenibacillus polymyxa strain 1151 16S ribosomal RNA gene, partial s | 878 | 878 | 97% | 0.0 | 98% | |
| FJ468006.1 | Paenibacillus polymyxa strain MS 0102 16S ribosomal RNA gene, part | 878 | 878 | 97% | 0.0 | 98% | |
| AY359628.1 | Paenibacillus polymyxa strain GBR-501 16S ribosomal RNA gene, part | 878 | 878 | 97% | 0.0 | 98% | |
| AY359623.1 | Paenibacillus polymyxa strain GBR-465 16S ribosomal RNA gene, part | 878 | 878 | 97% | 0.0 | 98% | |
| AY359634.1 | Paenibacillus polymyxa strain KCTC1761 16S ribosomal RNA gene, pa | 878 | 878 | 97% | 0.0 | 98% | |
| EU882855.1 | Paenibacillus polymyxa strain JSa-9 16S ribosomal RNA gene, partial | 876 | 876 | 97% | 0.0 | 98% | |
| EU982527.1 | Paenibacillus polymyxa strain 1244 16S ribosomal RNA gene, partial s | 874 | 874 | 97% | 0.0 | 98% | |
| EU982515.1 | Paenibacillus polymyxa strain 1208-3 16S ribosomal RNA gene, partia | 874 | 874 | 97% | 0.0 | 98% | |
| EU982501.1 | Paenibacillus polymyxa strain 1173 16S ribosomal RNA gene, partial s | 874 | 874 | 97% | 0.0 | 98% | |
| AB271758.1 | Paenibacillus polymyxa gene for 16S rRNA, partial sequence | 874 | 874 | 97% | 0.0 | 98% | |
| DQ435531.1 | Paenibacillus polymyxa strain AFR0406 16S ribosomal RNA gene, part | 874 | 874 | 97% | 0.0 | 98% | |
| AY838554.1 | Uncultured bacterium clone LE19 16S ribosomal RNA gene, partial se | 874 | 874 | 97% | 0.0 | 98% | |
| AY838538.1 | Uncultured bacterium clone LE03 16S ribosomal RNA gene, partial se | 874 | 874 | 97% | 0.0 | 98% | |
| AY359624.1 | Paenibacillus polymyxa strain GBR-472 16S ribosomal RNA gene, part | 874 | 874 | 97% | 0.0 | 98% | |
| AY359618.1 | Paenibacillus polymyxa strain GBR-325 16S ribosomal RNA gene, part | 874 | 874 | 97% | 0.0 | 98% | |
| AY359616.1 | Paenibacillus polymyxa strain GBR-180 16S ribosomal RNA gene, part | 874 | 874 | 97% | 0.0 | 98% | |

**Figure 4.** Blast result of Culture 12. Based on the 16s rDNA analysis, the culture 12 showed 98% similarity with *Paenibacillus sp.* (accession no: EU912456.1).



| | | | | | | |
|---|---|---|---|---|---|---|
| GQ178745.1 | Uncultured bacterium clone a_001_d04 16S ribosomal RNA gene, par | 231 | 231 | 47% | 2e-57 | 86% |
| EU475082.1 | Uncultured bacterium clone VWP_aaa01a02 16S ribosomal RNA gene | 231 | 231 | 49% | 2e-57 | 85% |
| HQ716120.1 | Uncultured bacterium clone T1WK15A53 16S ribosomal RNA gene, pa | 230 | 230 | 45% | 8e-57 | 86% |
| JF230712.1 | Uncultured bacterium clone ncd2646f04c1 16S ribosomal RNA gene, | 230 | 230 | 45% | 8e-57 | 86% |
| HQ236986.1 | Uncultured bacterium clone 383H06 16S ribosomal RNA gene, partial | 230 | 230 | 45% | 8e-57 | 86% |
| GU242418.1 | Uncultured bacterium clone 16saw139-3b04 16S ribosomal RNA gene | 230 | 230 | 45% | 8e-57 | 86% |
| GU982774.1 | Uncultured bacterium clone 3051bac1-87 16S ribosomal RNA gene, p | 230 | 230 | 45% | 8e-57 | 86% |
| GQ179602.1 | Uncultured bacterium clone c_007_b05 16S ribosomal RNA gene, par | 230 | 230 | 45% | 8e-57 | 86% |
| GQ179285.1 | Uncultured bacterium clone b_007_b03 16S ribosomal RNA gene, par | 230 | 230 | 45% | 8e-57 | 86% |
| GQ179150.1 | Uncultured bacterium clone b_004_a03 16S ribosomal RNA gene, par | 230 | 230 | 45% | 8e-57 | 86% |
| FJ264791.1 | Uncultured bacterium clone TS19_a03b09 16S ribosomal RNA gene, p | 230 | 230 | 45% | 8e-57 | 86% |
| FJ163378.1 | Uncultured bacterium clone 7LEL03 16S ribosomal RNA gene, partial | 230 | 230 | 45% | 8e-57 | 86% |
| EU777820.1 | Uncultured bacterium clone PB1_aai26f07 16S ribosomal RNA gene, p | 230 | 230 | 45% | 8e-57 | 86% |
| EU475152.1 | Uncultured bacterium clone VWP_aaa02h07 16S ribosomal RNA gene | 230 | 230 | 45% | 8e-57 | 86% |
| EU460547.1 | Uncultured bacterium clone PB2_aai22e04 16S ribosomal RNA gene, p | 230 | 230 | 45% | 8e-57 | 86% |
| EU460503.1 | Uncultured bacterium clone PB2_aai21q09 16S ribosomal RNA gene, p | 230 | 230 | 45% | 8e-57 | 86% |
| EU506488.1 | Uncultured bacterium clone MD19_aaa02q08 16S ribosomal RNA gene | 230 | 230 | 45% | 8e-57 | 86% |
| EU506414.1 | Uncultured bacterium clone MD19_aaa01d09 16S ribosomal RNA gene | 230 | 230 | 45% | 8e-57 | 86% |
| EF705407.1 | Uncultured Clostridium sp. clone MS161A1_B11 16S ribosomal RNA g | 230 | 230 | 45% | 8e-57 | 86% |
| EF700425.1 | Uncultured Clostridium sp. clone MS151A1_H05 16S ribosomal RNA g | 230 | 230 | 45% | 8e-57 | 86% |
| EF621463.1 | Uncultured bacterium clone f34 16S ribosomal RNA gene, partial sequ | 230 | 230 | 45% | 8e-57 | 86% |
| EF438216.1 | Uncultured Clostridia bacterium clone G1-1_10 16S ribosomal RNA ge | 230 | 230 | 45% | 8e-57 | 86% |
| DQ809633.1 | Uncultured bacterium clone RL184_aan81h03 16S ribosomal RNA gen | 230 | 230 | 45% | 8e-57 | 86% |
| DQ830173.1 | Uncultured bacterium clone CON3_B12 16S ribosomal RNA gene, part | 230 | 230 | 45% | 8e-57 | 86% |
| HQ176227.1 | Uncultured bacterium clone H2-plate9_G05 16S ribosomal RNA gene, | 228 | 228 | 50% | 3e-56 | 85% |
| HQ176219.1 | Uncultured bacterium clone H2-plate9_F07 16S ribosomal RNA gene, | 228 | 228 | 50% | 3e-56 | 85% |
| HQ259293.1 | Clostridium sordellii strain MA2 16S ribosomal RNA gene, partial sequ | 228 | 228 | 50% | 3e-56 | 85% |
| HQ259292.1 | Clostridium sordellii strain MA1 16S ribosomal RNA gene, partial sequ | 228 | 228 | 50% | 3e-56 | 85% |

**Figure 5.** Blast result of Culture 18. Based on the 16s rDNA analysis, the culture 18 showed 85% similarity with *Clostridium sordellii.* (Accession No: HQ259293.1)

**Figure 6.** Coir pith heaps accumulating in fiber extraction units.

sample was *Ochrobactrum* sp., which has several properties of importance. The genus *Ochrobactrum* was described first by Homes et al. (1988) and belongs to the α-2 subclass of the Proteobacteria (De Ley, 1992). *Ochrobactrum tritici* was identified as Bacterial strain 5bv11, isolated from a chromium-contaminated waste water treatment plant, which is resistant to a broad range of antibiotics and metals viz. Cr (VI), Ni (II), Co (II), Cd (II) and Zn (II) (Branco et al., 2004). Holmes et al. (1988) proposed *Ochrobactrum anthropi* as a sole and type species of *Ochrobactrum*, but they observed heterogeneities in geno- or phenotypic characters within the tested *O. anthropi* collection. *O. anthropi* strains have been isolated from samples originating from different continents. *Ochrobactrum sp.* contains root associated bacteria that enter bivalent interactions with plant and human hosts. Several members of these genera show plant growth promoting as well as excellent antagonistic properties against plant pathogens and were therefore utilized for the development of biopesticides (Weller, 1988; Whipps, 2001).

Most available *O. anthropi* isolates are from human clinical specimens (Lebuhn et al., 2000). *O. anthropi* LMG 5140 has also been isolated from arsenical cattle dipping fluid (Holmes et al., 1988). Moreover, there are some reports on the presence of *O. anthropi* in soil, on wheat roots and in internal root tissues of different plants (Anguillera et al., 1993; McInroy and Kloepper, 1994; Sato and Jiang, 1996).

More also, NF-4(*Lysinibacillus* sp.) could be isolated from the coir pith samples. It is described by Ahmed et al. (2007) as spore forming, Gram-positive, motile, rod shaped and boron-tolerant. A large number of Bacillus strains, including *B. fusiformis* capable of degrading different hydrocarbons, have been isolated from oil contaminated soils (Bento et al., 2003). It is also considered as a growth promoting agent. In addition to all the useful organisms, some pathogenic microorganisms could also be observed with 50% possibility.

## Conclusion

The results of the study indicated that coir pith is a source of nitrogen fixing bacteria and other useful microorganisms. Coir pith, which was considered as a problematic waste, has been found to harbor useful microorganisms with potential use as plant nutrient. Coir pith can be used as good organic manure after supplementing with efficient nitrogen fixing bacteria. It can therefore be concluded that the coir pith has great potential for use as a source of many useful microorganisms including nitrogen fixing bacteria.

## ACKNOWLEDGEMENTS

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# Coir Pith as Growth Medium for *Azotobacter Vinelandii* and *Azospirillum Brasilense*

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**Abstract**

Coir pith is a lignocellulosic biomass which is recalcitrant under ordinary conditions. Nitrogen fixation is commonly carried out in the soil and these soils acts as the medium for plant growth. This paper attempts to utilize coir pith as a substrate for two important nitrogen fixing organisms *viz. Azotobacter vinelandii* and *Azospirillum brasilense.* Coir pith was used as a source of carbon and energy by the bacteria and the ammonia produced during the process of nitrogen fixation was studied, the amount of ammonia produce indicates the fixation process by the bacteria. The present work succeeded in establishing the use of these two organisms to degrade the coir pith effectively and the resultant biodegraded material could be used as organic manure for plants.

**Key words:** Coir pith, Nitrogen fixation, Biodegradation, ammonia.

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## Introduction

Coir pith is the name given to the dust left behind after the industrially valuable long fiber of coir has been extracted from the coconut husk. It is a fluffy, spongy material with significant water holding capacity and is extremely compressible (Vijaya *et al*, 2008). Coconut husk is the basic raw material for the coir industry (Reghuvaran *et al*, 2009). Coir fibre extracted from husk is used in local beddings, mattings, rubberized coir mattress, yarns, in rope making etc. After extraction of coir fibre from husk, the remaining material (coir pith) remains as a byproduct. Coir pith constitutes as much as 70% of the coconut husk and has a high water holding capacity of 8 times of its weight. Interest in nitrogen fixation mainly in the sea has usually been focused on rates of nitrogen fixation, but information on the types of species present with the capability for nitrogen fixation can be important for predicting nitrogen fixation rates in situ (Zehr *et al*., 1998)

The coir pith contains about 35% lignin 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, but mainly by beta-aryl ether bonds. It is also present in the fibres and is responsible for the stiffness of coir. It is thought that the lignin has its origin in carbohydrates. It is mainly a hard substance and is almost free from degradation in ordinary conditions. It should be noted that coir pith is resistant to biodegradation due to the presence of lignin. Normally coir pith is dumped as agricultural waste and accumulates as a waste product as heaps of course and fine dust (Ghosh *et al*, 2007). The coir pith 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 (Ramalingam *et al*, 2004). High content of lignin in coir pith causes very slow decomposition following which it is used as raw organic manure for crops (Vinodhini *et al*, 2005).

The study of coir pith degradation with bacteria is very limited due to its poor degrading ability to degrade lignin present in it. Janshekar, H and Fiechter (1981) reported some bacterial culture was tested for the degradation of lignin which has the ability to degrade numerous phenols with structural relationship to lignin. The poor degradation of lignin was observed in all tested bacteria, they also commented that the poor degradation does not seem to be influenced by the medium composition and culture condition but is more probably due to the inability of the tested bacteria to degrade lignin to any considerable extent. Instead of this, for many years, the filamentous blue-green algae (Cyanobacteria) were believed to be primarily responsible for nitrogen fixation in oceanic waters because low or negligible in situ rates were observed in their absence and there was a correlation of in situ nitrogen fixation with light intensity (Stewart, 1974). Some Cyanobacteria were capable of degrading the coir pith. Some enhanced techniques show the use of coir pith based cyanobacterial biofertilizer in sustainable integrated agro ecosystems (Prabha *et al*, 2009). So, this method can are useful to promote the growth of plant and increase the quality and quantity of crop yield (Hume, 2007). Most recently, cellulolytic nitrogen fixing bacteria have been isolated in large numbers, apparently as a pure culture, from a specialized gland found in ship worms (Waterbury *et al*., 1983). The work conducted by Anandharaj (2007) also shows the coir pith can be partially decomposed through the action of cyanobacteria and can be used as biofertilizer for all varieties of food crops.

Nitrogen fixation is the reduction of atmospheric nitrogen to NH3 (ammonia). Free living prokaryotes with the ability to fix atmospheric dinitrogen (diazotrophs) are ubiquitous in soil. The free living diazotrophs are subclassified. Aerobic diazotrophs, of which there are over 50 genera, including Azotobacter, methane-oxidizing bacteria and cyanobacteria require oxygen for growth and fix nitrogen when oxygen is present. Azotobacter, some related bacteria and some cyanobacteria fix nitrogen in ordinary air but most members of this group fix nitrogen only when the oxygen concentration is low. Free living diazotrophs, which fix nitrogen

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only when oxygen is absent or vanishingly low, are wide spread. In natural ecosystems, biological nitrogen fixation is most important source of nitrogen. The contribution of free-living nitrogen fixing prokaryotes estimated to the nitrogen input of soil ranges from 0-60 kg/ha/year (Burgmann *et al*., 2003). It is also evident that dinitrogen nitrogen fixers microorganisms (diazotrophs) play important roles in ocean biogeochemistry and plankton productivity (Church *et al*., 1999).

Newton and Cavins, 2003 studied the nitrogen fixation (acetylene reduction) and ammonia liberation in a facultatively heterotrophic cyanobacterium. Autotrophically grown cells lost acetylene reduction activity when incubated under anaerobic conditions; the activity was maintained in the presence of methionine sulfoximine or by pretreatment of the cells with a carbon supply. Heterotrophically grown cells maintained acetylene reduction activity anaerobically in the absence of methionine sulphoximine. Another important observation by Hans Brintzinger, 1966 stated that the reduction of molecular nitrogen to ammonia by aerobic and anaerobic microorganisms occurs through combined mediation of two nonheme iron particles, one of which contains, besides iron, molybdenum. Gina *et al*, 1992 isolated two new nitrogen fixing bacteria from the rhizosphere of mangrove trees. Biological nitrogen fixation is catalyzed by the enzyme nitrogenase, which is possessed by diverse microorganisms representing virtually all phylogenetic groups. Nitrogenase is composed of two oxygen-labile metallo protein namely dinitrogenase and dinitrogenase reductase. Dinitrogenase is a 240-KDa alpha2-beta2 tetramer of the nifD and nifK gene products and dinitrogenase reductase is a 60-KDa alpha2 dimer of the nifH gene products that contains a single 4Fe-4S center coordinated between the two subunits (Rubio *et al*., 2005). Kennedy et al., 2004, also suggests understanding how fixed nitrogen regulates nitrogenase availability is necessary for devising strategies to increase the amount of ammonium synthesized by nitrogen fixing bacteria with the potential to be used in agriculture. In the case of *A.vinelandii* is complicated by the presence of three biochemically and genetically distant nitrogenase enzymes, each of which is synthesized under different conditions of metal supply. The regulation of conventional molybdenum nitrogenase, whose subunits are encoded by the Nif-HDK genes and which is similar to the enzymes purified from number of other nitrogen fixing organisms (Sabra *et al*., 2000).

Nitrogeneous fertilizers are one of the most widely used chemical fertilizers, as deficiency of nitrogen in the soil often limits crop yields (Sarita *et al*, 2008). Consumption of nitrogen fertilizer in Asia has increased from 1.5 to 47 million tonnes during the last 35 years (Dawe, 2000). Nitrogen fixation occurs in phyla from Archea and Eubacteria. These microorganisms possess the enzyme nitrogenase encoded by the *nifK*, *nifD* and *nifH* genes. These genes have been used to study phylogenetic relationship among nitrogen fixing bacteria (Young *et al*, 1992). Different techniques have been utilized to characterize the diversity of *nifH* gene pool identification from various environments, eg. Cloning of PCR-amplified products or by denaturing gradient gel electrophoresis, PCR based Restriction Fragment Length Polymorphism (RFLP), and flourescent labeled terminal restriction fragment length polymorphism (t-RFLP) (Poly *et al*, 2001).

From the earlier studies of the author states that the coir pith which is degraded with the combination of Mushroom species and Nitrogen fixing bacteria such as *Azotobacter* and *Azospirillum* cause definite reduction in the Lignin levels and increase the percentage of NPK in the coir pith. Thus the same can be used as effective organic manure. Of the various rhizosphere associated nitrogen fixing bacteria, *Azospirillum* species are probably the most studied and appear to have significant potential for commercial applications. These organisms, characterized by high nitrogen fixing ability, are found in abundant numbers in the rhizosphere as well as in the intercellular spaces of the roots of certain cereals and other plants (Bashan *et al*, 1997). Besides the nitrogen fixation, *Azotobacter* also produces Thiamine, Riboflavin, Indole

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acetic acid and Gibberelins. When Azotobacter is applied to seeds, seed germination is improved to a considerable extent, so also it controls plant diseases due to above substances produced by *Azotobacter* (Kader *et al*., 2002).

The present study estimates the efficacy of coir pith as a substrate for the growth of two important nitrogen fixing bacteria and thereby biodegrade coir pith for use as effective organic manure. This study therefore is an attempt to study the potential of this nitrogen fixing bacteria independent biodegradation, growth and action in coir pith. A consortium using this microflora with appropriate fungal species for composting helps in making coir pith a perfect soil conditioner and bio-organic manure for horticulture/Agriculture.

## Materials and Methods

### Collection of Samples

Coir pith samples were collected from accumulated stations of Alappuzha district, Kerala, India. The Bacterial samples were collected from Microbial Type Culture Collection (MTCC), Chandigarh (*Azotobacter vinelandii*, MTCC NO.124 and *Azospirillum brasilense*, MTCC NO.125) The composting process done in Rajiv Gandhi Chair in Contemporary Studies, School of Environmental Studies, Cochin University of Science and Technology (CUSAT). The biochemical estimations were carried out at Central Coir Research Institute, Alappuzha and Rajiv Gandhi Chair.

### Composting Process

Three lots of coir pith (1 kg each) in duplicates were laid as separate heaps. Nitrogen fixing bacteria such as *Azotobacter vinelandii* and *Azospirillum brasilense* were thoroughly mixed with the second and third lots of coir pith respectively and the first sample kept as untreated. Nitrogen fixing bacteria were added in the phosphate buffer for mixing in the sample. Moisture content (200%) was maintained and microbial growth has monitored regularly for 45 days, by drawing out samples at periodic intervals.

### Estimation of Ammonia

Estimation of Ammonia in coir pith sample is done by Neslerization method. The sample is digested and buffered. The ammonia in the sample is treated with Nessler's reagent and measured photometrically at 440 nm. Prepare a linear calibration curve using different concentrations of standard Ammonia solution which can be prepared from anhydrous ammonium chloride. 1 gm of the sample is digested with 1:1 HCl and filtered; add 6N NaOH solution to make pH of 10.5 and make up to a known volume. Then take 50ml of sample, add 2ml of Nessler's reagent and mix well. Let the reaction proceed for at least 10 min. Then measure photometrically at 440nm. Calculate the concentration from the calibration graph

### Estimation of Nitrogen

Nitrogen in the coir pith samples were estimated by Alkaline permanganate method using kjeldhal distillation unit (Vogel, 1961). The method includes Digestion, Distillation and Titration. Digestion process need following chemicals for every 0.2 gm coir pith samples. Conc. H2SO4-10 ml, Catalyst mixture ($K_2SO_4$ & $CuSO_4$) mixture in 50 and 10 gm proportions) take 3 gm each. Adjust the temperature 420°C and the digestion time is 1 to 1.5 hours. Distillation process was done with 40% NaOH and 4% Boric acid. After 9 minutes, the colour in the conical flask changes to pink colour to green. This is the end point of distillation. Then take conical flask for titration. Titration is done with 01 N H2SO4.Titrate with the distilled solution till the colour changes from green to permanent pale pink colour.

Percentage of Nitrogen =

$$\frac{14 \times \text{titrant value} \times \text{Normality of Acid} \times 100}{1000 \times \text{sample weight}}$$

### Estimation of Total Phosphorous

Total phosphorous in the coir pith sample were estimated with Vanado Molybdo phosphoric yellow colour method. Dried and powdered coir pith sample (0.5 g) is taken in boiling tube. 15 ml tri acid is added (mixture of Concentrated $HNO_3$, $H_2SO_4$ and Perchloric acid

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in the ratio 10:1:4) and kept overnight and digest. The digested sample is quantitatively transferred to 100 ml volumetric flask. It is make up to 100 ml with distilled water. Take 5 ml in a 25 ml volumetric flask and then 5 ml of Vanado molybdate reagent is added and make up to 25 ml with distilled water. Shake thoroughly and read in spectrophotometer (430 nm) after 10 min. Compare and estimate the value with standard curve.

Percentage of Phosphorous =

$$\frac{100\times25\times\text{ppm (dilution)}}{0.5\times5\times10{,}000}$$

**Estimation of Potassium**

It is done with Flame photometry. The estimation done by triple acid extract is diluted suitably to contain approximately 4-7 ppm. Read in the flame photometer after adjusting with standard (100 ppm, 50 ppm and 20 ppm).

$$\text{Percentage of Potassium} = \frac{\text{Reading}\times100}{0.5\times10{,}000}$$

Organic Carbon was estimated by Walkey and Black method (1934).

**Results and Discussion**

Nitrogen, Phosphorous, Potassium and Organic carbon in the coir pith before and after the biodegradation were estimated (Table 1 & 2). Ammonia reduction in the coir pith is the indication of Nitrogen fixation. The results of estimation of Ammonia are given in Table 3. The nitrogen in the composted coir pith (45 days) was observed to increase to 0.70% in the case of composting with *Azotobacter vinelandii* and to 0.61% in the case of *Azospirillum brasilense* from 0.19% nitrogen in raw coir pith. The values for each 10, 20 and 30 days have been tabulated in table 1 and 2. The Phosphorous also enhanced in the period of composting. Raw coir pith was 0.85% and when the course of composting it is enhanced to 1.77% and 2.08% for *Azotobacter vinelandii* and *Azospirillum brasilense* respectively. The increase of Potassium also observed to 0.22% and 0.19% for the two organisms from 0.04% which is for the raw coir pith. There was a reduction observed in the Organic Carbon content from 7.34% to 6.15% and 4.80% for both the nitrogen fixing organisms.

Nitrogen fixation is the reduction of atmospheric nitrogen to ammonia. The capacity for nitrogen fixation is widespread among bacteria and archaea. A reduction in ammonia in the compost is evident during nitrogen fixation and in the present study a definite increase of ammonia in the compost was observed. In raw coir pith 1261.16 mg/kg ammonia was present, but during composting the amount was enhanced to 2812.23 mg/kg for the period of 45 days composting in the case of *Azotobacter vinelandii* and for *Azospirillum brasilense*, it is 2927.81 mg/kg. There is a definite increase of ammonia, during composting of every 10, 20, 30 and 45days. The results confirm that nitrogen fixation is carried out very efficiently in the organisms which utilized the coir pith as substrate for growth.

The studies of Nitrogen fixation by the bacteria in soil are diverse but the same on other substrates is very limited. From the rhizosphere of mangrove areas, two new nitrogen fixing bacteria were isolated by Gina *et al*, 1992. Here in our studies, the efficacy of two nitrogen fixing bacteria viz. *Azotobacter vinelandii* and *Azospirillum brasilense* in the coir pith, were studied. Nitrogen fixation in *A.vinelandii* is complicated by the presence of three biochemically and genetically distant nitrogenase enzymes, each of which is synthesized under different conditions of metal supply. Besides nitrogen fixation, *A.vinelandii* also produces thiamine, riboflavin, indole acetic acid and gibberellins. When *Azotobacter* is applied to seeds, seed germination is improved to a considerable extent, so also it controls plant diseases due to above substances produced by *A.vinelandii* (Kader *et al*, 2002)

Any Organic fertilizer typically provides three primary macronutrients such as Nitrogen, Phosphorous and Potassium. Use of biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for

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**Table 1. Nitrogen, Phosphorous and Potassium content of raw and biodegraded coir pith with *Azotobacter vinelandii***

| Sl No | Samples | Composting Days | Nitrogen (%) | Phosphorous (%) | Potassium (%) | Organic Carbon (%) |
|---|---|---|---|---|---|---|
| 1 | Raw Coir pith | 0 | 0.19 | 0.85 | 0.04 | 7.34 |
| 2 | Coir pith + *A.vinelandii* | 10 | 0.29 | 1.48 | 0.13 | 6.94 |
| 3 | Coir pith + *A.vinelandii* | 20 | 0.38 | 1.52 | 0.15 | 6.64 |
| 4 | Coir pith + *A.vinelandii* | 30 | 0.68 | 1.71 | 0.19 | 6.34 |
| 5 | Coir pith + *A.vinelandii* | 45 | 0.70 | 1.77 | 0.22 | 6.15 |

**Table 2. Nitrogen, Phosphorous and Potassium content of raw and biodegraded coir pith with *Azospirillum brasilense***

| Sl No | Samples | Composting Days | Nitrogen (%) | Phosphorous (%) | Potassium (%) | Organic Carbon (%) |
|---|---|---|---|---|---|---|
| 1 | Raw Coir pith | 0 | 0.19 | 0.85 | 0.04 | 7.34 |
| 2 | Coir pith+*A.brasilense* | 10 | 0.38 | 0.89 | 0.06 | 6.41 |
| 3 | Coir pith+*A.brasilense* | 20 | 0.48 | 1.21 | 0.08 | 5.67 |
| 4 | Coir pith+*A.brasilense* | 30 | 0.50 | 2.02 | 0.15 | 4.85 |
| 5 | Coir pith+*A.brasilense* | 45 | 0.61 | 2.08 | 0.19 | 4.80 |

**Table 3. Ammonia content of raw and biodegraded coir pith with *Azotobacter vinelandii* and *Azospirillum brasilense***

| Sl no | Composting Days | Amount of Ammonia (mg/kg) | | |
|---|---|---|---|---|
| | | Raw coir pith | Coir pith + *Azotobacter vinelandii* | Coir pith + *Azospirillum brasilense.* |
| 1 | 0 | 1261.16 | 1261.16 | 1261.16 |
| 2 | 10 | 1261.16 | 1517.17 | 1621.12 |
| 3 | 20 | 1261.16 | 1981.22 | 2028.80 |
| 4 | 30 | 1261.16 | 2430.12 | 2442.86 |
| 5 | 45 | 1261.16 | 2812.23 | 2927.81 |

*Cord* 2011, **27** (2)

**Figure 1. Enhancement of Nitrogen in Composting Process**

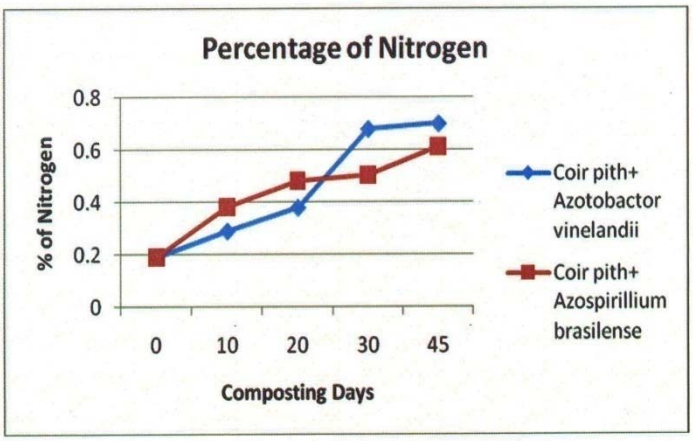


**Figure 2. Enhancement of Phosphorous Composting Process**

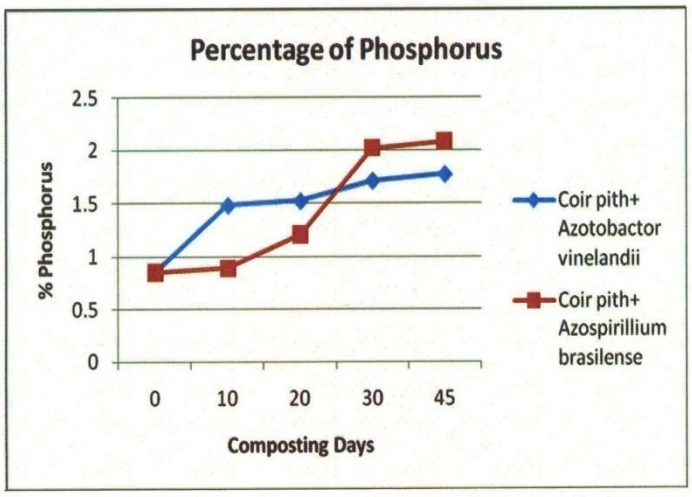

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**Figure 3. Enhancement of Potassium in Composting Process**

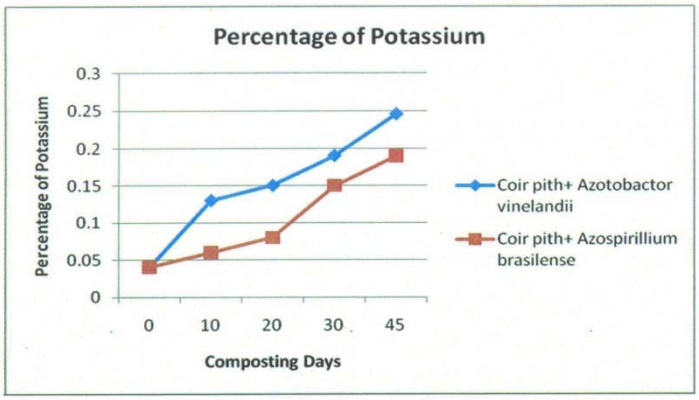
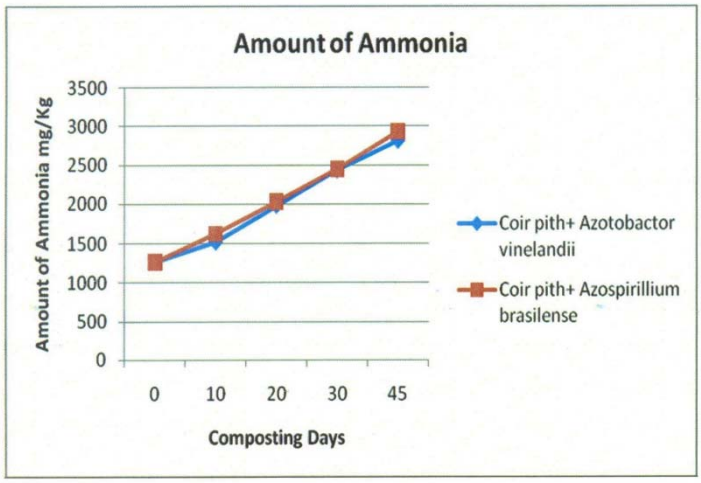



**Figure 4. Enhancement of Ammonia in Composting Process**



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sustainable agriculture (Mishra & Dadhach., 2010). In our studies, the enhancement of Nitrogen in coir pith after the composting is very evident. Nitrogen fertilizers are one of the most widely used chemical fertilizers, as deficiency of nitrogen in the soil often limits crop yields. Consumption of nitrogen fertilizer in Asia has increased from 1.5 to 47 million tons during the last 35 years (Dawe, 2000). In general, less than 50% of the added nitrogen is available to the plants. Biological nitrogen fixation, the enzymatic reduction of nitrogen to ammonia, replenishes the loss of nitrogen from soil-plant ecosystems. Soil diazotrophs are the main source of nitrogen input in primary production ecosystems (Cleveland *et al*, 1999)

The moisture content of coir pith were kept high (200%) in all the heaps during composting, which may help nitrogen fixing organism for their growth and action. Raw coir pith is very poor in nitrogen content, C: N ratio mounting to 112:1 (Nagarajan *et al*, 1985). Such a high C: N ratio is undesirable for any organic waste for application as organic manure in agricultural farms because it causes deleterious effects to the crops. The results of the present study find application of coir pith which acts as the substrate for the nitrogen fixing organisms; it can enhance the fixation process in coir pith itself and thereby enrich the coir pith with nitrogen. From the earlier studies, the use of these same organisms was reported to cause the reduction of lignin in coir pith. So, it could be established that the use of these nitrogen fixing agents, can enhance the content of NPK which enables its use as effective Organic manure.

## Conclusions

Nitrogen fixing bacteria are excellent and eco-friendly micro agents for the degradation of coir pith. From the earlier studies, coir pith is not an efficient medium for most of the microorganisms, but our study confirms the efficacy of coir pith for the growth of nitrogen fixing organisms, and thereby we can use the nitrogen fixing bacteria for the degradation of coir pith effectively. The final product also can be used for many plants as effective organic manure. This study will encourage the use of coir pith as organic manure for cultivation purposes and which will also help to minimize the problems relating to accumulation of this natural waste material in coir based industries.

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# The Effect of Different Mutagens on Amylase Activity of *Bacillus Spp* on Sago Waste.

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**ABSTRACT**

The sago waste, which are dumped in large quantities form a rich source of starch. The micro flora present in such wastes, especially *Bacillus spp* has the ability to produce amylase enzyme which are capable of degrading starch present in the sago wastes. The present study was undertaken with a view to understand the influence of mutagens on enzyme activity of *Bacillus spp*.The mutagens such as UV radiation (Physical), and Ethidium bromide (Chemical mutagen) was tested against *Bacillus spp* for their enzyme activity. The UV radiation enhances enzyme production while, Ethidium bromide (Conc: 0.5 mg/ml, 1.0 mg/ml and 2.5 mg/ml) reduces the enzyme activity of *Bacillus spp*.The results of the study thus revealed that the UV mutation caused genetic makeup of the organism and the resultant organism had enhanced the activity effectively, converted starch into glucose and the chemical mutation causes the reduced activity of the organism in the production of amylase. This biochemical activity of *Bacillus spp* as a function of mutagen helped in enhancing the processing of sago waste through strain improvement.

**Keywords:** Sago Waste, Mutagenesis, *α-amylase*, Ethidium Bromide.

## 1. INTRODUCTION

The residue obtained after the extraction of starch from the sago palm is starchy fibrous pith commonly called as sago waste. It is cheaply available and is abundant especially in Kerala and Tamil Nadu. The sago is a remedy for the diabetics and after its extraction the residue is merely dumped in open spaces. This cause serious environmental problem as it leached out in nearby streams during rainy season and hence contributes to pollution (Kasing *et al.*, 2000). The amylase action is of great industrial and biological importance. An enzyme is a protein molecule that speeds up chemical reactions. Each enzyme has a unique shape that determines its function. The enzyme has the capacity to acts on the carbohydrates and hydrolyses it into simpler molecules of sugar such as glucose and maltose. The carbohydrate present in plants refers as starch and sago (*Cycas circinalis*) is one of the rich sources of starch. The *Bacillus spp (esp Bacillus subtilis)* are the common micro flora in sago and they produce the amylase enzyme which hydrolyses the starch into glucose.

The raw sago pith waste (*Metroxylon sago* Rottb) was collected from the areas of Cochin where the wastes were dumped in large quantities. This was used as a source for the amylase enzyme. The micro flora present in the sample was isolated by serial dilution. The serially diluted sample was cultured to the Luria-Bertani (LB) broth and minimal M9 were used as the rich and defined media respectively. Tryptophan and threonine were used at 50μg ml and thiamine-HCL at 10μg ml final concentration. Solid media contained 2% agar. The cultured plates were incubated and when the colonies developed, they were sub-cultured and estimation was done. They were identified as gram positive bacilli, *Bacillus amyloliquefaceans* strain F(ATCC 23350) which were examined using glucose/maltose as the carbon source. While the cell growth was rapid and when glucose was used as the carbon source; higher cell mass, higher total and specific enzyme activities, and higher enzyme production rates were obtained when maltose was used as the carbon source. *B. amyloliquefaciens* has widely been reported to produce amylase enzymes (Abante *et al.*, 1999; Priest., 1977; Yoo *et al.*, 1988) but so far it has not been reported to produce cellulose enzyme, though other Bacillus species have been widely shown to produce several components of the cellulose enzymes (Pajni *et al.*, 1989). *B. amyloliquefaciens* is also known to produce many other enzymes such as alpha amylases, alpha acetolactase, decarboxylase, beta-glucanase, hemicellulase, maltogenic amylase, protease and xylanase on commercial basis (Luzmeira.com 2005) These can grow aerobically on nutrient agar medium, which were confirmed as amylase producing organisms as the culture exhibited clear zones around the colonies, when iodine solution was added.

Sago waste serves as feed for cattle, pigs and poultry. Yeong and Ali (1982) reported that it has no specific industrial or commercial use. According to Bintoro and Sainapar (1993) it can be used as a soil conditioner. Sago pith is composed mainly of starch and fiber, including a fair amount of minerals. The cellulose and starch components have a good potential for bioconversion into value added products. Ozawa *et al.* (1996) reported that

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the sago pith consist of starch (58%), cellulose (23%), hemicelluloses (9.2%), pectin (5.8%) and lignin (309%). As starch is resistant to easy digestion in cattle, it is important to degrade starch in to glucose. It is in this regard the biotechnology plays a significant role. Microbial strains (bacteria and fungi) are employed to degrade the sago waste in to cellulose and starch components (Coughlan, 1985). The breakdown of these components produces simple sugars which have many industrial uses.

Amylases from the *Bacillus spp* have several industrial applications such as in food, fermentation, textile and paper. α-amylases from *Bacillus spp* cleaves internal α-1, 4 linkages present in starch molecule in an endo fashion. The pure culture of the amylase producing microorganisms was then subjected to mutagenesis. Here the ultra-violet rays and chemical mutagens like EB (Ethidium Bromide) were used. The former was used with the intensity of 245 nm with different exposure times (5 min, 10 min and 20 min) and the latter used at concentrations of 5, 10 and 20 nM. The exposure to mutagens resulted in high mortality rate of the micro-organisms, which can be attributed to the harmful effect of the UV rays and those that survived showed varied enzyme activity which was estimated by various assay methods.

Some aspects of *Bacillus spp.*, that are of special interest for their industrial applications are (i) *B.subtilis* has no known pathogenic interactions with humans or animals. (ii) *B.subtilis* can secrete homologous and heterologous proteins; and (iii) *Bacillus* strains have many industrial applications, and systems are well developed for large scale cultivation. But there are limitations also, the prominent ones being the high levels of protease secreated, which cleaved the cloned gene products, and the high degree of structural instability of plasmids, which limits their use for production of recombinant proteins. (Vyas *et al*, 1994). The enzymatic hydrolysis of starch by α-amylase lead to the formation of smaller maltosaccharide fragments. Every starch polysaccharide molecule possess exactly one reducing end which can reduce Dinitro salicylic acid; therefore, the measurement of reducing sugars is a useful method to determine the molar concentration of starch molecules in solution The kinetics of liberation of reducing sugars in the course of enzymatic starch degradation are described with Michaelis-Menton kinetics (Heitmann *et al*, 1997).

Normally mutation influences the production of amylases in *Bacillus spp*. It has been reported that the morphological variations such as UV and X-rays influence the enzyme production. The mutagens such as UV rays, NTG, Ethidium Bromide, 5-Bromo uracil and ethyl methane sulphonate (EMS) are commonly employed in the mutagenic experiments. The development of such genetically engineered strain with the enhanced/reduced amylase activity can be achieved by the treatment of bacterial cells with these mutagens.

## 2. MATERIALS AND METHODS

### 2.1 Raw Sago Waste

The raw sago waste (*Cycas circinalis, Cycas revoluta*) obtained from the nearby areas where the wastes were dumped was used as source for the amylase enzyme. 1gm of sago waste which was mixed in 100 ml distilled water and was serially diluted. It was then then cultured in nutrient agar plates, screened and undertook starch hydrolysis test for amylase producers. The sample was sub cultured and assayed for enzyme activity.

(a) Media used: Luria-Bertani (LB) broth and minimal M9 were used as the rich and defined media respectively. Tryptophan and threonine were used at 50μg ml and thiamine-HCL at 10μg ml final concentration. Solid media contained 2% agar.

### 2.2 Mutagenesis

The UV mutagenesis was done with the UV lamp with different exposure times of 5 min, 10 min, and 15 minutes. After each exposure times; they are subcultured to another fresh culture medium. After the appropriate incubation times, it can taken for the enzyme assay. While the chemical mutagens were used in different concentrations of 0.5 mg/ml, 1 mg/ml and 2 mg/ml.

### 2. 3 Enzymes Assay

The enzyme activity was estimated by DNSA method and Starch Depletion Methods (Brooks *et al.*, 1972) in which the hydrolysis of starch to a significant extent resulting in the release of maltose was estimated by colorimetric method.

#### *I. Production of Amylase by using Bacillus Spp*

50 ml of liquid starch medium was prepared (50 ml) in 250 ml flask and sterilized at 121°C for 15 min in an autoclave. After cooling the medium, loopful of Bacillus spp was inoculated from slant culture ascetically and the flask was incubated in shaker at for 12-15 hours. Sample from the flask was used for the assay of enzyme.

#### *II. Preparation of Enzyme*

5 ml of culture suspension was taken and centrifuged at 4500rpm for 10 min at 5°C. After centrifugation the supernatant containing amylase enzyme was separated and preserved at 4°C. Supernatant containing amylase was used for the assay.

#### *III. Preparation of Standard Graph for Glucose*

Stock solution of glucose (1 mg/ml conc) was prepared in standard flask. Take 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml of stock solution of glucose in respective testtubes. Standard graph for glucose was prepared by Dinitrosalicylic method.

***IV. Assay of Amylase Activity***

1 ml of preserved supernatant of culture extract was taken in a test tube and 1 ml of substrate solution (Starch in phosphate buffer was added). The mixture was kept in water bath at 40°C for 30 min for the bioconversion takesplace. The preserved supernatant (2 ml) was boiled in a separate test tube to inactivate the enzyme present and was treated as blank. This was subjected to the same test as that of the above. Read the Optical density at 540 nm. This value was extrapolated in the standard graph and by the concentration of the glucose produced the activity of amylase was observed.

**2.4. Purification**

Purification of enzymes was done through-Ammonium sulphate precipitation and dialysis. The Ammonium Sulphate Precipitation was done by the addition of ammonium sulphate in small amount of enzyme homogenate. The mixture was then centrifuged at 10000 rpm for 15 minutes. The pellets were removed and supernatant collected. This led to 40% purification. It was again centrifuged at 10000 rpm for 15 min which resulted in 60% purification. It was repeated twice so as to get 100% purification.

The solution which was purified by Ammonium Sulphate Precipitation was then subjected to Dialysis. It is done for removal of salts from the proteins (enzymes). Special permeable membranes called Dialysis bags were used here, which allowed the low molecular weight components to pass through it, but not those with high molecular weight like proteins (enzymes). The dialysis bags filled with the enzyme sample were put into dialysis fluid (Phosphate buffer with pH-7). The repeated changes of the dialysis fluid helps the removal of more salts from the enzyme and become pure.

**2.5 Immobilization**

Immobilization was done by 1.5 ml of enzyme source which was mixed with 10 ml of 4% Sodium Alginate and mixed well. This mixture was drawn through a pipette to 2% Calcium Chloride solution. The beads are formed was then introduced to the 3% Hydrogen Peroxide solution. The enzyme get Immobilized.

**3. RESULTS**

**3.1 Isolation and Identification of Organism**

The sago waste, used as a source for the amylase was serially diluted from $10^{-1}$ to $10^{-7}$ in distilled water and they were plated in nutrient agar plate. After 24 hours of incubation in nutrient agar plate, the bacterial white colonies were obtained. The colonies obtained were about 120 in number in $10^{-7}$ dilutions. The colonies obtained were then identified by gram staining procedure. It showed, they are gram positive bacilli and these can grow aerobically on nutrient agar medium, which were confirmed as amylase producing organism as the culture exhibited clear zones around the colonies, when iodine solution was poured on it.

**3.2 Mutagenesis of Colonies**

With the exposure of mutagens, majority of the organisms died due to its harmful effect (Figure 1 and 2) and those that survived showed the varied enzyme activity which can be estimated by various assay methods. The results of wild and mutated strains are given in Table 1 and 2.

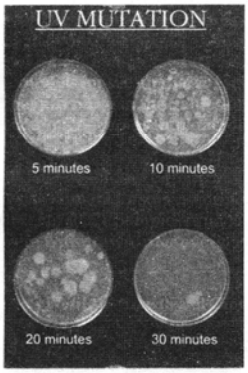



**Figure 1: Photograph of Showing Bacillus Colonies for Different Petri-Plates Time Exposures to U. V. Radiation.**

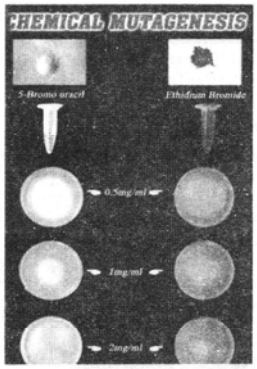



**Figure 2: Photograph of Petri-plates Showing Bacillus Colonies for Different Time Exposures to Chemical Mutagens.**

**Table 1**
**Standard Table for Glucose**

| Sl No | Particulars(ml) | Blank(B) | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---|---|---|---|---|---|---|---|
| 1 | Standard glucose | - | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 |
| 2 | Concentration | - | 200 | 400 | 600 | 800 | 1000 |
| 3 | Distilled Water | 4.0 | 3.8 | 3.6 | 3.4 | 3.2 | 3.0 |
| 4 | Dinitro Salicylic Acid | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| | | | *Heat for30 at 60-70°C* | | | | |
| 5 | OD at 540 nm | 0.00 | 0.20 | 0.27 | 0.42 | 0.57 | 0.71 |

**Table 2**
**Test Results of Wild, Ultra Violet and Chemical Mutated Strain**

| Sl No | Particulars(Ml) | Blank(ml) | Wild Strain (ml) | | | Ultra Violet Strain(ml) | | | Chemical Mutated Strain(ml) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Supernatant (unboiled) | - | 0.5 | 1.0 | 2.0 | 0.5 | 1.0 | 2.0 | 0.5 | 1.0 | 2.0 |
| 2 | Supernatant (boiled) | 2.0 | - | - | - | - | - | - | - | - | - |
| 3 | Starch Solution | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| | | | *Incubate for 10 min* | | | | | | | | |
| 4 | Dinitro Salicylic Acid | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 5 | Optical Density at 540 nm | 0.00 | 0.06 | 0.11 | 0.18 | 0.18 | 0.22 | 0.25 | 0.09 | 0.02 | 0.01 |

### 3.3 Enzyme Assay

**Dinitro Salicylic Acid Method (DNSA Method.)**

The standard graph is plotted with the concentration of glucose on X-axis and Optical density on Y-axis. Tabulation for Standard graph and test value is given in Table 1 and 2.

#### *(a) Wild Strain*

The DNSA method, that is based on the hydrolysis of starch (amylase and amylopectin). To a significant extends resulting in the release of maltose was estimated by colorimetric method. Here the method is performed with the different volumes of enzyme and getting the enzyme activities 40µg, 125µg and 245µg respectively. It can be represented in the the Table 2.

#### *(b) U.V. Mutated Strain*

The enzyme solution which produced by organisms after U.V. mutation were subjected to the DNSA method. The 3 different kinds of samples are issued, viz 5 minute, 10 minutes and 20 minute exposed culture. The optical density which given after the assay was somewhat increased when compared with the wild type. for the 5 min exposed culture gives the enzyme activity 245µg, the 10 min exposed one gives 300µg and the 20 min exposed sample gives 340µg. The result is given in Table 2.

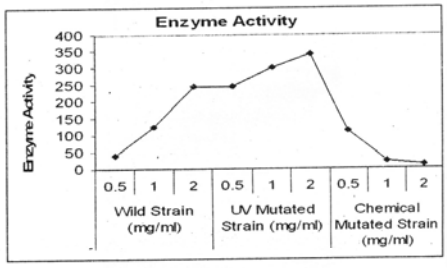
#### *(c) Chemical Mutated Strain (EtBr)*

After the DNSA method of chemical mutated strain, it was observed the enzyme activity 110µg (.5 mg/ml Concentration) and values are lower than the same of the wild strain. The EtBr mutated strain gives the activity 20µg, instead which gives the 125µg in the case of wild strain. The Table 2 gives its tabulation.

The enzyme activity can also be represented in the form of a graph. The graph-1 represents the activity of the enzymes by DNSA method, by plotting the different concentration of samples in X-axis and enzyme activity on Y-axis. From the graph it is clear that the increased enzyme activity by the action of ultra violet mutation and reduced activity by Chemical mutagen.

Their enzyme activity can be represented in the form or Graph also in Graph-I . The different samples are plotted in the X-axis and enzyme activity on Y-axis.

**Graph I Enzyme Activity**




### 4. DISCUSSION

*Bacillus spp* was isolated from sago waste and checked for amylase production. The colony reporting positive result

was maintained in nutrient agar and used for enzyme production. Gashaw *et al*, 1999 reported the presence of two extra cellular amylases designated as Amy I and Amy II were purified from the cell free culture supernatant of *Bacillus spp*. So for only one type of amylases has been reported from *Bacillus stearothermophiles*.

Khajeh *et al*, 2001a & 2001b proved that upon modification there is a dramatic enhancement of activity of amylase from *Baillus litcheniformis* determined at 37°C. Mutagens at particular concentrations increased enzyme activity. Bernard Tao (1989) reported that directed mutagenesis i.e. the promise of tailors made enzymes and proteins that have engineered properties. In the present study, random mutagenesis of the amylase producer gave a substantial alteration in enzyme activity measured by enzyme assay.

Enhancement of operational stability with retention of catalytic activity of enzyme technology today. Efforts were aimed at elucidation of alteration in enzyme activity due to mutagenesis has been focused. Here the efforts were done to improve the enzyme activity of amylase by inducing the Mutagenesis and thereby increase the quality of cattle feed.

While these advances are positive steps towards fulfilling the promise of creating designer enzyme protein engineering is still not yet a reality. We are still in a phase of observation and explanation rather than prediction and creation. Current research has established a few rudimentary guidelines of enhanced crude enzyme activity.

The action of ethidium bromide is quite well studied and estimated. Ethidium bromide is a phenantridine dye which binds to nucleic acids by intercalation (Le Pecq & Paoletti, 1967). The mode of binding alters the tertiary structure of DNA and in particular that of circular DNA (Waring, 1970). So the changes in the enzyme activity and production are assumed as the alteration in the DNA level of the species to produce the enzyme. The results of wild and mutated strains are given below in Table 1. A highly effective mutagen may not necessarily show high efficiency and vice versa (Jayakumar and Selvaraj, 2003). Both the physical and chemical mutagens were found to make the changes in these activities. Here, the physical mutagen was found to have a positive effect and thereby enhancing the enzyme activity but the chemical mutagen (EB) showed negative effect by decreasing the enzyme production. Enzyme activity was estimated by Dinitro Salicylic Acid (DNSA) method. The activity of enzyme was measured by extrapolating the optical density value in the standard graph. The enzyme activity for wild strain was estimated as 40µg, 125µg and 245µg for 5, 10 and 20 ml of the enzyme solution respectively. The same for the ultraviolet-treated samples showed the enzyme activity to be 245µg, 330µg and 340µg for 1 ml each at 5 min, 10 min and 20 min exposure respectively, and the chemically mutated species showed enzyme activity at 110µg, 20µg and 10µg for 5 mM, 10 mM and 20 mM concentration for 1 ml of sample. This observation is in harmony with the works of Kavithamani *et al*. (2008), who studied the mutagenic effectiveness and efficiency of gamma rays and EMS in soybean.

From this study it could be concluded that under the selected conditions, ultraviolet rays proved to be effective in enhancing the enzyme activity where as the chemical mutagen caused decreased production of amylase. Hence, this method of strain improvement may prove to be an economic and effective help in the processing of sago waste. By the action of amylase, the starch gets degraded and converted in to lower sugar moieties such as glucose, which are easily digestible to the ruminants. This aspect of the possibilities of sago waste being put to better use than reduced to being an environmental menace warrants further study so that sago waste can be put to use as a cheaper and quality-enriched cattle feed.

## 5. CONCLUSION

The sago waste, which is dumped on the village areas and road premises, is a rich source of the amylase enzyme and this enzyme done the function of degrading starch and thereby it acts as a good feed for cattles and other ruminants. It is seen that the mutagens (Ultra-violet light, Ethidium Bromide) have a key influence in the Bacillus organisms for he production of amylase enzymes. It causes the enhancement and the reductions of the enzyme action at specific concentrations of the mutagens.

In the present work, only Ultra-violet mutagen greatly enhances enzyme activity and such a way of strain improvement may be helpful in processing of sago waste. But this aspect not been verified and in future, if such strains are developed and used in degradation of waste that is used as cattle feed, it could bring a chance in the quality of cattle feed.

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# USE OF COIR PITH COMPOST AS AN EFFECTIVE CULTIVATING MEDIA FOR ORNAMENTAL, MEDICINAL AND VEGETABLE PLANTS

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## ABSTRACT

Coir pith which is considered as a problematic waste in coir fibre extraction units can be converted to effective organic manure with use of fungus (*Pleurotus sajor caju*) and nitrogen fixing bacteria (*Azotobacter vinelandii* and *Azospirillum brasilense*) and which can be used for the cultivation of all kinds of plants. Twenty ornamental, ten medicinal and five vegetable plants were subjected for the investigation. From the studies, it is confirmed that all the plants show enhanced growth in coir pith compost compared with garden soil. The composted coir pith can be used for an effective cultivating media for all kinds of plants including ornamental, medicinal and vegetables. Thus the use of coir pith for cultivation of plants can be entertained in homes, nurseries and fields; thereby minimize the environmental pollution caused by coir pith.

**Keywords: Compost, Ornamental Plants, Organic Manure, Carbohydrate, Chlorophyll**

## INTRODUCTION

Coir pith is known as coir dust and is the major by-product of the coir fiber extraction industries **[1]**. Normally coir pith is dumped as agricultural waste and accumulates as heaps of course and fine dust. After the extraction of coir fibre from the husk it is a ligocellulosic waste material consists of lignin 20 to 40%, cellulose 40 to 50%, hemicelulose 15 to 35% and protein 2.04% **[2]**. Coconut (*Cocos nucifera*) is cultivated in tropical

countries. The fibrous mesocarp of coir is used to make ropes. The waste of coir yarn industry (coir pith) gets accumulated in large quantities making their disposal difficult, though it is used as soil conditioner **[3]**. India is the leading producers of coconut. It is an important oil seed and cash crop grown in south Indian states especially in Kerala, Tamil Nadu and Karnataka.

The plants that concern man can be divided into three general categories: (1) agricultural plants that are grown for food or fiber; (2) weedy plants that grow where they are not wanted; (3) ornamental, or amenity, plants. The latter group is grown mainly for its aesthetic qualities but may have certain secondary benefits such as providing shade, privacy, wind protection, etc. In general, the term "ornamental plant" or "ornamental" is used to describe those species primarily cultivated for their aesthetically-pleasing characteristics such as form, bark, leaves, flowers, fruit or some combination thereof.

In the United States there is currently a growing interest in ornamentals. The expanded use of these plants has caused their cash value to rival, and in some instances exceed, the value of food and fiber crops. In 1984, nursery production in three north-eastern states (Maine, New York and Pennsylvania) accounted for more than $230 million **[4]**; the value of white potatoes in the same three states in 1982 was $188 million (USDC, 1984).

All the experimental medicinal plants are well known for its efficiency for medicinal values. Brahmi (*Bacopa monnieri*), a well known prostrate herb, is distributed in damp, marshy areas throughout India **[1]**. *Piper longum* can be cultivated successfully in organic matter rich fertile well drained forest soils. It is originated in South Asia and is found almost all over India. It is a component of Indian medicine reported as remedy for treating several diseases including gonorrhea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infections, chronic gut related pain, arthritis and alleviation of anxiety **[5]**. *Phyllanthus niruri* originated in India, usually occur as a winter weed throughout the hotter parts. *P. niruri* is a herb of Euphorbiaceae family that grows upto 60 cm. In recent years, more and more people are complimenting their treatment with natural supplements. Kalmegh (*Andrographis paniculata*) as ethanolic extract has an insulin sensitizing effect **[6]**. Coconut coir dust is being marketed as a soilless medium substitute for sphagnum peat moss that inhibits fungus gnat (*Bradysia* sp.) development **[7]**. Several other important studies also conducted on antioxidant activity

of some medicinal plants grown under organic farming conditions **[8]**. Growth of *A. paniculata* in vermicomposted coir pith has suggested that vermicomposted coir pith could be helpful for reclamation of soils from industrial sites for cultivation of *A. paniculata* in a small scale nursery **[9]**. Aqueous extract of leaves of *A. paniculata* has traditionally been used for treatment of various liver disorders and jaundice **[10]**. Vegetable farming is an important cultivation practice of peoples in Kerala. But after several times of cultivation the fertility of soil has been lost due to the reduction of nutrients in the soil. Use of coir pith in soil helps to reclaim the nutrients in the soil and also helpful for enhancing the water holding capacity of the soil.

This study presents the use of coir pith compost with white rot fungus and nitrogen fixing bacteria for cultivation of ornamental, medicinal and vegetable plants.

## MATERIALS AND METHODS

### Experimental Section

Coir pith was collected from Kalavoor, Alapuzha district, India. Microbial species get from Microbiology division of CCRI, Alappuzha and Agro biotech, Kottayam. Experimental protocol, field experiment and biochemical analysis of coir pith was carried out at School of Environmental Studies, CUSAT and CCRI, Kalavoor.

### Composting Process

Two lots of coir pith (1 kg each) in duplicate were laid as separate heaps. *P. sajor caju* (12 g) spawn and N-fixing bacteria (*Acetobactor vilendii and Azospirillum brasilense*) were thoroughly mixed with first lot of coir pith. Second sample was kept as untreated. Moisture content was maintained and study was monitored regularly for 45 days, by drawing out samples at periodic intervals.

### Field Experiment

The coir pith compost degraded by *Pleurotus sajor caju, Azotobacter vinelandii* and *Azospirillum brasilense* were compared with control (garden soil). Two sets of 35 pots were used for cultivation of twenty ornamental, ten medicinal and five vegetable plants. Each set of pot was maintained for each species of experimental plant. One plant is cultivated in garden soil and the other is in compost. After 45 days, measurements were made of leaf number, shoot and root length. Aqueous extract of leaf was analyzed for carbohydrate **[11]**, protein **[12]**, and chlorophyll **[13]**.

### Constituent Analysis of Coir Pith and Soil

Analysis of lignin was carried out by Modefied Klason Lignin assay, N by Kjel dahl method, organic carbon by Walkey and

Black method, P by Spectrophotometer and K by Flame photometer.

**RESULT AND DISCUSSION**

Lignin content, organic carbon and NPK of garden soil sample and coir pith before and after decomposition with *P. sajor caju* and N-fixing bacteria were estimated **(Table 1)**. Lignin content of raw coir pith (32%) varied after biodegradation with a combination of organisms [*P. sajor caju* and N- fixing bacteria (*Acetobacter* and *Azospirillum*)]. Decomposition was up to 17% with the combination. *Pleurotus spp.* is known to decompose and utilize various agricultural wastes **[9]**. From current results, an increase in nitrogen component of substrate, and a proportionate decrease in carbon were also observed as also reported in earlier works **[3]**. A salient finding observed is that concentration of nitrogen in spent substrates increased with reduction in C: N ratio25. In all treatments, a reduction was observed of lignin content, which confirms action of microbial agents effectively. There was a definite reduction in organic carbon content of raw coir pith when treated with mushroom and bacteria. The growth physiology observed by the pot/grow bag culturing of all the plants shown in **Table 2**-7. A pot culture experiment was carried out to study effect of coir pith based potting mixture with garden soil on twenty different ornamental plants, ten medicinal plants and five vegetable plants. Maximum plant growth (leaf number, shoot length and root length), carbohydrate, protein and chlorophyll were achieved in plants grown in soil and composted coir pith. In both the samples the control (soil) shows no significant increase in both root and shoot length. But in plants cultivated in composted coir pith there is a definite increase of shoot and root were observed. All kinds of plants including ornamental, medicinal and vegetables were showed enhanced growth in coir pith compost comparing with garden soil. The authors already studied the efficiency in growth of medicinal plants in coir pith compost **[1]**, but the present study show light to the efficiency of all kinds of plants including ornamental, medicinal and vegetable plants growing in coir pith compost. All the twenty plants were grown well in both the samples and the results were tabulated in **Table 2**. In case of ornamental plants; the leaf number is more in plants grown in compost than garden soil. The shoot and root length also show variations. **[14]** observed coir pith showed a spectacular increase of the water holding capacity of the potting mixture when tomato plants on coir pith based potting mixture grown. From the results, it is observed that an increase in nitrogen and

decrease in carbon content was observed in all the samples and this reduction in C: N ratio resulted from the decomposers using *Pleurotus sajor caju* and nitrogen fixing bacteria. A salient finding observed is that the plants grown in coir pith compost is well grown with more lengthy shoots and more number of leaves. This would have confirmed that coir pith compost can be used as an effective potting media for all kind of plants including Ornamental, Medicinal and Vegetables.

The protein content of ornamental plants in the range of 1.39 (*Hibiscus rosasinensis*) to 4.57 (*Fabiana imbricate*) in plants cultivated in soil and it is 3.12 (*Brugmansia suaveolens*) to 5.98 (*Fabiana imbricate*) in plants cultivated in coir pith compost. In case of medicinal and vegetable plants also have no fluctuations in the previous results. Therefore it is confirmed that composted coir pith itself can be used as an effective potting medium for all kinds of plants including ornamental, medicinal and vegetable plants. Carbohydrate and chlorophyll content also show similar variations. It is observed that the amount of carbohydrate is high in plants grown in the coir pith compost while the same in the soil show reduced values. In ornamental plants, *Rhododendron peridymenoides* (0.36 mg/g) shows the highest value of carbohydrates and the lowest one is *Hydechium coronarium* (0.20 mg/g). In case of medicinal plants, *Bacopa monnieri* (0.36 mg/g) have the highest amount of carbohydrate which is grown in coir pith compost and the same in garden soil shows the value 0.26 mg/g. All the medicinal plant shows the carbohydrate content more than 0.30 mg/g while the same plants grown in soil indicate the range of 0.20 mg/g to 0.26 mg/g. There are not many differences in the case of vegetable plants. *Solanum melongata* shows the higher value (0.36 mg/g) of carbohydrate content and the lowest is *Vigna unguiculata* which is cultivated in garden soil. Chlorophyll is one of the important indicator for the growth of plants. Chlorophyll content in the ornamental plants show a definite difference when cultivated in coir pith compost while in comparison there are not much changes during cultivated in garden soil. The chlorophyll content is between 0.16 mg/g (*Hibiscus rosasinensis*) to 0.26 mg/g (*Nyctanthus arbortristis*) in compost and in garden soil it is in range of 0.11 mg/g (*Brugmansia suaveolens* and *Rhododendron peridymenoides*) to 0.20. Almost similar results were reported that high percentage of rooting of acalypha and bougainvillea was observed in coir dust medium when compared to sand or soil+organic manure. In case of

medicinal plants, almost similar kinds of results were observed. The plants grown in the coir pith compost show increased chlorophyll value and the same in garden soil have low values in comparison with compost. Vegetable plants also have no definite fluctuation from the pattern of ornamental and medicinal plants. **[9]** suggested that vermicomposted coir pith with *Eudrilus eugeniae* and *Lampito mauritii* were used to prepare coir pith based compost in different ratios combining with cow dung. They grow *Abelmoschus esculentus* and study their growth physiology such as shoot length, root length, carbohydrate, protein and chlorophyll. The byproduct of the coir industry, coir pith can be converted to effective coir pith manure by the use of fungus and nitrogen fixing bacteria and use this manure for almost all kinds of plants including ornamental, medicinal and vegetable plants. All the experimental plants such as twenty ornamental, ten medicinal and five vegetable plants were grown in coir pith compost much better than garden soil. Thus the present study confirms that composted coir pith can be used as an effective growing medium for all kinds of plants without the use of garden soil.

## CONCLUSION

Coir pith compost is as excellent and eco-friendly option for cultivation of all kind of plants. Composted coir pith based potting medium for cultivation of ornamental, medicinal and vegetable plants suggested that this compost can be used in reclamation of soils for its enhanced production, and also possibly almost all the other crops. This study will encourage those involved in cultivation of ornamental plants in homes and nurseries, cultivation of medicinal plants and vegetables organically, economically and using environmental friendly ways.

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Figure 1: Plants in Garden Soil and Compost Grow Bags

Table 1: Lignin, Organic Carbon and NPK Contents on Garden Soil and Compost

| Treatment | Lignin (%) | | Organic carbon(%) | | Nitrogen(%) | | Phosphorous(%) | | Potassiu | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 (Days) | 45 (Days) | 0 (Days) | 45 (Days) | 0 (Days) | 45 (Days) | 0 (Days) | 45 (Days) | 0 (Days) | 4 |
| Garden Soil | - | - | 5.98 | 5.63 | 0.43 | 1.08 | 0.25 | 0.44 | 0.056 | |
| mposted Coir pith with *P. sajor caju*, *A. vinelandii* and *A. brasilense* | 32 | 17 | 7.13 | 6.27 | 0.44 | 0.67 | 0.22 | 0.52 | 0.033 | |

Table 2: Leaf Number, Shoot and Root Length of Medicinal Plants

| Plant Name | Leaf Number | | | | | | Shoot Length(cm) | | | | | | Root Length(cm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Soil | | | Compost | | | Soil | | | Compost | | | Soil | | | Cor | |
| | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 | 60 | 0 | |
| *Andrographis paniculata* | 8 | 18 | 48 | 10 | 19 | 52 | 10 | 14 | 18 | 10 | 13 | 20 | 6 | - | 4 | 6 | |
| *Phyllanthus niruri* | 11 | 16 | 19 | 11 | 16 | 22 | 10 | 13 | 17 | 11 | 12 | 18 | 8 | - | 14 | 7 | |
| *Bacopa monnieri* | 8 | 19 | 30 | 8 | 12 | 31 | 8 | 10 | 12 | 8 | 10 | 12 | 8 | - | 13 | 6 | |
| *Piper longum* | 6 | 12 | 18 | 7 | 12 | 18 | 10 | 17 | 19 | 10 | 17 | 19 | 6 | - | 12 | 8 | |
| *Kaempferia galanga* | 10 | 13 | 16 | 10 | 13 | 19 | 9 | 17 | 18 | 9 | 16 | 20 | 8 | - | 12 | 7 | |
| *Vitex negundo* | 12 | 17 | 19 | 12 | 16 | 20 | 12 | 16 | 19 | 11 | 18 | 22 | 6 | - | 14 | 6 | |
| *Eupatorium triplinerve* | 11 | 14 | 33 | 12 | 15 | 36 | 12 | 16 | 31 | 12 | 19 | 34 | 6 | - | 14 | 9 | |
| *Inula recemosa* | 11 | 20 | 35 | 10 | 22 | 37 | 11 | 20 | 29 | 12 | 21 | 31 | 5 | - | 15 | 6 | |
| *Euphorbia nivulia* | 6 | 21 | 31 | 6 | 21 | 34 | 12 | 15 | 16 | 13 | 15 | 16 | 8 | - | 11 | 5 | |
| *Ocimus kiliandscharium* | 13 | 30 | 40 | 13 | 30 | 41 | 10 | 19 | 41 | 12 | 20 | 42 | 10 | - | 12 | 10 | |

Table 3: Leaf Number, Shoot and Root Length of Ornamental Plants

| Plant Name | Leaf Number | | | | | | Shoot Length(cm) | | | | | | Root Length(cm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Soil | | | Compost | | | Soil | | | Compost | | | Soil | | | Compo | |
| | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 |
| *Brugmansia suaveolens* | 8 | 20 | 90 | 8 | 28 | 101 | 10 | 16 | 45 | 20 | 21 | 50 | 8 | - | 12 | 8 | - |
| *Rhodendron peridymenoides* | 9 | 32 | 100 | 9 | 31 | 109 | 9 | 12 | 15 | 8 | 12 | 18 | 7 | - | 13 | 6 | - |
| *Nyctanthus arbortristis* | 10 | 18 | 48 | 11 | 17 | 52 | 11 | 22 | 34 | 11 | 18 | 35 | 10 | - | 11 | 9 | - |
| *Dahlia juarezii* | 8 | 43 | 101 | 7 | 42 | 112 | 10 | 19 | 24 | 9 | 14 | 25 | 2 | - | 14 | 7 | - |
| *Hydrangea arborescens* | 10 | 16 | 25 | 10 | 15 | 27 | 10 | 18 | 28 | 10 | 21 | 30 | 5 | - | 14 | 5 | - |
| *Hydechium coronarium* | 6 | 16 | 30 | 6 | 16 | 32 | 5 | 8 | 10 | 5 | 8 | 10 | 5 | - | 15 | 6 | - |
| *Polypodium glycyrrhiza* | 7 | 12 | 14 | 6 | 11 | 17 | 11 | 16 | 21 | 11 | 17 | 25 | 6 | - | 12 | 8 | - |
| *Averrhoa bilimbi* | 6 | 14 | 20 | 8 | 14 | 23 | 10 | 15 | 20 | 12 | 17 | 20 | 8 | - | 12 | 8 | - |
| *Bellis perennis* | 10 | 25 | 80 | 11 | 25 | 83 | 8 | 11 | 14 | 8 | 12 | 15 | 2 | - | 11 | 2 | - |
| *Spathiphyllum wallisii* | 7 | 13 | 20 | 7 | 12 | 24 | 5 | 6 | 9 | 5 | 9 | 10 | 5 | - | 10 | 5 | - |
| *Rosa rugosa* | 11 | 16 | 26 | 11 | 16 | 28 | 11 | 16 | 20 | 11 | 14 | 22 | 9 | - | 10 | 8 | - |
| *Petunia hybrida* | 10 | 17 | 22 | 12 | 18 | 26 | 10 | 12 | 14 | 10 | 14 | 16 | 9 | - | 11 | 9 | - |
| *Brunfelsia australis* | 8 | 15 | 24 | 8 | 15 | 25 | 8 | 14 | 17 | 8 | 15 | 18 | 8 | - | 12 | 9 | - |
| *Hibiscus rosasinensis* | 10 | 17 | 35 | 10 | 18 | 37 | 10 | 20 | 24 | 11 | 19 | 25 | 5 | - | 10 | 5 | - |
| *Tumera ulmifolia* | 10 | 15 | 21 | 10 | 16 | 24 | 9 | 10 | 10 | 9 | 11 | 14 | 6 | - | 7 | 6 | - |
| *Ixora coccinae* | 11 | 20 | 21 | 11 | 20 | 26 | 11 | 31 | 40 | 11 | 30 | 42 | 8 | - | 10 | 2 | - |
| *Jasminum officinale* | 8 | 20 | 40 | 8 | 21 | 42 | 10 | 20 | 29 | 10 | 21 | 31 | 8 | - | 10 | 6 | - |
| *Fabiana imbricata* | 7 | 17 | 18 | 6 | 18 | 22 | 15 | 30 | 48 | 15 | 26 | 50 | 6 | - | 10 | 6 | - |
| *Bauhinia purpurea* | 9 | 13 | 20 | 9 | 13 | 20 | 12 | 18 | 30 | 12 | 21 | 32 | 6 | - | 11 | 5 | - |
| *Tabernaemontana divaricata* | 10 | 20 | 21 | 12 | 20 | 24 | 10 | 16 | 21 | 10 | 20 | 27 | 8 | - | 11 | 9 | - |

Table 4: Leaf Number, Shoot and Root Length of Vegetable Plants

| Plant Name | Leaf Number | | | | | | Shoot Length(cm) | | | | | | Root Length(cm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Soil | | | Compost | | | Soil | | | Compost | | | Soil | | | Compost | |
| | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 |
| *Momordica charantia* | 0 | 18 | 50 | 0 | 20 | 60 | 0 | 92 | 190 | 0 | 98 | 250 | 0 | - | 15 | 0 | - |
| *Abelmoschus esculentus* | 0 | 20 | 50 | 0 | 25 | 52 | 0 | 41 | 90 | 0 | 45 | 98 | 0 | - | 11 | 0 | - |
| *Lycopersicon esculentum* | 0 | 31 | 49 | 0 | 30 | 55 | 0 | 42 | 81 | 0 | 46 | 91 | 0 | - | 14 | 0 | - |
| *Solanum melongena* | 0 | 38 | 59 | 0 | 32 | 62 | 0 | 30 | 70 | 0 | 36 | 78 | 0 | - | 15 | 0 | - |
| *Vigna unguiculata* | 0 | 28 | 55 | 0 | 30 | 58 | 0 | 100 | 180 | 0 | 96 | 180 | 0 | - | 11 | 0 | - |

Table 5: Carbohydrate, Chlorophyll and Protein Content of Ornamental Plants

| No. | Plant Name | Biochemical Parameters | | | | | |
|---|---|---|---|---|---|---|---|
| | | Carbohydrate (mg/g) | | Chlorophyll(mg/g) | | Protein(mg/g) | |
| | | Soil | Compost | Soil | Compost | Soil | Compost |
| 1 | *Brugmansia suaveolens* | 0.28 | 0.30 | 0.11 | 0.26 | 2.41 | 3.12 |
| 2 | *Rhodendron peridymenoides* | 0.21 | 0.36 | 0.11 | 0.21 | 3.62 | 4.95 |
| 3 | *Nyctanthus arbortristis* | 0.23 | 0.31 | 0.18 | 0.25 | 2.52 | 4.12 |
| 4 | *Dahlia juarezii* | 0.25 | 0.35 | 0.12 | 0.21 | 4.53 | 5.10 |
| 5 | *Hydrangea arborescens* | 0.21 | 0.29 | 0.19 | 0.20 | 2.41 | 3.85 |
| 6 | *Hydechium coronarium* | 0.20 | 0.28 | 0.20 | 0.21 | 4.48 | 6.90 |
| 7 | *Polypodium glycyrrhiza* | 0.21 | 0.31 | 0.18 | 0.25 | 3.80 | 4.79 |
| 8 | *Averrhoa bilimbi* | 0.25 | 0.30 | 0.19 | 0.19 | 3.56 | 4.80 |
| 9 | *Bellis perennis* | 0.23 | 0.28 | 0.18 | 0.18 | 2.14 | 3.81 |
| 10 | *Spathiphyllum wallisii* | 0.22 | 0.27 | 0.18 | 0.21 | 2.46 | 3.78 |
| 11 | *Rosa rugosa* | 0.22 | 0.30 | 0.17 | 0.22 | 1.45 | 3.66 |
| 12 | *Petunia hybrida* | 0.21 | 0.27 | 0.19 | 0.19 | 2.51 | 2.78 |
| 13 | *Brunfelsia australis* | 0.21 | 0.28 | 0.16 | 0.18 | 2.47 | 2.75 |
| 14 | *Hibiscus rosasinensis* | 0.25 | 0.31 | 0.15 | 0.16 | 1.39 | 3.85 |
| 15 | *Tumera ulmifolia* | 0.21 | 0.35 | 0.18 | 0.19 | 3.46 | 4.90 |
| 16 | *Ixora coccinae* | 0.20 | 0.31 | 0.18 | 0.18 | 4.56 | 5.11 |
| 17 | *Jasminum officinale* | 0.26 | 0.30 | 0.19 | 0.21 | 3.61 | 4.12 |
| 18 | *Fabiana imbricata* | 0.25 | 0.30 | 0.15 | 0.18 | 4.57 | 5.98 |
| 19 | *Bauhinia purpurea* | 0.20 | 0.22 | 0.15 | 0.19 | 4.51 | 8.81 |
| 20 | *Tabernaemontana divaricata* | 0.20 | 0.28 | 0.15 | 0.21 | 4.50 | 5.40 |

Table 6: Carbohydrate, Chlorophyll and Protein Content of Medicinal Plants

| S. No. | Plant Name | Biochemical Parameters | | | | | |
|---|---|---|---|---|---|---|---|
| | | Carbohydrate(mg/g) | | Chlorophyll(mg/g) | | Protein(mg/g) | |
| | | Soil | Compost | Soil | Compost | Soil | Compost |
| 1 | *Andrographis paniculata* | 0.20 | 0.30 | 0.19 | 0.25 | 2.42 | 4.01 |
| 2 | *Phyllanthus niruri* | 0.21 | 0.31 | 0.19 | 0.21 | 3.51 | 4.75 |
| 3 | *Bacopa monnieri* | 0.26 | 0.36 | 0.18 | 0.20 | 3.46 | 3.00 |
| 4 | *Piper longum* | 0.25 | 0.30 | 0.17 | 0.26 | 2.45 | 2.81 |
| 5 | *Kaempferia galanga* | 0.21 | 0.35 | 0.17 | 0.19 | 2.49 | 2.96 |
| 6 | *Vitex negundo* | 0.26 | 0.31 | 0.18 | 0.18 | 2.46 | 3.02 |
| 7 | *Eupatorium triplinerve* | 0.25 | 0.31 | 0.19 | 0.21 | 1.52 | 3.89 |
| 8 | *Inula recemosa* | 0.22 | 0.30 | 0.20 | 0.21 | 2.61 | 4.98 |
| 9 | *Euphorbia nivulia* | 0.21 | 0.31 | 0.19 | 0.19 | 4.50 | 5.96 |
| 10 | *Ocimus kiliandscharium* | 0.20 | 0.32 | 0.18 | 0.18 | 4.40 | 6.95 |

Table 7: Carbohydrate, Chlorophyll and Protein Content of Vegetable Plants

| S. No. | Plant Name | Biochemical Parameters | | | | | |
|---|---|---|---|---|---|---|---|
| | | Carbohydrate(mg/g) | | Chlorophyll(mg/g) | | Protein(mg/g) | |
| | | Soil | Compost | Soil | Compost | Soil | Compost |
| 1 | *Momordica charantia* | 0.22 | 0.31 | 0.18 | 0.19 | 2.32 | 4.21 |
| 2 | *Abelmoschus esculentus* | 0.22 | 0.32 | 0.17 | 0.18 | 2.41 | 4.07 |
| 3 | *Lycopersicon esculentum* | 0.28 | 0.31 | 0.17 | 0.17 | 3.56 | 4.10 |
| 4 | *Solanum melongena* | 0.21 | 0.36 | 0.17 | 0.18 | 3.52 | 4.00 |
| 5 | *Vigna unguiculata* | 0.20 | 0.35 | 0.18 | 0.19 | 4.58 | 5.80 |

# Bioconversion of coir pith as effective soil less media for roof gardening

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## ABSTRACT

Experiments were conducted to evaluate the effect of biodegraded coir pith (BCC) treated with white rot fungi (*Pleurotus sajor caju*) and bacterial species (*Azotobacter vinelandii and Azospirillum brasilense*) for the cultivation of garden plants *viz. Rosa rubiginosa, Jasmine sambae, Aralia elata, Gardenia jasminoides* and *Chrysalidocarpus lutescens.* Plants potted with composted coir pith showed an increase in growth when compared to that of garden soil. Protein, Carbohydrate and Chlorophyll content of the plants grown in BCC was also higher, since BCC supports sufficient plant growth and the weight of the roof garden using this compost is less, it can be used as a compost for roof gardening. Coir pith composted with (*Pleurotus sajor caju* and *Azotobacter vinelandii)* and (*Pleurotus sajor caju* and *Azospirillum brasilense*) were marketed at a lower rate with the brand name AZOTO-V and AZOSPIRIL-B respectively (**Keywords**: Lignocellulose, roof garden, soilless media, *P. sajor - caju*).

## INTRODUCTION

Coconut is one of the oldest crops in India is being grown at present in 1.5 million hectares with an approximate production 10,000 million nuts/year. India ranks third in the world, in the production next to Indonesia and Philippines. The production showed a marked increase due to the applications of newer and improved technologies in farming, composting and irrigation.

Coir pith/coir dust is a major byproduct of coir fiber extraction industries (Reghuvaran and Ravindranath, 2010) and coconut coir pith, an agro waste from coir industry and it is a renewable resources as compost. The elastic cellular cork like pithy material forming the non fibrous tissue of the coconut husk generally accounts for 50 % - 60 % of the total weight of husk. Eight hundred tons of coir pith is produced for every 10,000 husks used in the coir industry. Chemical analysis has revealed that coir pith contains three major constituents- cellulose, hemicelluloses and lignin. Cellulose, a polymeric chain of anhydrous glucose units exists mainly in crystalline form. Hemicellulose made up of mixed polymers of various pentose and hexose sugars is amorphous in nature. Lignin, an amorphous polymer of phenyl propane surrounds the cellulose in the cell wall. Lignin exists in situ as large molecular weight component of indefinite size. The major properties of coir pith are water holding/retention capacity of higher degrees (6-8 times of its weight), slow degradation (due to high lignocellulose content), high porosity, storing and releasing of nutrients over extended periods of time, acceptable electrical conductivity (EC), pH, cation exchange capacity (CEC), aeration and oxygenation. These properties allow enhanced root penetration, greater physical resiliency that can withstand compression better. Being a poor conductor of heat, the coir helps to keep soil temperature low and being considered as an ideal medium for plant growth.

Accumulation of coir pith in large quantities causes pollution problems. During monsoon, rain leaching of tannins and salts from the hillocks of coir pith leads to ground water pollution and may affect the food chain to the levels that are harmful for wild life and humans as the pollutants from this source are extremely difficult to be removed by treatment methods. Biodegradation of coir pith is essential to control the pollution (Warrier and Moudil, 1947). Since coir pith is composed of lignocellulosic compounds, the degradation of these compounds was earlier considered as an impossible task. Coir pith undergoes a slow decomposition due to low carbon (pentosan) to lignin ratio (less than 0.5 %), which is the minimum required amount for the slow decomposition of organic matter in the soil. Coir pith, though a problematic waste, it can be

converted as a potential and valuable organic manure by microbial degradation.

The practical problem associated with the direct application of raw coir pith as manure has been solved using earth worm degraded coir pith (vermicomposting) or Pith Plus-a mushroom culture. Oyster mushrooms belonging to the *Pleurotus* species have the ability to degrade lignin slowly under favorable conditions (Reghuvaran *et al*., 2009). At present, composted coir pith with organic supplements is widely being used for many crops like Horticulture and Floriculture. Composted coir pith has advantages in improving crop productivity of plants, in the management of certain root diseases, and also in increase the capability of the soils to store moisture and nutrients (Vinodhini *et al*., 2005) and can be used as manure in crop plantation.

Roof gardens have gained increased attention in recent years, as an urban horticulture alternative (Boivin *et al*., 2001) with advantages. The plant material destroyed during the construction phase can be restored at the top of the building and may reduce the adverse effects of urbanization (Osmundson, 1999). It has also been known that there is 50 % reduction in the heat flux into buildings (Onmura *et al*., 2001; Eumorfopoulou and Aravantinos, 1998; Lukenga and Wessels, 2001; Theodosiou, 2003), due to reduction of solar irradiance by roof gardens. It is guessed that the life span of the building may be increased due to the protection of the various roof components by the roof gardens from the UV, high temperatures, and rapid fluctuation of temperature damages (Lukenga and Wessels, 2001; Stein, 1990). The quantity of runoff water entering into municipal storm water may be reduced (Kolb, 2004; Liesecke, 1998, 1999; Rowe *et al*., 2003; Shade, 2000; U. S. EPA, 2003). Although these benefits have been identified long ago, information on the quantification of these benefits and sustainability of various types of green roofs are lacking (Michael *et al*., 2005) and roof gardens have not yet been considered as a common practice (Panayiotis *et al*., 2003).

The main reasons may be the cost towards the initial construction and the excess load (substrate weight) exerted on the frame of the building (Scrivens, 1990). In extensive roof gardens, the minimal substrate depth is constituted with light weight materials (Fischer and Jauch, 1995). Intensive roof gardens for growing shrubs or trees contain soil as the basic substrate, in order to support plant growth and provide sufficient anchorage to the plants (FLL, 1995).

The present study has aimed 1) to evaluate the effect of biodegraded coir pith (BCC) treated with white rot fungi (*Pleurotus sajor caju*) and bacterial species (*Azotobacter vinelandii* and *Azospirillum brasilense*) for the cultivation of garden plants, 2) To investigate its usage coir pith for roof gardening, 3) To study the potency of this potting medium for commercial marketing.

## MATERIALS AND METHODS

### *Collection of samples*

Coir pith was collected from the coir industries of Kattukada, Alappuzha, Kerala. White rot fungi, pith plus were procured from the Central Coir Research Institute (CCRI), Kalavoor, Alappuzha. The Bacteriae -*Azotobacter vinelandii*, MTCC No. 124 and *Azospirillum brasilense*, MTCC No. 125 were procured from Microbial Type Culture Collection (MTCC), Chandigarh. The experimental studies were conducted in the Rajiv Gandhi Chair in Contemporary Studies, School of Environmental Studies, Cochin University of Science and Technology (CUSAT). The biochemical estimations were carried out at the Central Coir Research Institute, Alappuzha and Rajiv Gandhi Chair, CUSAT.

Plant saplings such as *Rosa rubiginosa, Jasmine sambae, Aralia elata, Gardenia jasminoides* and *Chrysalidocarpus lutescens* were procured from Horticulture division, Kerala Agricultural University (KAU), Mannuthy, Trissur, Kerala.

### *Composting*

The experiments were carried out in 2 sets, mounted in 5 kg quantities of coir pith heaps in triplicate. The first lot was supplemented with both fungus (*Pleurotus sajor caju*) and bacteria (*Azotobacter vinelandii*). The second lot was supplemented with *Pleurotus sajor caju* and *Azospirillum brasilense*. The experimental coir pith heaps was moistened and monitored for 30 days. A control composting experiment using the conventional method was also carried out simultaneously.

***Analysis of Coir pith***

Analysis of lignin, Nitrogen, Carbon, Phosphorous and Potassium were carried out by modified Klason lignin assay, Kjel dahl mehod, Walkey and Black method, Spectrophotometer and Flame photometer respectively.

***Formulation of the biodegraded coir pith compost as AZOTO-V and AZOSPIRIL-B***

The biodegraded coir pith obtained after 30 days could be packed and marketed as AZOTO-V (coir pith composted with combination of *Pleurotus sajor caju* and *Azotobacter vinelandi*) and AZOSPIRIL-B (Coir pith composted with combination of *Pleurotus sajor caju* and *Azospirillum brasilense*).

***Use of the compost as Roof gardening medium***

The pots were filled with AZOTO-V and AZOSPIRIL-B separately and saplings of the ornamental plants *viz.*, *Rosa rubiginosa, Jasmine sambae, Aralia elata*, *Gardenia jasminoides* and *Green palm* were planted in the medium without addition of soil. The medium was moistened to enable growth of the plants.

***Estimation of physical and chemical characteristics of plants***

The physical characteristics of the plants such as shoot length and root length grown on AZOTO-V and AZOSPIRIL-B were measured with a measuring scale. The bio-chemical characteristics were analysed by estimating the Carbohydrate (Lee and Tournsean, 1958), Protein (Lowry *et al.*, 1951) and Chlorophyll (Arnon, 1949).

**Table 1**. Lignin, Organic carbon and NPK content of garden soil and composted coir pith

| S. No | Composting experimental sets | Lignin (%) | Organic carbon (%) | Nitrogen (%) | Phosphorous (%) | Potassium (%) |
|---|---|---|---|---|---|---|
| 1 | Garden soil (Control) | - | 7.66 | 1.08 | 0.44 | 0.078 |
| 2 | Coir pith + *P.sajor caju* + *A. vinelandii* | 19 | 6.65 | 0.71 | 0.48 | 0.036 |
| 3 | Coir pith + *P.sajor caju* + *A. brasilense* | 20 | 5.34 | 0.75 | 0.52 | 0.039 |

For details, please refer Materials and Methods

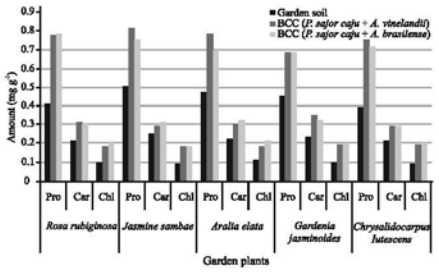


**Figure 1.** Contents of protein, carbohydrate and chlorophyll content in garden plants

For details, please refer Materials and Methods

## RESULTS AND DISCUSSION

The study of the biodegraded coir pith included analysis of Lignin, Organic carbon and NPK of samples treated with *Pleurotus sajor caju* in combination with *Azotobacter vinelandii* and *Azospirillum brasilense* before and after the decomposition (Table 1). The lignin content in the biodegraded coir pith with the combination of organisms varied in comparison with that of the raw coir pith. The decomposition was observed to be 17 % in samples treated with *P. sajor caju* and *A. vinelandii*. It has been shown that the Pleurotus species are efficient in decomposition of various agricultural wastes (Mandhare *et al.*, 2003). While the levels of nitrogen, phosphorous and potassium increased, the level of organic carbon decreased. In both the treatments a reduction was observed in the lignin content, which may facilitated the action of microorganisms. Composting with the mushroom

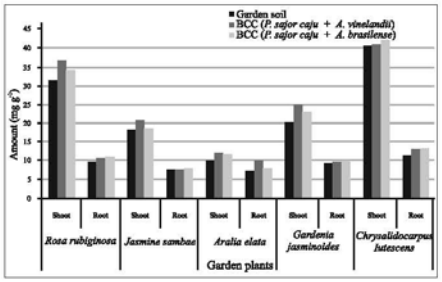


**Figure 2.** Shoot and root length in roof garden plants

For details, please refer Materials and Methods

**Figure 3.** *Chrysalidocarpus lutescens* in coir pith compost

**Figure 4**. *Aralia elata* cultivated in coir pith compost

**Figure 7.** *Jasmine sambae* in coir pith compost

**Figure 8.** Coir pith compost in different pots

species enhanced the content of NPK, transforming the coir pith compost as anenriched organic manure (Reghuvaran and Ravindranath, 2012).

The pot culture experiment was carried out to study the effect of degraded coir pith based potting media for garden plants *viz*., *Rosa rubiginosa, Jasmine sambae, Aralia elata, Gardenia jasminoides* and *Chrysalidocarpus lutescens.* The study was conducted for a period of 2 months, as the plants grow to a standard size within the period. Plants grown in the coir pith compost showed an increase in the shoot and root length (Figure 2). The shoot and root length in BCC with (*P. sajor caju* and *A. vinelandii*) an (*P. sajor caju* and *A. brasilense)* of *Rosa rubiginosa* were 30.6 cm and 9.8 cm and 32.8 cm and 9.4 respectively. Whereas, the shoot and root length were 28 cm and 8.6 cm for the same species of the plants grown in the garden soil. Rise in shoot and root lengths were also observed in BCC for other plants used in the experiments. The increase in the growth of the plants with composted coir pith may be

**Figure 5.** *Rosa rubiginosa* cultivated in coir pith compost

**Figure 6.** *G. jasminoides* in coir pith compost

due to the availability of NPK. Biodegraded coir pith was shown as an effective medium for the cultivation of medicinal plants such as *Phyllanthus amaranthus*, *Andrographis paniculata*, *Bacopa monneiri* and *Piper longum* (Reghuvaran and Ravindranath, 2010). A considerable increase in growth of rice plants has also been observed using coir pith based cyanobacterial biofertilizer (Krishna Veni., 1999; Lavanyapriya, 1997). In the present study, potting medium consisting of biodegraded coir pith appears to be an efficient cultivating medium for garden plants.

Levels of protein, carbohydrate and chlorophyll content of the plants also varied for garden soil and biodegraded coir pith (Figure 1). In case of *Rosa rubiginosa*, the protein content was 0.41 mg $g^{-1}$ in garden soil medium. The protein content was 0.77 mg $g^{-1}$ and 0.78 mg $g^{-1}$ in *Rosa rubiginosa* grown in coir pith composted with *P. sajor caju* and *Azotobacter vinelandii* and with *P. sajor caju* and *Azospirillum brasilense* respectively. The results of the other four species showed almost similar fluctuations. The chlorophyll content and carbohydrate content showed similar variations. In the case of garden soil, the carbohydrate and chlorophyll contents were 0.10 mg $g^{-1}$ and 0.21 mg $g^{-1}$ respectively for *Rosa rubiginosa* and elevated levels were seen with the biodegraded coir pith with *P. sajor caju* and *Azotobacter vinelandii* and *P. sajor caju* and *Azospirillum brasilense.* Similar results were obtained for *Jasmine sambac.* Studies elsewhere reported similar observations with other plants with the medium (Lokesha *et al*., 1988; Baskaran and Saravanan, 1997; Reghuvaran *et al*., 2012). The use of coir pith as a soil conditioner in tropical farming is well documented whereas that of utilization of coir pith as


**Figure 9.** Packed Coir pith composted with *Pleurotus sajor caju* and *A. brasilense*


**Figure 10.** Packed Coir pith composted with *Pleurotus sajor caju* and *A. vinelandii*

potting medium remain to be explored (Saravanan and Nambisan, 1995). The present study has indicated that the coir pith composted with *P. sajor caju*, *Azotobacter vinelandii* and *Azospirillum brasilense* may be useful as soil less potting media for roof gardening.

Coir pith composted with (*Pleurotus sajor caju* and *Azotobacter vinelandii*) and (*Pleurotus sajor caju* and *Azospirillum brasilense*) were marketed as AZOTO-V (Figure 8) and AZOSPIRIL-B (Figure 7) as soil free media in 1 kg packets for cultivation of ornamental plants in roof gardening.

## CONCLUSION

The soil less coir pith composted with the combination of fungus (*Pleurotus sajor caju*) and nitrogen fixing bacteria may constitute as a better potting media for experimental garden plants than the garden soil. This alternate can replace garden soil in roof gardening practices; which has the advantage of reducing the weight of the gardens on the terrace. The products are being commercialized as AZOTO-V and AZOSPIRIL-B at a cheaper cost for growing ornamental plants.

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