COMPARATIVE EFFICIENCY OF LIGNOCELLULOLYTIC FUNGI FOR BIOCONVERSION OF COIRPITH

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DECLARATION

I hereby declare that this thesis entitled "**Comparative efficiency of lignocellulolytic fungi for bioconversion of coirpith**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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Dedicated to

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LIST OF ABBREVIATIONS

^{0}C	Degree Celsius
%	Per cent
N	Nitrogen
P	Phosphorus
K	Potassium
PDA	Potato Dextrose Agar
OA	Oatmeal Agar
YEA	Yeast Extract Agar
CA	Carrot Agar
CDA	Czapek's Dox Agar
RM	Richards' Medium
EC	Electrical Conductivity
m	Metre
µm	Micrometre
cm	Centimetre(s)
g	Gram
mm	Millimetre
viz.	Namely
et al.	And others
m ha	Million hectare
Fig.	Figure
kg	Kilogram
S	Seimens
d	Deci
C : N ratio	Carbon : Nitrogen ratio
MWHC	Maximum Water Holding Capacity
N	Normal
CD	Critical difference
h	Hour
@	At the rate of
sp.	Species

INTRODUCTION

1. INTRODUCTION

India is the major coconut producing country in the world and during 1999-2000 an area of 1.78 m ha was under coconut cultivation with an annual production of 12,252 million nuts (Singh, 2002). In India during 1999-2000, Kerala ranked first in coconut production with a share of 42.17 per cent of total production (Rethinam and Thampan, 2002).

The coconut husk is mainly used for extracting coir fibre. To yield one tonne of coir fibre 10,000 husks are required and from this another one tonne coir pith is generated as waste material (Nagarajan *et al.*, 1985). According to Arumughan and Damodharan (1993) there are about 84,000 retting and coir extracting units located in Kerala producing white fibre. Power extraction units which utilize the fresh husks have also become popular for extraction of the brown fibre.

Coirpith dumped out of the coir factories is increasing every year. The tannins that ooze from the dump yard during monsoon create environmental pollution problems. According to Doraisamy and Ramasamy (1994) coirpith is a recalcitrant complex molecule causing solid waste pollution problems.

Annual production of coirpith in India is about 7.5 million tonnes (Kamaraj, 1994), out of which 11 lakh metric tonne is from Kerala alone.

Coirpith can become an important source of organic manure. It has got very good water holding capacity also. But we cannot directly apply coirpith to the field crops, because it has got wider C : N ratio (112 : 1) coupled with low nitrogen content, presence of soluble tannin related phenolic compounds (8-12 per cent), its low and difficult biodegradability due to high lignin content (Fan *et al.*, 1982). According to Ramasamy *et al.* (1985) coirpith is a recalcitrant agroresidue containing high amount of

lignin and cellulose which resist decomposition by microorganisms under natural conditions.

Thus, if coirpith can be converted into a usable organic manure it is total wealth from waste. This is done effectively by a number of fungi like *Pleurotus sajor-caju*, *Trichoderma* spp., *Aspergillus* sp. etc. (Savithri and Khan, 1994). We have to find out the most efficient native strain of lignocellulolytic fungi for degrading coirpith.

The present study was carried out with the following objectives :

- Isolation and characterization of native strains of lignocellulolytic fungi, which are capable of degrading coirpith.
- Determine the ability of fungi in degrading coirpith by estimating the organic carbon, total nitrogen, total phosphorus, total potassium, cellulose and lignin content of retted and non retted coirpith samples before and after composting
- Find out the number of days taken for maximum bioconversion of coirpith by each isolate
- Measure the reduction in volume and weight of the coirpith after composting and find out the most efficient fungi in degrading coirpith.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Lignocellulosic substances are the most abundant naturally occurring organic polymers in the biosphere. They are the main sources of the naturally occurring cellulose generated in agriculture, food processing, municipal service etc. (Bisaria *et al.*, 1987). However, lignocellulosic materials are not easily degraded by cellulases due to crystallinity, lignin content and the complex structure of cellulose matrix (Fan *et al.*, 1982). The organisms which are capable of producing both cellulolytic and lignolytic enzymes are capable of degrading lignocellulosics. Fungi belonging to class Hymenomycetes especially mushroom fungi are capable of producing both cellulolytic enzymes (Kannan and Oblisami, 1990) and lignolytic enzymes (Munoz *et al.*, 1997).

Wood *et al.* (1988) reported that mushrooms degrade all major components of lignocellulosics such as lignin, cellulose and hemicellulose and have advantage over other fungi in degrading the native lignocellulosics without much pretreatments. Among the various white rot basidiomycetes, the oyster mushroom *Pleurotus* spp. are reported to be the most efficient colonizers and degraders of lignocellulosics (Rajarathnam *et al.*, 1987; Chang and Miles, 1989).

2.1 COLLECTION, ISOLATION AND IDENTIFICATION OF LIGNOCELLULOLYTIC FUNGI

Pegler (1976) reported that the genus *Pleurotus* is known to contain 50 species, out of which about 25 species are known to occur in India. Nair (1990) developed a key for the identification of mushroom.

2.1.1 *Pleurotus eous* (Berk.) Sacc.

This mushroom was first collected from tree trunks in West Bengal, Sikkim and Nepal areas during the last century and subsequently they were identified and described by Berkeley (1850; 1852; 1854).

P. eous was reported from Sikkim (Pegler, 1976); Mysore (Singh and Rajarathnam, 1977) and from Bankura, West Bengal (Roy and Samajpati, 1980). Suharban (1987) conducted a detailed monographic study on *Pleurotus* species and *P. eous* was one among the 20 species of *Pleurotus* reported from Kerala. Desai *et al.* (1991) reported a pink coloured oyster mushroom, growing in clusters on dead wood of Hyga tree from Karnataka. Anitha (1998) reported *P. eous* from Kerala. Balakrishnan *et al.* (2002) also reported the pink coloured oyster mushroom, *P. eous* growing on dead decaying woods of *Jatropha curcas* and *Erythrina indica* in the forests of Wayanad in Kerala.

Purkayastha and Chandra (1985) had given the morphological description of the species.

Sporophores aggregate in tufts, usually lignicolous, sometimes growing on dead portion of living trees. Usually sessile and imbricate. Pileus 3.5-9 cm in diameter, initially spathulate, flabelliform when old, pink or flesh or cream coloured, coriaceous, glabrous, margin incurved, gills decurrent, whitish or creamish, narrow, thin, lamellulae of four different length, stipe small or absent. Basidia clavate with four sterigmata. Basidiospores hyaline, cylindrical, thin walled, 6.0 – 8.0 x 2.5 – 3.5 µm. spore print white.

2.1.2 *Pleurotus squarrosulus* (Mont.) Sing.

Bose and Bose (1940) recorded *P. squarrosulus* (Mont.) Sing. from West Bengal. This mushroom was reported from Kadala and Bombay (Chopra and Chopra, 1955); Calcutta and West Bengal (Chandra, 1974); Nilgiri District of Tamil Nadu (Pegler, 1975); Kerala (Devi, 1982; Suharban, 1987; Balakrishnan, 1994). This mushroom was growing profusely on mango trees and dead stumps during rainy season (Natarajan and Manjula, 1978).

Purkayastha and Chandra (1985) had given the morphological description of this species.

Sporophores generally growing in clusters on logs, usually centrally stipitate, white to cream coloured. Pileus usually 2.0 – 8.0 cm wide, sub infundibuliform, with a deep centric depression, white to cream coloured, coriaceous and flexible when fresh, becoming stiff on drying, minutely scaly, becoming smooth on drying, margin glabrous sometimes with minute scales. Gills crowded, decurrent, unequal, white to cream when young, brownish with age, edge serrate, stipe central, sometimes eccentric, cylindrical, 4.5 cm long, base not hollow, without ring and volva, flesh white. Basidia clavate, tetrasterigmatic, 13.6 – 18.7 x 3.4 – 4.2 µm. Basidiospores hyaline, oblong, elliptical, smooth, thin walled 4.2 – 6.8 x 3 – 3.4 µm. Spore print white.

Cultural characters of *P. squarrosulus* was described by Natarajan and Manjula (1978).

The fungus grew well in malt extract agar medium, covering a nine centimetre petriplate in 10 days. The advancing zone of the culture was even, hyaline and appressed. The mat of the colony was first mostly appressed and white, after two weeks growth becoming cottony or wooly and brown coloured in patches. Abortive fruit-bodies were produced in the petriplates on malt extract agar medium after 15 days of inoculation and kept in room temperature without any special light treatment.

2.1.3 *Calocybe indica* P. & C.

Purkayastha and Chandra (1974) recorded this fungus from Calcutta and West Bengal. It was first cultivated by Purkayastha and Nayak (1979). Use of various agricultural wastes as basal substrate for the cultivation of *C. indica* was attempted by Purkayastha and Nayak (1981); Purkaystha *et al.* (1981) and Doshi *et al.* (1987). Enzyme related

biodegradative potential of *C. indica* was studied by Krishnamoorthy *et al.* (2002).

Purkayastha and Chandra (1985) described the characters of *C. indica* P. & C. :

Sporophore usually growing solitary in soil, robust in size, centrally stipitate, fleshy, white or pale coloured. Pileus 10.0 – 14.0 cm in diametre, at first convex, later expanded and flattened, white, non-hygrophanous, cuticle easily peeled, mat polished, sometimes appressed, scales present at or around the centre, margin regular, incurved, smooth, non striate. Gills distinctly formed, crowded, white, separable, unequal, pliable, not thin, attenuated towards the margin of the pileus.

Stipe central, sometimes eccentric, cylindrical with sub bulbous base, upto 10.0 cm long, white, cartilaginous, surface dry and fibrillose, base not hollow, without annulus and volva, flesh white. Hymenophoral trama regular but for a slight divergency below the subhymenium. Basidia with carminophilic granules, clavate, tetrasterigmatic 25.5 – 30.6 x 6.8 – 8.5 µm. Basidiospores hyaline, broadly ellipsoidal, thin walled, without ornamentation, with prominent apiculus, non-amyloid, 5.9 – 6.8 x 4.2 – 5.1 µm. spore print-white.

2.1.4 *Coprinus comatus* (Mull. ex Fr.) S.F. Gray

Bose and Bose (1940) reported this mushroom from West Bengal for the first time in India. This mushroom was also reported from Calcutta (Banerjee, 1947); Baroda (Moses, 1948); Punjab and Uttar Pradesh (Chopra and Chopra, 1955); Nagpur (Trivedi, 1972); Kashmir valley (Kaul and Kachroo, 1974); Sanatnagar, Sreenagar and Kashmir (Watling and Gregory, 1980).

Purkayastha and Chandra (1985) described this fungus :

Sporophores showing singly, scattered or in clumps, centrally stipitate, usually oblong, characterized by shaggy appearance of the

pileus, almost white at maturity, fruiting body autodigesting. Pileus 6.0 – 15.0 cm long, 2.5 – 5.0 cm wide, cylindrical or oblong when young and tan or purplish tan when old. Surface covered with distinct shaggy brown scales, pileus splitting at the margin. Gills distinctly formed, crowded, free, white when young, then pink, finally black, rather broad, cap and gill deliquesing into an inky fluid. Stipe centrally placed, tapering at the top, 3.5 – 8.0 cm long, 0.6 – 1.5 cm thick, whitish, smooth, hollow with a thin loose ring around the stipe disappearing quickly without volva. Flesh white, fragile. Basidiospores black, smooth, elliptical, 12.0 – 17.0 x 6.0 - 7.0 µm. Spore print-black.

2.1.5 *Schizophyllum commune* Fr.

Watling and Gregory (1980) reported the fungus from Sanatnagar, Srinagar and Jammu-Tawi. It is distributed all over India. Biodegradation of wood by *S. commune* was reported by Lalitha and Thirupathiah (1991).

Purkayastha and Chandra (1985) described this fungus.

Sporophores growing in groups on branches or trunks of trees, on old wood, usually coriaceous, tough, pliant when fresh, attached laterally to the substratum. Pileus upto 4.0 cm in diametre, semicircular or circular, greyish white, surface tomentose, margin incurved, lobed in larger fruit-bodies. Gills distinctly formed, white or greyish white, radiating from the point where the fruit-body is attached, branched, edge split and revolute. Stipe if present, is rudimentary often absent. Flesh grey, pliable when moist and fresh, brittle when dry. Basidia four spored, basidiospores hyaline, oblong with obtuse ends, smooth, non amyloid 5.5 – 7.0 x 2.5 – 3.5 µm. Spore print-white.

2.2 CULTURAL STUDIES OF LIGNOCELLULOLYTIC FUNGI

2.2.1 Growth of Lignocellulolytic Fungi under Different Solid Media

Jandaik and Kapoor (1975) reported that Potato Dextrose Agar (PDA) fortified with yeast extract supported maximum growth of *P. sajor-caju*.

Oats Agar was found to be the best medium for the growth of *Pleurotus* spp. followed by PDA (Suharban, 1987). Geetha (1982) reported that growth of *Coprinus lagopus* was maximum on PDA. Balakrishnan (1994) tested four solid media *viz.*, PDA, Oats Agar, Carrot Agar and modified Oats Agar medium to find out the best medium for the growth of different *Pleurotus* spp. Oats Agar blended with 40 per cent coconut milk (modified Oats Agar) has supported the maximum mycelial growth for all the species tested, followed by common Oats Agar medium.

2.2.2 Effect of Temperature on the Growth of Lignocellulolytic Fungi

Jandaik and Kapoor (1975) observed that *P. sajor-caju* failed to grow at temperature 10°C or below or 35°C or above and the maximum growth was recorded at 25°C. Similar observation was made by Rangad and Jandaik (1977) working with *P. cornucopiae* and *P. ostreatus* (Grag). Unlike *P. sajor-caju*, *P. ostreatus* (florida) supported highest growth at 30°C. Optimum temperature for maximum growth of *Pleurotus sajor-caju*. was found to be 25°C (Suharban, 1987).

Chandra and Purkayastha (1977) reported that *Calocybe indica* preferred a temperature of 30°C for its optimum growth. Yungchang and Yee (1977) reported that *Volvariella volvacea* and *Coprinus cinereus* grew well at a temperature range of 30°C to 35°C and when the temperature rose to 40°C growth of *V. volvacea* was retarded. According to Geetha (1982) optimum temperature for the growth of *Coprinus lagopus* was 35°C.

2.2.3 Effect of pH on the Growth of Lignocellulolytic Fungi

Rangad and Jandaik (1977) reported that at pH 5.6 the maximum growth of *Pleurotus* spp. was observed, while they failed to grow at pH below 4 or above 7. Optimum pH for growth of *Pleurotus* spp. was 5.5 and minimum 4.5 (Suharban, 1987). Balakrishnan (1994) reported that pH 6

was found to be most ideal for all the *Pleurotus* species tested. This was followed by pH 6.5 and 7. Below 5.5 and above 7.5 the rate of growth was found to be drastically decreased.

pH 5 was found to be optimum for the growth of *Calocybe indica* (Chandra and Purkayastha, 1977). Yungchang (1977) reported that *V. volvacea* grew best at pH 7 and *C. cinereus* preferred an acidic medium. According to Geetha (1982) *C. lagopus* grew on a wide range of pH from acidic to alkaline. The fungus attained maximum growth at pH 5 and as the pH increased above 6, a gradual decrease in dry weight was noticed.

In *P. sajor-caju* for the action of Cx cellulase enzyme on cellulose the optimum pH required was 5.8 and for laccase activity pH 6 was found to be the optimum (Thayumanavan, 1982). Brajeshorikoijam *et al.* (2000) reported that the excretion of extracellular cellulase occur only with in the narrow pH range of 4.0 – 5.0. Above and below this range there is a drastic fall in the cellulase production.

2.3 CHARACTERS OF COIRPITH

The pith material forming nonfibrous tissues of the husk is generally referred to as coirpith or as coco-peat (Bhowmic and Debnath, 1985). According to Menon (1987) coirpith is a waste product obtained during the extraction of the coir fibre from retted, partially retted or unretted husk and constitute upto 70 per cent of the husk itself. Coirpith can become an important source of organic manure and has very good water holding capacity. It can absorb eight times its weight of water (Menon, 1987).

Coirpith has got wide C : N ratio and has 8-12 per cent tannin related phenolic compounds. Low and difficult biodegradability is due to high lignin content (Fan *et al.*, 1982). Savithri and Khan (1994) reported that the composition of coirpith vary depending upon various factors *viz.*,

fertility status of coconut garden, method of extraction, disposal, time of collection and other environmental factors.

2.3.1 Pre-composting Physico-Chemical Analysis of Coirpith Samples

2.3.1.1 pH

The pH of coirpith samples collected from different parts of Tamil Nadu ranged between 6.2 to 7.1 (Savithri *et al.*, 1997). While the pH of coirpith collected from various parts of Karnataka were 5.8 (Ravichandran, 1988); 6.68 (Srikanth, 1997); 6.12 (Anand, 1998); 6.25 (Kadalli and Nair, 2000). Gopal and Gupta (2001) reported that the pH of coirpith was 5.9.

2.3.1.2 Electrical Conductivity (EC)

The salt content of coirpith was dependent on the quality of water used for retting. EC of raw coirpith samples collected from different parts of Tamil Nadu ranged between 0.12 – 2.13 dSm^{-1} (Savithri *et al.*, 1997). While the studies conducted in Karnataka revealed that the E.C of coirpith was 1.5 (Ravichandran, 1988); 1.26 (Srikanth, 1997); 1.26 (Anand, 1998); 1.54 (Kadalli and Nair, (2000); 1.63 dSm^{-1} (Amlan and Suseeladevi (2001).

2.3.1.3 Maximum Water Holding Capacity (MWHC)

The unique property of coirpith is its high water holding capacity. The maximum water holding capacity of raw coirpith was reported to be 624 (Ravichandran, 1988; Rajanna, 1988); 400 to 600 (Savithri and Khan, 1994); 775.31 (Anand, 1998); 801.06 per cent (Kadalli and Nair, 2000).

2.3.1.4 Organic Carbon Content

The organic carbon content of raw coirpith varied with the sample. It was reported to be 37.1 (Satyanarayana *et al.*, 1984); 29 (Nagarajan *et al.*, 1985); 40.6 (Ravichandran, 1988); 29.05 (Theradimani and Marimuthu (1992); 45.1 (Nallathambi and Marimuthu, 1993); 48.12 (Anand, 1998);

29.88 (Biddappa *et al.*, 1998); 28.97 (Ramamoorthy *et al.*, 1999); 28.94 (Ramamoorthy *et al.*, 2000); 48.72 (Kadalli and Nair, 2000); 55.65 (Amlan and Suseeladevi, 2001); 26 per cent (Gopal and Gupta, 2001).

2.3.1.5 Nutrient Status

Nutrient status of coirpith varied with the sample. The content of total nitrogen, phosphorus and potassium reported by different workers are: 0.90, 0.05, 0.90 (Joachim, 1930); 0.26, 0.01, 0.78 (Nagarajan *et al.*, 1985); 0.68, 0.03, 0.36 (Ravichandran, 1988); 1.03, 0.09, 1.20 (Jothimani, 1994); 0.42, 0.09, 0.82 (Anand, 1998); 0.29, 0.02, 0.22 (Biddappa *et al.*, 1998); 0.47, 0.02, 0.62 (Kadalli and Nair, 2000); 0.46, 0.19, 0.63 (Amlan and Suseeladevi, 2001); 0.28, 0.01, 0.78 per cent (Gopal and Gupta, 2001). The total nitrogen content of coirpith reported as 0.28 (Theradimani and Marimuthu, 1992); 0.32 (Nallathambi and Marimuthu, 1993); 0.28 per cent (Ramamoorthy *et al.*, 1999).

2.3.1.6 C : N Ratio

The C : N ratio of raw coirpith reported by different workers are : 112:1 (Nagarajan *et al.*, 1985); 60:1 and 58 :1 (Ravichandran, 1988); 104:1 (Theradimani and Marimuthu, 1992); 141:1 (Nallathambi and Marimuthu, 1993); 90:1 (Srikanth, 1997); 114.81:1 (Anand, 1998); 103:1 (Biddappa *et al.*, 1998; Ramamoorthy *et al.*, 1999); 103.65:1 (Kadalli and Nair, 2000); 120.9:1 (Amlan and Suseeladevi, 2001).

2.3.1.7 Cellulose Content

The cellulose content of raw coirpith recorded by different workers are : 35.00 (Pillai and Warrier, 1952); 26.50 (Nagarajan *et al.*, 1985); 27.13 (Theradimani and Marimuthu, 1992); 40.00 to 45.00 (Pavithran, 1993); 32.00 (Jothimani, 1994); 27.50 to 36.10 (Savithri *et al.*, 1997); 34.11 (Anand, 1998); 27.13 (Ramamoorthy *et al.*, 1999); 35.51 (Kadalli and Nair, 2000); 17.08 (Amlan and Suseeladevi, 2001); 26.8 per cent (Gopal and Gupta, 2001). Ansu (1999) reported that the cellulose content

of retted coirpith and non-retted coirpith were 36.50 and 25.50 per cent respectively.

2.3.1.8 Lignin Content

The lignin content of raw coirpith reported by different workers are : 33.00 (Joachim, 1930); 25.20 (Pillai and Warrier, 1952); 30.00 (Nagarajan *et al.*, 1985); 37.00 (Ravichandran, 1988); 31.00 (Theradimani and Marimuthu, 1992); 40.00 to 45.00 (Pavithran, 1993); 29.00 (Jothimani, 1994); 24.90 to 31.10 (Savithri *et al.*, 1997); 51.33 (Anand, 1998); 28.25 (Ramamoorthy *et al.*, 1999); 48.91 (Kadalli and Nair, 2000); 66.15 (Amlan and Suseeladevi, 2001); 34.80 per cent (Gopal and Gupta, 2001). Ansu (1999) reported that the lignin content of 24.23 and 16.80 per cent were observed in raw, retted and non-retted coirpith respectively.

2.4 COMPOSTING OF COIRPITH

Smith and Elliot (1990) reported that any organic material having wider C : N ratio offer stiff resistance to microbial degradation and the crop growth was also adversely affected temporarily. According to Thambirajah and Kuthubutheen (1989) composting has been found to be the most useful method for narrowing down the C : N ratio.

According to Ramasamy *et al.* (1985) coirpith is a recalcitrant agro residue containing high amount of lignin and cellulose resisting decomposition by microorganisms under natural conditions. Organisms especially basidiomycetous fungi like mushrooms which are capable of producing cellulolytic (Kannan and Oblisami, 1990) and lignolytic (Munoz *et al.*, 1997) enzymes are capable of degrading coirpith.

Among the various mushrooms, *Pleurotus* spp. are the most versatile capable of colonizing and degrading a variety of lignocellulosic waste materials (Chang and Miles, 1989).

Thayumanavan (1982) reported that the extracellular enzymes of the fungus play a major role in the degradation of the structural elements such

as cellulose, hemicellulose, lignin and pectin present in natural substrates. The enzyme system of *Pleurotus* spp. involved in degradation of lignocellulosic substrates are endogluconase, β-glucosidase, xylanase, laminarinase, laccase, polyphenol oxidase on cereal straw (Madan and Bisaria, 1983; Rajarathnam *et al.*, 1987).

Some species of *Pleurotus* had the capability of producing laccase which degraded part of the cellulose and lignin present in coirdust (Reddy, 1985). Nagarajan *et al.* (1985) reported that *Pleurotus* spp. were able to degrade coirpith. Theradimani and Marimuthu (1991) assessed the efficacy of seven species of *Pleurotus, Volvariella volvacea* and one *Polyporus* sp. on composting of coirpith. Among the mushrooms tested *Pleurotus platypus* was found to be an efficient degrader of coirpith.

Biodegradation of coirpith using the oyster mushroom *P. sajor-caju* was reported by Vijayakumari *et al.* (1991); Suharban *et al.* (1997); Ansu (1999).

Nallathambi and Marimuthu (1993) reported *P. platypus* to be a potent mushroom for organic recycling of agricultural wastes. Bioconversion of agrowastes by *P. florida* has been reported by Veenasavalgi *et al.* (1994). The degradation potential of oyster mushrooms *viz.*, *P. djamor* and *P. citrinopileatus* on various lignocellulosic materials were studied by Geetha and Sivaprakasam (1998b).

Ramamoorthy *et al.* (1999) assessed the efficacy of degradation of coirpith by *Pleurotus* species *viz.*, *P. djamor*, *P. citrinopileatus* and *P. eous* and they found that among the fungi tested *P. djamor* degraded the coirpith to the maximum level by decreasing the cellulose, lignin and organic carbon content. Sharma *et al.* (1999) studied the biological efficiency and cellulase activities of early and late fruiting *Pleurotus* spp. *viz.*, *P. djamor* and *P. ostreatus* on paddy straw and found that both were capable of degrading lignin, cellulose and hemicellulose components of straw. Ouseph *et al.* (2001) assessed the lignocellulose degradation by

oyster mushroom and found that *P. sajor-caju* was the most efficient degrader of cellulose and lignin. Vadivel *et al.* (2002) reported the potentialities of *P. sajor-caju* in recycling of agro wastes.

Ramamoorthy *et al.* (1999) assessed the efficacy of *Calocybe indica* in degrading coirpith. Enzyme related biodegradative potential of *C. indica* was studied by Krishnamoorthy *et al.* (2002).

Decomposition of rice straw by *Coprinus cinereus* was assessed by Yungchang (1977). It can decompose hemicellulose and cellulose present in rice straw.

Biodegradation of wood by *Schizophyllum commune* was reported by Lalitha and Thirupathiah (1991).

2.4.1 N and C : N Ratio

Alexander (1977) reported that during microbial utilization of plant substrates nitrogen became mobilized in the cells of the colonizers while most of the carbon was released as CO_2. Lignocelluloses are highly deficient in nitrogen and mushroom respond favourably to addition of various nitrogenous materials for enzyme production, degradation and yield (Wang, 1982; Zadrazil and Kurtzman, 1982). Basidiomycetes are reported to differ vastly in their N requirement form as well as level not only for growth but also for enzyme production (Yungchang and Yee, 1977).

Stimulation by urea and suppression by ammonium sulphate of endogluconase production was also reported by Wang (1982). Stimulatory effect of urea on cellulase production in *P. sajor-caju* has been observed (Hong *et al.*, 1985). Nitrogen in the substrates after cultivation of *P. sajor-caju* was higher than that in the starting substrates (Bisaria *et al.*, 1987).

2.4.2 Cellulose Degradation

Cellulose, the most abundant carbohydrate produced by plants in the biosphere, is a linear polymer of glucose units linked by β-1,4 glycosidic linkages (Erikson, 1978). The enzyme cellulase plays a major role in the degradation and recycling of cellulose (Beguin and Aubert, 1985).

Cellulose degrading and utilizing ability of *P. sajor-caju* was reported by Bhandari and Singh, 1981; Thayumanavan, 1982; Hong *et al.*, 1985; Nagarajan *et al.*, 1985.

Joseph *et al.* (1991) recorded the yield potential and cellulolytic enzyme production potential of three species of oyster mushroom and reported that *P. florida* recorded maximum yield as well as celluloytic enzyme production.

Rai and Saxena (1992) reported that due to association with lignin and hemicellulose degrading ability, the cellulase in *Pleurotus* spp. would have greater access to the cellulose in the native lignocelluloses.

Theradimani and Marimuthu (1992) assessed the efficacy of *P. platypus* on degradation of cellulose present in coirpith. Among the fungi tested highest activity of endogluconase was observed with Solan strain of *P. sajor-caju* and maximum activity of exogluconase was noticed in Coimbatore strain of *P. sajor-caju* followed by Solan strain.

Reduction of cellulose content of the substrate following the growth of *Pleurotus* spp. was reported by Geetha (1994). Cellulose degrading ability of *P. djamor* was assessed by Geetha and Sivaprakasam (1998b).

Among the different fungi tested by Ansu (1999) *P. sajor-caju* brought about maximum degradation of cellulose and maximum cellulase production. Ramamoorthy *et al.* (1999) reported that *P. djamor* produced maximum endocellulase (C_x) and exocellulase (C_1) activity followed by *P. citrinopileatus* and *P. eous*.

Enzyme related biodegradative potential of *C. indica* was studied by Krishnamoorthy *et al.* (2002). It produced cellulase, laccase and polyphenol oxidase during their growth on the lignocellulosic substrates.

Yungchang and Yee (1977) reported the ability of *Volvariella volvacea* and *Coprinus cinereus* in degrading cellulose and hemicellulose fraction present in rice straw.

2.4.3 Lignin Degradation

It is estimated that the planet currently contains 3×10^{11} metric tons of lignin with an annual biosynthetic rate of approximately 2×10^{11} tons (Argyropoulos and Menachem, 1997).

Lignin is a complex structure formed by the oxidative polymerization of coumaril, coniferyl and synapyl alcohol (Kirk and Farrel, 1987; Boominathan and Reddy, 1992). Lignin degradation is important in the global recycling of carbon because of the greater abundance of lignin in the biosphere and also because it is an important factor delimiting the degradation of cellulose and other polysaccharides (Kirk and Farrell, 1987).

Several basidiomycetous fungi are known to degrade lignin and produced laccase *in vitro* (Tien and Kirk, 1984; Ramasamy *et al*, 1985; Ramasamy *et al.*, 1989). Ability of *P. sajor-caju* to degrade lignin, cellulose and hemicellulose has been demonstrated by Kandasamy and Ramasamy (1978). Thayumanavan (1982) reported that the enzymes frequently associated with lignin degradation were phenol oxidases in nature and include tyrosinase and laccase. He also isolated the enzyme laccase from the culture filtrate of *P. sajor-caju*.

Enzymology of lignin degradation in coir dust by *P. sajor-caju* was studied by Reddy (1985). He observed that some species of *Pleurotus* had the capacity to produce laccase and degraded part of the cellulose and lignin present in coir dust.

P. sajor-caju degraded the lignin present in coir pith (Nagarajan *et al.*, 1985; Ramasamy *et al.*, 1989; Ansu, 1999). Ramamoorthy *et al.* (1999) tested the efficacy of different *Pleurotus* spp. and *Calocybe indica* in degrading coirpith and found that *P. djamor* was very effective in degrading lignin content of coirpith.

Theradimani and Marimuthu (1992) reported that *P. platypus* showed maximum cellulolytic and lignolytic activity under *in vitro* conditions and enzyme activity of the fungus was positively correlated with its ability to degrade coirpith. Savithri and Khan (1994) reported that cellulose and hemicellulose present in coirdust supported the initial growth of fungus and these two were acting as co-substrate for lignin degradation. Geetha and Sivaprakasam (1998b) observed that *P. citrinopileatus* recorded higher rate of lignin degradation compared to *P. djamor*.

Enzyme related biodegradative potential of *C. indica* was reported by Krishnamoorthy *et al.* (2000). *C. indica* produced cellulases, laccase and polyphenol oxidase during their growth on the substrates. They also observed that inoculation of *C. indica* resulted in narrowing down the cellulose : lignin ratio in the substrates tested.

Yungchang (1977) reported that *Coprinus cinereus* was not an efficient degrader of lignin.

2.5 POST COMPOSTING CHEMICAL ANALYSIS

Composting narrowed down the C : N ratio and reduced lignin and cellulose content.

2.5.1 C : N Ratio

According to Alexander (1997) a C : N ratio of less than 20 : 1 served as an indicator of the maturity and stability of organic substrate. Cappaert *et al.* (1976) recommended a C : N ratio of 25 to 35 : 1 to be the optimum for composted organic matter. Nagarajan *et al.* (1985) reported that coirpith having a C : N ratio of 24 : 1 could be used as a good source

of organic matter for field crops. They also found that organic carbon content was reduced from 29 per cent to 24.5 per cent while the nitrogen content increased from 0.26 per cent to 1.06 per cent during decomposition. Biddappa *et al.* (1998) reported that C : N ratio of coirpith was reduced to 13.04 : 1 from 103.4 : 1 after composting with *P. sajor-caju* for a period of two months. During this period nitrogen content of coirpith increased from 0.29 per cent to 1.92 per cent and organic carbon content decreased from 29.88 to 25.24 per cent.

C : N ratio of compost was considered as an index for assessing the maturity of compost (Theradimani and Marimuthu, 1992). They tested the efficacy of seven different species of *Pleurotus*, one species of *Polyporus* and *V. volvacea* on the degradation of coirpith and found that among the fungi tested *P. platypus* was the most efficient degrader of coirpith. It could narrow down the C : N ratio from 104 : 1 to 18 : 1. Nallathambi and Marimuthu (1993) tested the decomposing efficacy of five *Pleurotus* species and found that *P. platypus* was very efficient in reducing organic carbon and increasing nitrogen content of the substrates after 15 days of inoculation. Organic carbon content reduced from 45.1 to 36.6 per cent and nitrogen increased from 0.32 to 1.19 per cent.

Ramamoorthy *et al.* (1999) evaluated the efficacy of *P. djamor*, *P. citrinopileatus*, *P. eous* and *C. indica* on degradation of coirpith. Among the four fungi tested *P. djamor* decreased the organic carbon content to the maximum i.e., 23.14 per cent and increased the N content to maximum level i.e., 1.15 per cent. C : N ratio of the compost was reduced to 20 : 1 by *P. djamor*. Kadalli and Nair (2000) reported that while composting with *Pleurotus* spp. the C : N ratio of raw coir dust was reduced to 34.19 per cent from 112.9 per cent.

Ramamoorthy *et al.* (2000) studied the efficacy of native microflora *viz.*, *Aspergillus niger*, *Penicillium* spp., *Fusarium* spp., *Streptomyces* spp., *Bacillus* spp. etc. in degrading coconut coir pith and found that

compared to all the organisms *A. niger* brought about maximum degradation. C : N ratio *viz.*, 39 : 1 from 103 : 1. According to Maheswarappa *et al.* (2000) the C : N ratio of coirpith enriched with poultry droppings was reduced from 93 : 1 to 10 : 1. Organic carbon content was reduced from 26 to 18.6 per cent and nitrogen content was increased from 0.28 to 1.85 per cent.

2.5.2 Nutrient Status

Nagarajan *et al.* (1985) reported that potassium content of coirpith increased from 0.78 to 1.2 per cent after composting. Similarly phosphorus content in the compost increased six fold i.e., from 0.01 per cent to 0.06 per cent. Similar results were obtained by Biddappa *et al.* (1998) who found that phosphorus content increased from 0.02 to 0.03 per cent and potassium content from 0.22 to 0.39 per cent.

Kadalli and Nair (2000) reported that phosphorus and potassium content of coir dust based enriched super compost increased from 0.02 to 2.16 and 0.62 to 0.91 per cent respectively. An almost similar result was observed by Maheswarappa *et al.* (2000) who found that phosphorus and potassium content of coirpith enriched with poultry droppings was increased from 0.01 to 2.04 and 0.78 to 1.87 per cent respectively.

2.5.3 Cellulose Degradation

Composting of coirpith with *Pleurotus* spp. reduced the cellulose content to 10.1 per cent from 26.5 per cent (Nagarajan *et al.*, 1985). Working with *Pleurotus* spp. Theradimani and Marimuthu (1992) found that the maximum reduction of cellulose was noticed after 35 days of incubation on coirpith. *P. platypus* brought about 58.6 per cent reduction of cellulose over control.

According to Geetha and Sivaprakasam (1998a) *P. djamor* elaborated more amount of cellulases and laccase and degraded higher amount of cellulose as compared to *P. citrinopileatus*.

Ansu (1999) reported that the cellulose content in retted and non-retted coirpith reduced to 11.26 and 22.50 per cent from 25.50 and 36.30 per cent respectively after 30 days of incubation. The maximum per cent of cellulose reduction was recorded in retted coirpith on 30th day of incubation with *P. sajor-caju*.

Ramamoorthy *et al.* (1999) tested the cellulase enzyme production and cellulose degradation by different species of *Pleurotus* and *C. indica*. Among the mushrooms tested *P. djamor* showed maximum endocellulases (C_1) and exocellulase (C_x) enzyme activity. *C. indica* showed least activity. *P. djamor* reduced cellulose content to 62.22 per cent over control. *C. indica* was least effective in reducing cellulose. It brought about only 6.89 per cent reduction over control. Kadalli and Nair (2000) reported that cellulose content of coirpith reduced from 35.51 per cent to 18.81 per cent after an incubation period of 120 days. Maheswarappa *et al.* (2000) reported that the cellulose content of coirpith enriched with poultry droppings was reduced to 15 per cent from 28.6 per cent in raw coirpith.

2.5.4 Lignin Degradation

According to Cortez *et al.* (1996) lignin : nitrogen ratio and lignin content are the best predictors of litter decay rate. Since coirpith is a high lignin product, lignin content can be used as a marker to study degree of decomposition. Anand *et al.* (1998) also reported that lignin : nitrogen ratio could be used to assess the maturity of the coirdust based compost.

Nagarajan *et al.* (1985) recorded 84 per cent reduction in lignin content after composting raw coirpith with *Pleurotus* spp. Lignin content reduced from 30 per cent to four per cent. Theradimani and Marimuthu (1991) tested the efficacy of seven *Pleurotus* spp., *V. volvacea* and *Polyporus* sp. in degrading lignin content in coirpith. *P. sajor-caju* brought about 82.26 per cent reduction in lignin content. Geetha and Sivaprakasam (1998a) also observed high lignin degrading ability of *P. citrinopileatus*.

Ansu (1999) obtained maximum per cent lignin degradation by *P. sajor-caju* in retted coirpith on 30th day of SSF. Ramamoorthy *et al.* (1999) tested the efficacy of *P. djamor*, *P. citrinopileatus*, *P. eous* and *C. indica* on degradation of lignin present in coirpith and found that among the mushrooms tested *P. djamor* decreased the lignin content to 6.25 per cent i.e., 77.87 per cent reduction over control. *C. indica* was least effective in degradation of lignin content in coirpith. Kadalli and Nair (2000) reported that after composting with *Pleurotus* spp. the lignin content was reduced to 24.01 per cent from 48.91 per cent. Maheswarappa *et al.* (2000) also working with *Pleurotus* spp. reported that the lignin content of coirpith enriched with poultry droppings was reduced from 34.8 per cent to 12.2 per cent.

2.6 REDUCTION IN WEIGHT AND VOLUME OF COMPOST

Weight reduction caused by white rot fungi on solid state fermentation has been reported by Merill *et al.* (1964); Abraham *et al.* (1992) and Biddappa *et al.* (1998). Azizi *et al.* (1990) recorded 25.5 per cent reduction in weight of sugar cane bagasse on cultivation of *P. sajor-caju*. Ansu (1999) reported that there was weight reduction in paddy straw (22.74 per cent), retted coirpith (3.09 per cent) and non retted coirpith (9.07 per cent) after inoculation with *Pleurotus* spp. under solid state fermentation.

Nagarajan *et al.* (1985) reported that the volume of coirpith reduced from 1 m^3 to 0.52 m^3 after composting with *P. sajor-caju*. Particle size of coirpith was reduced after composting (Savithri and Khan, 1994). Volume of coirpith compost was reduced to half of its original volume (Doraisamy and Ramasamy, 1994). According to Sivaprakasam and Seetharaman (1995) about 40 per cent reduction in volume of raw coirpith was observed after composting. Ansu (1999) reported that there was reduction in height of paddy straw, retted and non retted coirpith inoculated with *Pleurotus* spp. under solid state fermentation. Reduction in volume of coirpith after composting was also reported by Krishnakumar and Jawahar (2001).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 COLLECTION, ISOLATION AND IDENTIFICATION OF NATIVE STRAINS OF LIGNOCELLULOLYTIC FUNGI

A survey was conducted in different parts of Thiruvananthapuram district during May-June 2001 for collecting mushrooms. Two species of *Pleurotus viz., P. eous* (Berk.) Sacc., *P. squarrosulus (Mont.) Sing.*; two strains of *Calocybe indica* P. & C. *viz.*, strain 1 and 2; *Coprinus comatus* (Mull. ex Fr.) S.F. Gray; *Schizophyllum commune* Fr.; *Volvariella* sp., *Mycena* sp., *Lepiota* sp., *Termitomyces* spp. and some unknown species of mushrooms were collected. From these collections six lignocellulolytic fungi *viz., P. eous, P. squarrosulus*, two strains of *Calocybe indica*, *Coprinus comatus*, *S. commune* and three species of *Pleurotus* [*P. sajor-caju, P. florida, P. florida* (Holland)] obtained from AICRP Centre on Mushrooms, College of Agriculture, Vellayani were used for the study.

Isolation and maintenance of these cultures were carried out by adopting tissue culture method. Healthy, medium sized mushroom sporocarp was taken and its surface was wiped with cotton dipped in 70 per cent alcohol. The mushroom was split longitudinally and a small tissue from the newly exposed split surface was scooped out with the help of a sterile forceps or an inoculation needle and then transferred to Potato Dextrose Agar (PDA) slants under aseptic conditions in front of the flame and incubated under room temperature (28 ± 4°C) for four days. These isolates were then purified by the hyphal tip method and maintained on PDA slants by periodic subculturing.

Identification of the native strains of lignocellulolytic fungi were carried out following the procedure outlined by Nair (1990). Comparison of the morphological characters was done following the published works

in literature (Natarajan and Manjula, 1978; Purkayastha and Chandra, 1985; Suharban, 1987; Balakrishnan, 1994; Anitha, 1998).

Specimens were collected from the field at different stages of development and general observations like locality, type of substrate, date of collection etc. were recorded in the field itself. The specimens were then taken to the laboratory after wrapping in waxed paper sheet for further studies. The collections were serially numbered. The detailed characters were recorded following the techniques and proforma developed by Nair (1990). The proforma is given in Appendix I.

Spore prints were prepared by removing the stipe of the fruit-body and pileus was placed on a piece of white/black paper with the gills facing downwards and kept under a bell-jar for two hours at room temperature. After two hours the pileus was removed carefully, the spore print dried and sealed within a plastic cover and characters studied.

The micro characters were studied with free hand sections mounted in lactophenol and by tissue maceration.

Macrochemical and metachromatic reactions of various parts of the basidiocarps were studied following the methods perfected by Watling (1971). The test was carried out on the surface context of pileus, stipe apex and base. For this fresh tissue (one cm square) was dissected out and placed in the depression in a porcelain plate. A few drops of Melzer's reagent were applied and the reaction indicated by colour changes was recorded. Melzer's reaction of spore mass was detected following the method of Watling (1971). Small portion of spore print was transferred to a clean microscopic slide and mounted in Melzer's solution and colour change noted under microscope. The reaction was graded as amyloid if the spores were coloured blue black to dark violet and non-amyloid if the colour change was yellow brown. All the microscopic characters were recorded by the drawings using a camera lucida attached to the microscope.

3.2 CULTURAL STUDIES OF LIGNOCELLULOLYTIC FUNGI

3.2.1 Growth of Lignocellulolytic Fungi on Solid Media under Laboratory Condition

Six different solid media [Potato Dextrose Agar (PDA), Oat meal Agar (OA), Yeast Extract Agar (YEA), Carrot Agar (CA), Czapek's Dox Agar (CDA) and Richards' Medium (RM)] were used to find out the best medium for the growth of nine lignocellulolytic fungi. The composition of the media used are given in Appendix II. The media were prepared and sterilized by autoclaving at 1.02 kg cm^{-2} for 15-20 minutes. The cooled media before solidification were poured into sterile petridishes of 9 cm diametre and allowed to solidify. Culture disc of 5 mm diametre cut out from seven day old culture was placed in the centre of the medium and incubated at room temperature. Colony diametre was measured when the growth was completed in any one of the petridishes for each fungus. The experiment was replicated thrice.

3.2.2 Effect of Different Temperature on the Growth of Various Lignocellulolytic Fungi

In order to assess the optimum temperature for maximum growth of nine lignocellulolytic fungi, five millimetre culture disc of actively growing seven day old culture of these fungi were inoculated in 50 ml PDA broth and incubated at 25, 30, 35 and 40°C. After 10 days of incubation the mycelial mat was filtered, dried at 70°C and dry weight was taken till the two consecutive weights were equal. Three replications were kept for each treatment.

3.2.3 Effect of pH on the Growth of Lignocellulolytic Fungi

The pH of PDA broth was adjusted to 4, 5, 6, 7 and 8 by using 0.1 *N* HCl and 0.1 *N* NaOH. Fifty ml of each medium was taken in 100 ml conical flask and autoclaved at 1.02 kg cm^{-2}. The medium was inoculated by a five millimetre culture disc of seven day old culture of the nine

different species of lignocellulolytic fungi and incubated at room temperature for 10 days. The mycelial mat was filtered, dried at 70°C and dry weight was taken till constant weight was obtained. Three replications were maintained.

3.3 COLLECTION OF COIRPITH SAMPLES

The waste produced after extraction of coir fibre from the coconut husk which composed of coconut coirpith along with small bits of coir fibres were collected from the dumping yards of coir factories. Retted coirpith was collected from Vazhamuttam and Thiruvallam areas of Thiruvananthapuram district of Kerala State. The non retted coirpith for *in vitro* studies was collected locally and for field level trials it was collected from Thenkasi and Nagarcoil areas of Tamil Nadu.

3.3.1 Pre-Composting Physico-Chemical Analysis of Retted and non-Retted Coirpith Samples

3.3.1.1 pH

To find out pH of the coirpith one gram of air dried 2mm sieved coirpith sample was taken in a 100 ml beaker. Twenty ml distilled water was added to it (1 : 20 coirpith / water suspension) and stirred at regular intervals for half an hour. pH was determined using Elico digital pH meter (Jackson, 1973).

3.3.1.2 Electrical Conductivity (EC)

The EC content of coirpith sample was determined by the supernatant solution of 1 : 20 coirpith/water suspension using conductivity meter (Jackson, 1973).

3.3.1.3 Maximum Water Holding Capacity (MWHC)

Maximum water holding capacity of coirpith sample was determined using Keen Raczkowski box as described by Piper (1966).

A filter paper was placed at the perforated bottom of the Keen Raczkowski box and weighed. The coirpith sample was filled gradually with continuous tapping to get natural compaction and filled till the box was full. It was then levelled and water was added slowly till the sample was completely wet. The box was then submerged in water upto a depth of one cm for one hour. The box was taken out, weighed and placed in an oven at 105°C for several hours to obtain constant weight. Water holding capacity of sample was thus calculated.

3.3.1.4 Organic Carbon

The organic carbon content of the coirpith samples was estimated following Walkley and Black's rapid titration method (Jackson, 1973).

One hundred milligram of coirpith sample passed through 0.5 mm sieve was taken in 500 ml Erlenmeyer flask. Twenty ml potassium dichromate (1 *N*) and 40 ml concentrated sulphuric acid were added and kept aside for 30 minutes for completing the reaction. Two hundred ml distilled water was added to stop the reaction and the content of the flask was back titrated with standard ferrous sulphate solution using ferroin indicator. A blank titration was simultaneously carried out and the volume of ferrous sulphate consumed for the blank was determined.

3.3.1.5 Total Nitrogen

The total nitrogen content of the coirpith samples was estimated by modified Microkjeldahl method (Jackson, 1973).

This method involves two steps.

1. Digestion of sample to convert organic form of nitrogen to ammonia
2. Determination of ammonium in digest (distillation)

Five hundred mg ground coirpith sample was digested with 10 ml concentrated sulphuric acid and catalyst "Kjeltabs Cu/3.5" (3.5 g K_2SO_4 +

0.4 g $CuSO_4$. $5H_2O$) in a Kjeltech system. The digested sample was made upto 100 ml. From that 10 ml was taken for distillation in the Kjeltech distillation unit. The liberated ammonia was collected in 20 ml of four per cent boric acid with two to three drops of mixed indicator. After distillation the content of the flask was titrated against 0.01 *N* hydrochloric acid.

3.3.1.6 Total Phosphorus

The total phosphorus in coirpith sample was determined in the Nitric/ Perchloric acid digest of coirpith by the colorimetric method (Jackson, 1973).

Five hundred mg coirpith sample passed through 0.5 mm sieve was digested with 15 ml of diacid mixture (HNO_3 : $HClO_3$ in the ratio 4 : 1). The digested sample was made upto 100 ml and from that 10 ml was taken and 10 ml of Barten's reagent was also added and the volume was made upto 100 ml. A reagent blank was also prepared. Phosphorus content of digested sample was estimated by Vanadomolybdate yellow colour method using Klett Summerson Photoelectric Colorimeter with blue filter. A standard curve was also prepared by plotting concentration of P standard on X axis and corresponding colorimetric readings on Y axis. Concentration of the unknown samples were found out from the standard graph.

3.3.1.7 Total Potassium

The total potassium content of the diacid digested coirpith sample was estimated using Flame Photometer (Jackson, 1973).

Five hundred mg coirpith sample passed through 0.5 mm sieve was digested with 15 ml of diacid mixture (HNO_3 : $HClO_3$ in the ratio of 4 : 1). The digested sample was made upto 100 ml. Potassium content of the digested sample was estimated using flame photometer and concentration was found out from the standard curve.

3.3.1.8 Cellulose

Cellulose content of coirpith sample was estimated by adopting the method described by Updegraff (1969).

One hundred mg of oven dried sample was mixed with three ml acetic: nitric reagent (150 ml (80 %) : 15 ml) in a test tube. The tube was placed in a water bath at 100°C for 30 minutes. The contents of the tube was centrifuged at 8000 rpm for 15 minutes. The supernatant was discarded and the residue washed in distilled water. Ten ml of 67 per cent sulphuric acid was added to the residue and allowed to stand for one hour. From this one ml was taken and diluted to 100 ml. Ten ml of anthrone reagent was added to one ml of this diluted solution and kept in a boiling water bath for 10 minutes. After cooling the transmission was measured in a spectrophotometer at 630 nm. Anthrone reagent with distilled water served as blank.

A standard curve with cellulose was prepared. One hundred mg cellulose was taken in a test tube and added 67 per cent sulphuric acid, allowed to stand for one hour. From this one ml was taken and diluted to 100 ml. From this a series of volumes (0.4 to 2 ml corresponding to 40 – 200 µg of cellulose) was taken. 10 ml anthrone reagent was added, kept in boiling water bath for 10 minutes, cooled and transmission was measured at 630 nm.

3.3.1.9 Lignin

The lignin content of coirpith sample was determined as per the procedure outlined by Prasad and Govindarajan (2001).

Two hundred mg ground coirpith sample was weighed out and one ml of 72 per cent sulphuric acid was added for each 100 mg of sample. Then the mixture was placed in a water bath at 30 ± 5°C and stirred frequently. After one hour the sample was diluted with 28 ml of water per ml of acid and the contents were transferred to 125 ml flask and

hydrolysed again by autoclaving at 120°C for one hour. The hot solution was filtered through a tared Gooch crucible. Then the Klason lignin residue was washed with water to remove the acid. Crucibles containing samples were dried to constant weight at 105°C and the lignin content was expressed as per cent of the original sample.

3.4 *IN VITRO* TESTING OF FUNGUS FOR COIRPITH DECOMPOSITION

3.4.1 Spawn Preparation

Paddy grain was used as the spawn substrate. The paddy grain was presoaked overnight and then boiled in tap water. Boiling was stopped when the outer husk of the grain was just split. The grains were then drained and cooled by spreading over a gunny piece. Then the substrate was mixed with calcium carbonate @ 50 g for one kg grain substrate. The processed grain substrate was then filled in saline drip bottles or polypropylene packets. The bottles and packet filled with the substrate were sterilized in an autoclave at 1.02 kg cm^{-2} pressure for two hours. After sterilization the spawn bottles were inoculated with pure culture of the fungus or mother spawn and incubated at 28 ± 2°C. One month old spawn was used for composting (Plate 1).

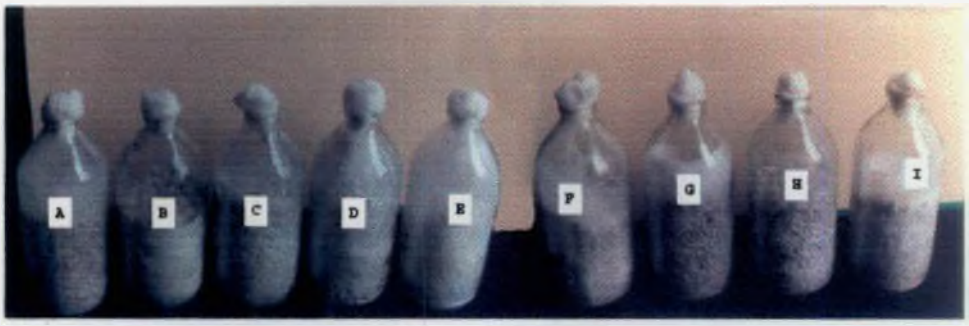
3.4.2 *In Vitro* Composting of Coirpith

The experiment was conducted in Completely Randomised Design (CRD) with 60 treatment combinations of three factors. First factor consisted of two different types of coirpith. Second factor consisted of different species of fungi and third factor days after inoculation for composting with three replications each.

Factor A – Type of coirpith

A_1 – Retted coirpith

A_2 – Non retted coirpith



A. *Pleurotus eous*
B. *Pleurotus squarrosulus*
C. *Pleurotus sajor-caju*
D. *Pleurotus florida*
E. *Pleurotus florida* (Holland)
F. *Calocybe indica – 1*
G. *Calocybe indica - 2*
H. *Schizophyllum commune*
I. *Coprinus comatus*

Plate. 1 Growth of lignocellulolytic fungi in spawn bottles

Plate 2. Field level trials on coirpith composting

Factor B – Different species of lignocellulolytic fungi

B_1 – *Pleurotus eous* (Berk.) Sacc.

B_2 – *P. squarrosulus* (Mont.) Sing.

B_3 – *P. sajor-caju* (Fr.) Singer

B_4 – *P. florida* Eger

B_5 – *P. florida* Eger (Holland)

B_6 – *Calocybe indica* P. & C. –1

B_7 – *C. indica* P. & C. –2

B_8 – *Schizophyllum commune* Fr.

B_9 – *Coprinus comatus* (Mull. ex Fr.) S.F. Gray

B_{10} – Control (Coirpith alone)

Factor C – Days after inoculation

C_1 – 20 days

C_2 – 30 days

C_3 – 45 days

For the experiment retted and non retted coirpith samples were taken in 500 ml conical flask after moistening with distilled water to about 200 per cent moisture. Then it was sterilized in an autoclave at 1.02 kg cm^{-2} for one hour at a temperature of 121.5°C. One month old spawn prepared in paddy grains was used for inoculation and after inoculation it was incubated under room temperature. Three replications were maintained for each treatment. Samples were taken at 20^{th}, 30^{th} and 45^{th} day after inoculation.

3.4.3 Chemical Analysis of Composted Coirpith Samples

The composted coirpith samples were dried in hot air oven at 70°C for 24 h. The oven dried samples were powdered in a Willey mill and

analysed for organic carbon, total nitrogen, total phosphorus, total potassium, cellulose and lignin content as per the procedures described under section 3.3.1.

3.5 EVALUATION OF EFFICIENCY OF SELECTED ISOLATES ON THE COMPOSTING OF COIRPITH UNDER FIELD CONDITION

Lignocellulolytic fungi *viz.*, *P. eous*, *P. squarrosulus*, *P. sajor-caju*, *P. florida*, *C. indica*.-2, *S. commune* found to be efficient in degrading coirpith under *in vitro* studies were taken for field level trials (Plate 2).

Factor A type of coirpith

A_1 – Retted coirpith

A_2 – Non retted coirpith

Factor B – Different species of lignocellulolytic fungi

B_1 – *P. eous*

B_2 – *P. squarrosulus*

B_3 – *P. sajor-caju*

B_4 – *P. florida*

B_5 – *C. indica*-2

B_6 – *S. commune*

B_7 – Control (coirpith alone)

Factor C – Days after inoculation

C_1 – 20 days

C_2 – 30 days

C_3 – 45 days

Three kg of coirpith was spread uniformly (75 x 45 cm) inside the compost shed. One month old spawn @ 1.5 g/kg of coirpith was applied for each treatment over the coirpith layer. Another 3 kg of coirpith was

spread over the first layer and urea was applied @ 5 g/kg coirpith as suggested by Nagarajan *et al.* (1985). This sandwiching process was repeated to make a heap of 30 kg coirpith. Water was sprinkled to maintain the moisture level at 200 per cent. The heap was allowed to decompose for 45 days. Three replications were maintained for each treatment. Representative samples were collected from each treatment at 20, 30 and 45th day after inoculation. The samples were collected in brown paper cover and dried in a hot air oven at 70°C for 24 h. Then the dried samples were powdered and analysed for pH, EC, maximum water holding capacity, organic carbon, total nitrogen, phosphorus, potassium cellulose and lignin as per the procedures descried under section 3.3.1.

Temperature in compost beds were recorded regularly with the help of a soil thermometer.

3.6 REDUCTION IN VOLUME AND WEIGHT OF THE COMPOST

Polythene bag of 45 cm width and 60 cm length were used for composting the coirpith (Theradimani and Marimuthu, 1992). Five kg coirpith was used for the study. Five hundred gram of coirpith was taken as the base layer in the polythene cover. Mushroom spawn was spread over the first layer @ 1.5 g / kg coirpith and a second layer of coirpith was also added. Over this urea was applied @ 5 g/kg coirpith. This sandwiching process was repeated until 10 layers of coirpith were reached. Moisture content was maintained at 200 per cent. The holes in the polythene bag were plugged with cotton thus providing aeration. Then the polythene bag was tied tightly. The weight, height and diametre of each treatment were noticed on the first and 45th day. From these observations reduction in volume and weight of coirpith after composting were calculated.

RESULTS

4. RESULTS

4.1 COLLECTION AND DESCRIPTION OF NATIVE STRAINS OF LIGNOCELLULOLYTIC FUNGI

A survey was conducted in different parts of Thiruvananthapuram district during May-June 2001 and several mushrooms were collected. The collected mushrooms were described and identified as per the procedure outlined by Nair (1990) and brought them into pure culture (Table 1 and 2).

Table 1 Native strains of lignocellulolytic fungi collected

Sl. No.	Name of mushroom species collected	Substrate	Location and period of collection
1	*Pleurotus eous* (Berk.) Sacc.	Dead stumps of cocoa plants	Kalliyoor, May 2001
2	*P. squarrosulus* (Mont.) Sing.	Dead stumps of mango tree	Kunnukuzhi, June 2001
3	*Calocybe indica* P.& C.-1	Soil	Peroorkada June 2001
4	*Calocybe indica* P.& C.-2	Soil	Varkala June 2001
5	*Schizophyllum commune* Fr.	Dead woods	Vellayani June 2001
6	*Coprinus comatus* (Mull. ex Fr.) S.F. Gray	Mushroom bed	Vellayani May 2001

Table 2 Lignocellulolytic fungi included in the study

Sl. No.	Name of species	Source
1	*Pleurotus eous* (Berk.) Sacc.	Native isolate
2	*P. squarrosulus* (Mont.) Sing.	Native isolate
3.	*P. sajor-caju* (Fr.) Singer	AICRP on mushroom, Vellayani
4.	*P. florida* Eger	"
5.	*P. florida* Eger (Holland)	"
6.	*Calocybe indica* P. & C.-1	Native isolate
7.	*Calocybe indica* P. & C.-2	"
8.	*Schizophyllum commune* Fr.	"
9.	*Coprinus comatus* (Mull. ex Fr.) S.F. Gray	"

4.1.1 Morphological Characters of Newly Collected Native Isolates

Macro characters (Plate 3) and micro characters (Plate 4 and 5) of the newly collected native species of lignocellulolytic fungi were studied in detail as explained under materials and methods. Explanation of the scientific terms were given in Appendix III.

4.1.1.1 Pleurotus eous (Berk.) Sacc.

Collected from Kalliyoor panchayat of Thiruvananthapuram district during May 2001.

Habitat : Sporophores in groups on cocoa tree, caespitose, imbricate.

Pileus : Pink or flesh coloured, 3-9 cm in diametre. Initially spathulate, flabelliform when old, coriaceous, glabrous, margin incurved, wavy, fleshy textured, veil absent.

Gills : Crowded, decurrent, creamish or pinkish, narrow, thin, lamellulae of four different length, 16-22 /cm, gill edge incurved.

Stipe : Absent, even if present very small, eccentric, 1-1.5 cm length and 1-2 cm diametre. Volva and annulus absent.

Spores : Basidiospores hyaline, cylindrical, thin walled, 6.0 – 8.0 x 2.5 – 3.5 µm, amyloid. Basidia narrowly clavate, bearing four sterigmata, hymenophoral trama irregular, hyaline with interwoven hyphae. Spore print – white.

4.1.1.2 P. squarrosulus (Mont.) Sing.

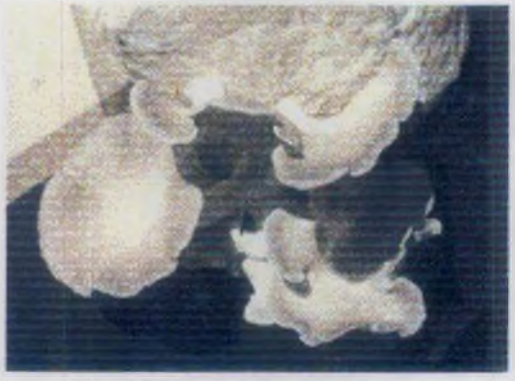
Collected from Kunnukuzhi area of Thiruvananthapuram district during June 2001.

Habitat : Sporophores in clusters on mango trees, and on dead mango stumps.

Pileus : Circular or subinfundibuliform with a deep centric depression, 2-8 cm wide, white to cream coloured, same on wetting, turning somewhat brownish with age, coriaceous and flexible when fresh, becoming stiff on drying, squamose to squarrose with concentrically arranged innate scales, margin thin, regular or lobed.

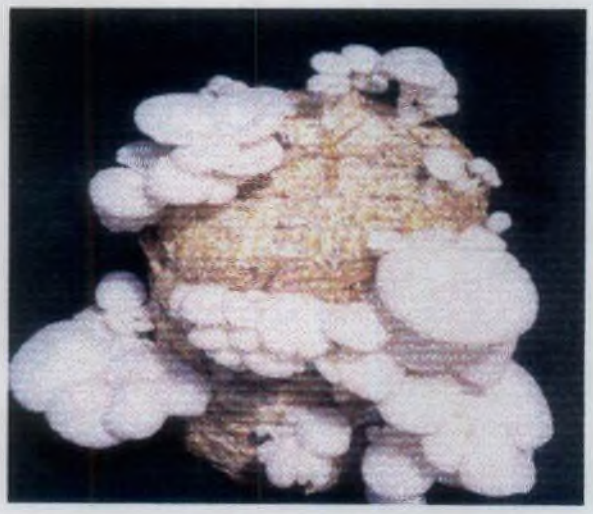
A. *Pleurotus eous*

B. *Pleurotus squarrosulus*



C. *Pleurotus sajor-caju*

D. *Pleurotus florida*



E. *Pleurotus florida* (Holland)

Plate 3. Lignocellulolytic fungi used for the study

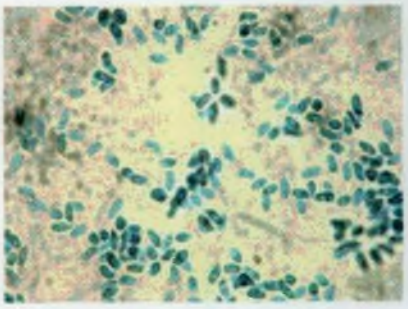
F. *Calocybe indica – 1*

G. *Calocybe indica – 2*

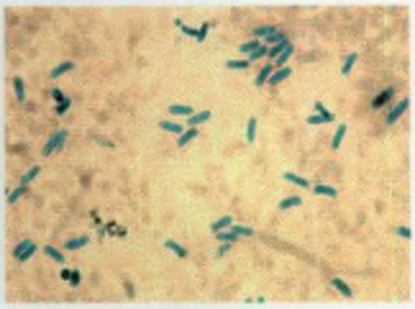
H. *Schizophyllum commune*

I. *Coprinus comatus*

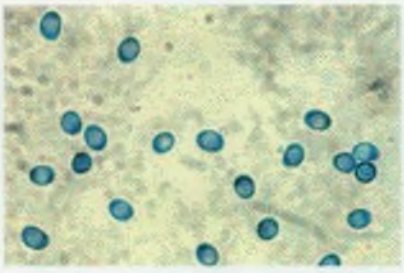
Plate 3-continued



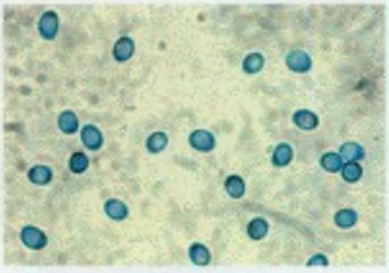
A. *Pleurotus eous*



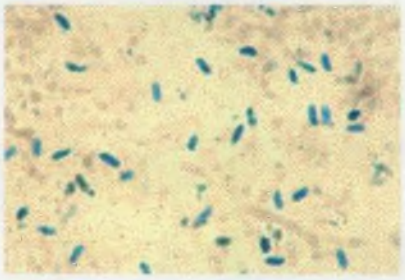
B. *Pleurotus squarrosulus*



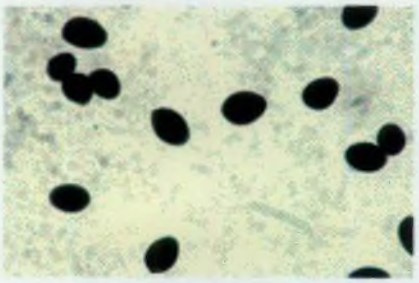
C. *Calocybe indica – 1*



D. *Calocybe indica – 2*



E. *Schizophyllum commune*



F. *Coprinus comatus*

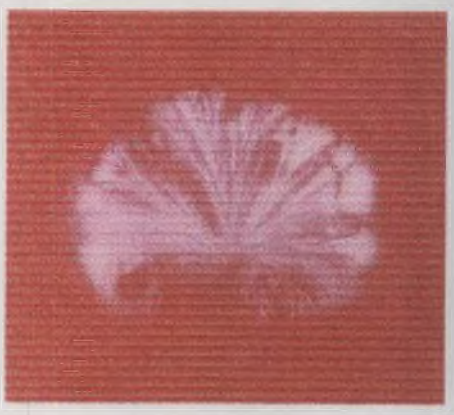
Plate 4. Basidiospores of native isolates

A. *Pleurotus eous*

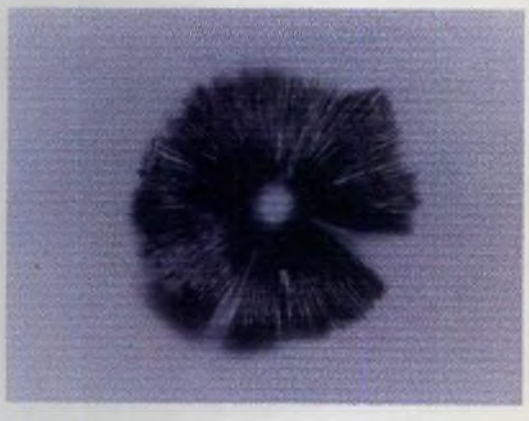
B. *Pleurotus squarrosulus*

C. *Calocybe indica – 1*

D. *Calocybe indica – 2*



E. *Schizophyllum commune*



F. *Coprimus comatus*

Plate 5. Sporeprint of native isolates

Gills : Crowded, decurrent, white to pale buff, distinctly formed, unequal, pliable, edge serrate, 25-30 /cm.

Stipe : Typically central, rarely eccentric, cylindrical, 4-5 cm length and 2-3 cm diametre, white to cream coloured, volva and annulus absent.

Flesh white, hymenophoral trama not completely irregular, but with a distinct axial arrangement.

Spores : Basidiospores hyaline, oblong, cylindrical, smooth, thin walled, 4.2 – 6.8 x 3.0 – 3.4 µm Basidia clavate, tetrasterigmatic, 13.6 x 18.7 – 3.4 x 4.2 µm. Spore print – white.

4.1.1.3 Calocybe indica P. & C. – 1 and 2

Collected from Peroorkada and Varkala areas of Thiruvananthapuram district.

Habitat : Sphorophores usually growing solitary in soil, robust in size, centrally stipitate, fleshy, white or pale coloured.

Pileus : 5-14 cm in diametre, at first convex, later expanded and flattened, white, non hygrophanous, cuticle easily peeled, mat polished, sometimes appressed, scales present at or around the centre, margin regular, incurved, smooth, non striate.

Gills : Distinctly formed, crowded, separable, white, unequal, attenuated towards margin of pileus, 17-20 /cm.

Stipe : Central, sometimes eccentric, cylindrical with subbulbous base, upto 10 cm long, white, cartilaginous, surface dry and fibrillose, without annulus and volva, base not hollow. Flesh white, hymenophoral trama regular.

Spores : Basidiospores hyaline, broadly ellipsoidal, thin walled without ornamentation, non amyloid, 5.9 – 6.8 x 4.2 – 5.1 µm. Basidia clavate, tetrasterigmatic 25.5 – 30.6 x 6.8 – 8.5 µm. Spore print - white.

4.1.1.4 Schizophyllum commune **Fr.**

Collected from College of Agriculture, Vellayani.

Habitat : Sporophores growing in groups on branches or trunks of trees, on old wood, usually coriaceous, tough, attached laterally to the substratum.

Pileus : Upto 4 cm in diametre, semicircular, greyish white, surface tomentose, margin incurved, lobed in larger fruit bodies.

Gills : Distinctly formed, white or greyish white, radiating from the point where fruit body attached, edge split, 26-27/cm.

Stipe : Usually sessile, if present is rudimentary and concolorous.

Flesh grey, pliable when moist and fresh, brittle when dry. Hymenophoral trama not bilateral, non-amyloid.

Spores : Basidia four spored, basidiospores hyaline, oblong with obtuse ends, smooth, 5.52 – 7.0 x 2.5 – 3.5 µm, spore print - white.

4.1.1.5 Coprinus comatus **(Mull. ex Fr.) S.F. Gray**

Collected from Vellayani.

Habitat : Sporophores seen in mushroom beds as weed fungi.

Pileus : First cylindrical or oblong, campanulate or expanded when fully grown, first covered with dense dirty white wooly coating which breaks up into patches, appear as distinct shaggy brown scales which eventually falls away leaving the pileus shiny, 6-8 cm long and 2.5 – 3.0 cm in diametre.

Gills : Distinctly formed, crowded, free, white when young, then pink, finally black, gills deliquescing into an inky fluid, 20-25/cm.

Stipe : Centrally placed, tapering at the top, whitish, smooth, hollow with a delicate white cord of mycelium. Annulus narrow, movable. 3.5 to 8 cm long and 0.6- 1.5 cm thick.

Spores : Smooth, black, elliptical, 12-17 x 6-7 µm in size, spore print – black.

4.2 CULTURAL STUDIES OF LIGNOCELLULOLYTIC FUNGI

4.2.1 Effect of Different Solid Media on the Growth of Lignocellulolytic Fungi

In general, all the lignocellulolytic fungi prefer natural media for their growth and growth in synthetic media was very poor. Among the natural media these fungi grew best in OA, PDA and its growth in YEA and CA was less than that observed in PDA and OA. The growth in these two media was thin and superficial (Table 3, 4 and Plate 6).

The growth of *Pleurotus eous* at the end of seven days in PDA and CA was on par.

Growth rate of *P. squarrosulus* was same in PDA, OA and CA. But on CA the mycelial growth was thin compared to PDA and OA. From the fourth day onwards a brownish colour started appearing from margin in PDA while similar colouration was observed in OA only from the tenth day. The brown colour gradually covered the entire growth. This colouration was not noticed in the growth on other media tried. The fruit body primordia appeared in OA after 15 days of growth.

P. sajor-caju grew best in OA and produced fruit-bodies after 12 days.

PDA favoured better growth of *P. florida* and the mycelium of the fungus in this medium was white and fluffy. In oat meal agar eventhough linear growth was less white fluffy mycelium was observed.

The linear growth of *P. florida* (Holland) was maximum in Yeast Extract Agar. However its growth was very thin. Eventhough the linear growth was less it produced a thick white fluffy growth in PDA.

PDA was the best medium for the growth of *Calocybe indica*, as it supported a white fluffy and thick mycelial growth. Eventhough CA also supported some linear mycelial growth of the fungus the mycelium on the medium was sparse and thin compared to that observed in PDA.

Table 3 Growth of lignocellulolytic fungi on different solid media after seven days of incubation (diameter in cm)

Sl. No.	Fungus	Potato Dextrose Agar	Oatmeal Agar	Yeast Extract Agar	Carrot Agar	Czapek's Dox Agar	Richards' Medium	Mean
1	*Pleurotus eous*	9	8.1	8.47	9	7.9	5.23	7.79
		(3)	(2.85)	(2.91)	(3)	(2.81)	(2.29)	(2.81)
2	*P. squarrosulus*	9	9	8.63	9	7.1	6.37	8.15
		(3)	(3)	(2.94)	(3)	(2.66)	(2.52)	(2.85)
3	*P. sajor-caju*	8.06	8.8	6.99	2.26	8.23	6.56	6.57
		(2.84)	(2.97)	(2.64)	(1.56)	(2.87)	(2.56)	(2.56)
4	*P. florida*	7.87	5.08	5.6	3.56	5.6	5.16	5.41
		(2.8)	(2.25)	(2.37)	(1.89)	(2.37)	(2.27)	(2.33)
5	*P. florida* (Holland)	4.07	1.33	5.7	2.23	3.1	2.29	2.96
		(2.02)	(1.15)	(2.39)	(1.49)	(1.76)	(1.51)	(1.72)
6	*Calocybe indica*-1	5.63	4.17	4.84	5.53	3.4	0.64	3.76
		(2.37)	(2.04)	(2.20)	(2.35)	(1.84)	(0.8)	(1.94)
7	*C. indica*-2	5.69	4.22	4.38	5.55	3.80	0.86	4.02
		(2.39)	(2.05)	(2.09)	(2.36)	(1.95)	(0.93)	(2.00)
8	*Schizophyllum commune*	8.56	7.33	6.8	5.63	6.63	6.2	6.83
		(2.93)	(2.71)	(2.61)	(2.37)	(2.58)	(2.49)	(2.61)
9	*Coprinus comatus*	2.6	4.17	6.59	7.5	5.33	1.592	4.36
		(1.61)	(2.04)	(2.57)	(2.74)	(2.31)	(1.26)	(2.09)
	Mean	6.46	5.48	6.37	5.29	5.52	3.41	
		(2.55)	(2.34)	(2.52)	(2.30)	(2.35)	(1.85)	

Values in parentheses are square root transformed values

CD (0.05 level) : Fungus 0.067, media 0.058, fungus x media 0.164

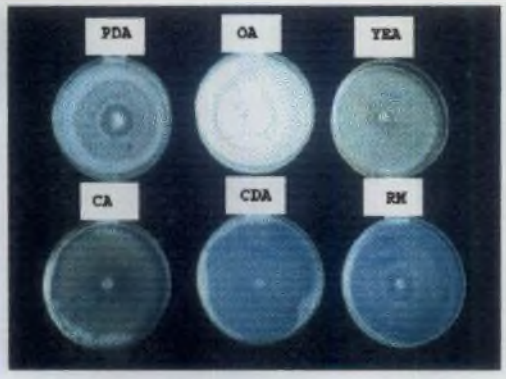
Table 4 Growth characters of lignocellulolytic fungi on different solid media after seven days of incubation

Sl. No	Fungus	Character	Solid media					
			PDA	OA	YEA	CA	CDA	RM
1	Pleurotus eous	1	White	White	White	White	White	White
		2	Thin	Thin	Thin	Thin	Sparse	Sparse
		3	No	No	No	No	No	No
2	*P. squarrosulus*	1	White turn brown 4th day onwards	White, cottony turn brown 10th day onwards	White	White	White	White
		2	Thick	Thick	Thin	Thin	Sparse	Sparse
		3	No	15th day of incubation	No	No	No	No
3	*P. sajor-caju*	1	White fluffy	White fluffy	White	White	White	White
		2	Thick	Thick	Thin	Thick	Sparse	Sparse
		3	No	12th day of incubation	No	No	No	No
4	*P. florida*	1	White fluffy	White fluffy	White	White	White	White
		2	Thick	Thick	Thin	Thick	Sparse	Sparse
		3	No	No	No	No	No	No
5	*P. florida* (Holland)	1	White fluffy	White fluffy	White	White	White	White
		2	Thick	Thick	Thin	Thick	Sparse	Sparse
		3	No	No	No	No	No	No
6	*Calocybe indica*-1	1	White fluffy	White fluffy	White	White	White	White
		2	Thick	Thick	Thin	Thin	Sparse	Sparse
		3	No	No	No	No	No	No
7	*Calocybe indica*-2	1	White fluffy	White fluffy	White	White	White	White
		2	Thick	Thick	Thin	Thin	Sparse	Sparse
		3	No	No	No	No	No	No
8	*Schizophyllum commune*	1	White leathery	White fluffy	White	White	White	White
		2	Thick	Thick	Sparse	Thin	Sparse	Sparse
		3	No	10th day of incubation	No	No	No	No
9	*Coprinus comatus*	1	Pale	White fluffy	White	White	White	White
		2	Thin	Thick	Thin	Thin	Sparse	Sparse
		3	No	10th day of incubation	No	No	No	No

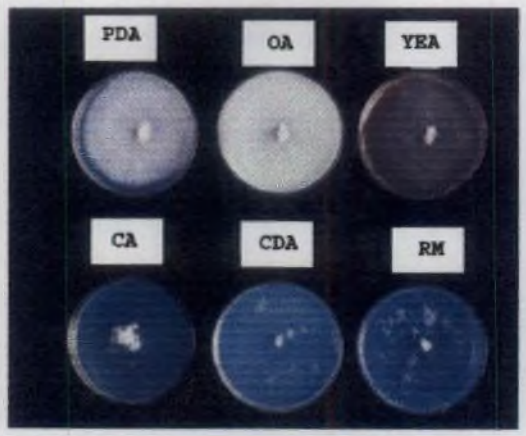
1- Colour of mycelium
2- Growth pattern
3- Fruit body production

No – Not observed

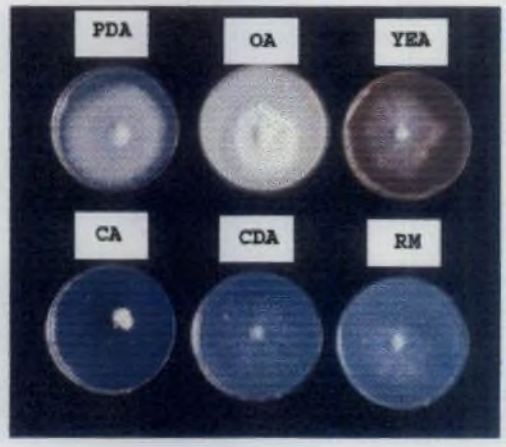
A. *Pleurotus eous*



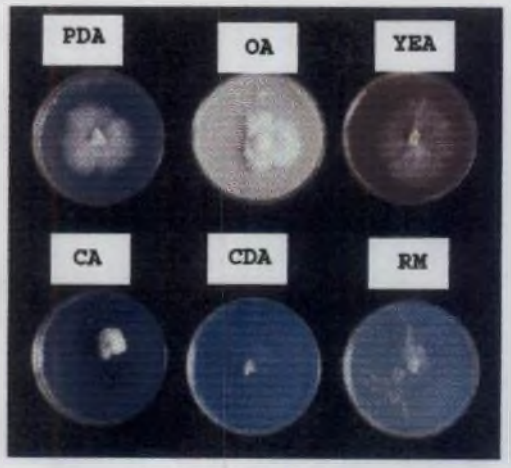
B. *Pleurotus squarrosulus*



C. *Pleurotus sajor-caju*

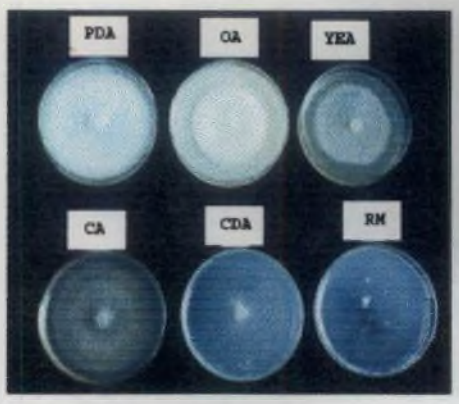


D. *Pleurotus florida*

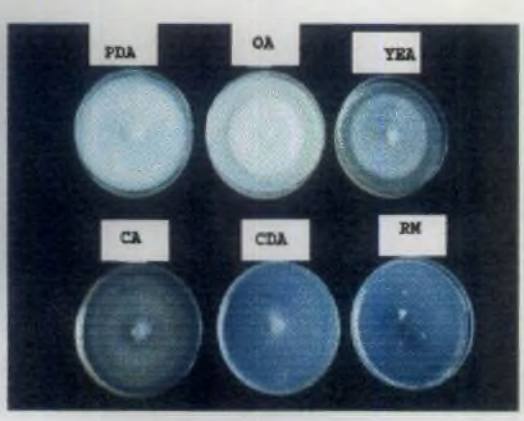


E. *Pleurotus florida* (Holland)

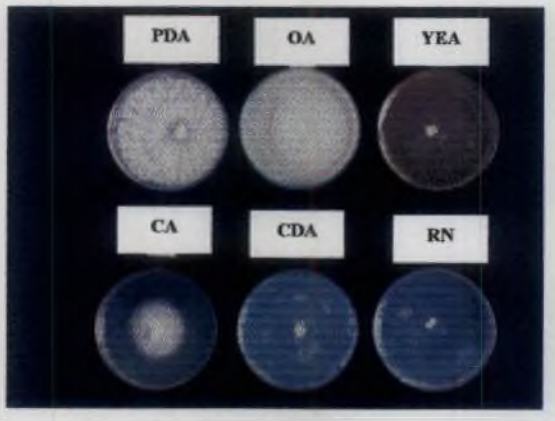
Plate 6. Effect of solid media on the growth of lignocellulolytic fungi



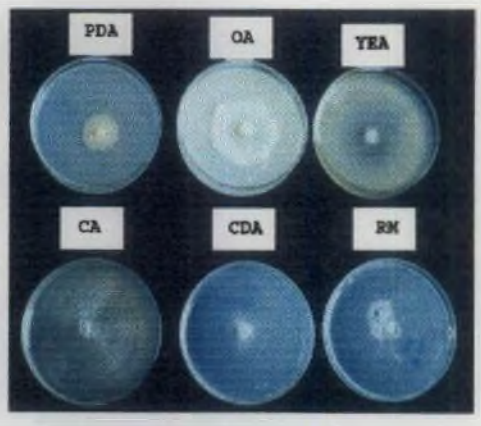
F. *Calocybe indica – 1*



G. *Calocybe indica – 2*



H. *Schizophyllum commune*



I. *Coprinus comatus*

Plate 6. Continued

For *S. commune* PDA was found to be the best medium for the growth. White leathery mycelial growth completely covered the petridish after 10th day of incubation. This was followed by OA. Fruiting bodies started appearing after 10th day of incubation in OA.

Linear mycelial growth was highest in CA for *Coprinus comatus*. But OA was found to be the best medium for growth and fruit body formation. White fluffy mycelial growth was observed on OA. 10th day after inoculation fruit body primorida appeared on this media.

4.2.2 Effect of Temperature on the Growth of Lignocellulolytic Fungi

Based on the effect of temperature on the growth of nine lignocellulolytic fungi they could be grouped into three categories. Three species of lignocellulolytic fungi *viz.*, *P. florida* and *Calocybe indica* strain 1 and 2 preferred a temperature of 30°C for maximum mycelial growth. *P. eous* and *P. florida* (Holland) preferred 25 and 30°C for their optimum growth. *P. squarrosulus, S. commune* and *Coprinus comatus* preferred a higher temperature of 35°C for its best growth. While *P. sajor-caju* gave the maximum growth at a temperature of 25°C, growth of all the fungi drastically reduced as the temperature was increased to 40°C (Table 5 and Plate 7).

4.2.3 Effect of pH on the Growth of Lignocellulolytic Fungi

Nine lignocellulolytic fungi were grown in PDA broth of pH 4, 5, 6, 7 and 8. All these fungi preferred neutral to acidic pH. Growth of all the nine fungi were significantly poor in pH 8 compared to lower pH values.

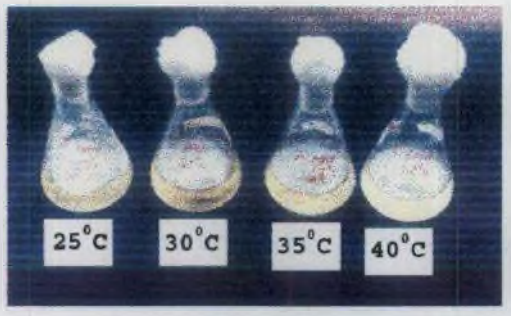
P. eous and *P. florida* grew best at pH 5 and 6. The growth of *P. sajor-caju* did not differ significantly when it was grown at pH varying from 4 to 6. Holland strains of *P. florida* preferred a pH of 4 for its optimum growth. *Calocybe indica* strains 1 and 2, *S. commune* and *Coprinus comatus* showed best mycelial growth at pH 6. The best growth of *P. squarrosulus* was noticed at neutral pH (Table 6 and Plate 8).

Table 5 Growth of lignocellulolytic fungi in Potato Dextrose broth at different temperatures after ten days of incubation (dry weight in grams)

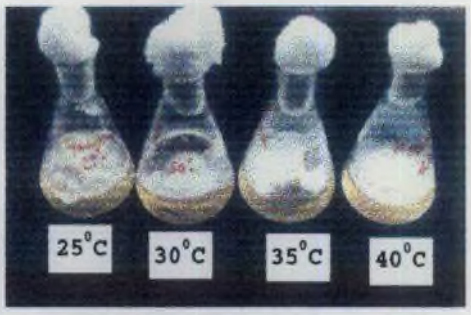
Sl. No.	Fungus	Temperature °C				
		25	30	35	40	Mean
1	*Pleurotus eous*	0.56	0.61	0.37	0.13	0.39
		(0.75)	(0.78)	(0.60)	(0.37)	(0.63)
2	*P. squarrosulus*	1.14	1.67	1.8	0.76	1.31
		(1.07)	(1.29)	(1.34)	(0.87)	(1.14)
3	*P. sajor-caju*	0.6	0.39	0.12	0.12	0.27
		(0.77)	(0.62)	(0.35)	(0.34)	(0.52)
4	*P. florida*	0.32	0.46	0.11	0.1	0.22
		(0.57)	(0.68)	(0.34)	(0.31)	(0.47)
5	*P. florida* (Holland)	0.22	0.25	0.11	0.09	0.15
		(0.47)	(0.50)	(0.33)	(0.3)	(0.4)
6	*Calocybe indica*-1	0.53	0.60	0.53	0.12	0.42
		(0.73)	(0.77)	(0.73)	(0.34)	(0.65)
7	C. *indica*-2	0.56	0.64	0.58	0.12	0.45
		(0.75)	(0.80)	(0.76)	(0.34)	(0.66)
8	*Schizophyllum commune*	0.56	0.98	1.8	0.74	0.98
		(0.75)	(0.99)	(1.35)	(0.86)	(0.99)
9	*Coprinus comatus*	0.12	0.21	0.36	0.12	0.19
		(0.34)	(0.46)	(0.6)	(0.34)	(0.44)
	Mean	0.47	0.59	0.51	0.20	
		(0.69)	(0.77)	(0.72)	(0.45)	

Values in parentheses are square root transformed values

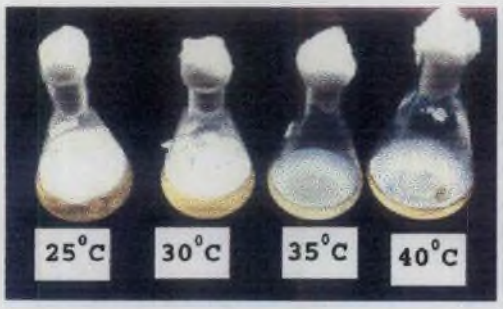
CD (0.05 level) : Fungus 0.016, Temperature 0.012, Fungus x Temperature 0.033



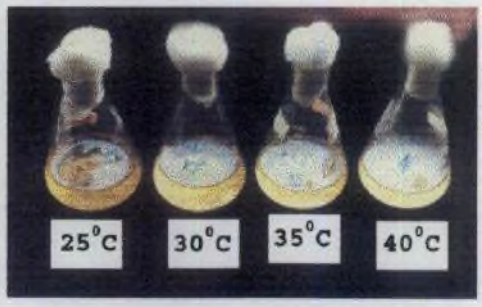
A. *Pleurotus eous*



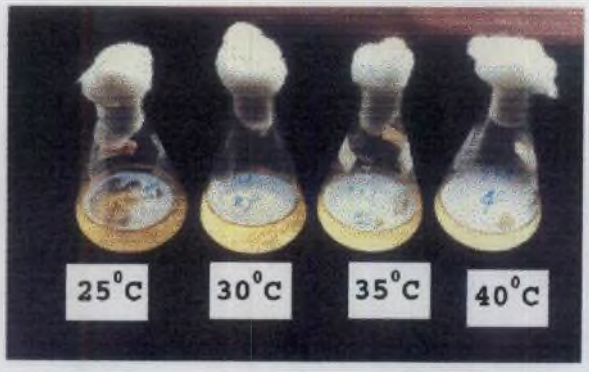
B. *Pleurotus squarrosulus*



C. *Pleurotus sajor-caju*

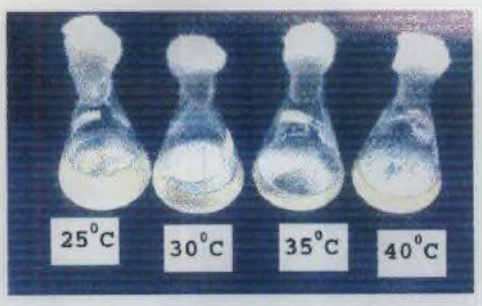


D. *Pleurotus florida*

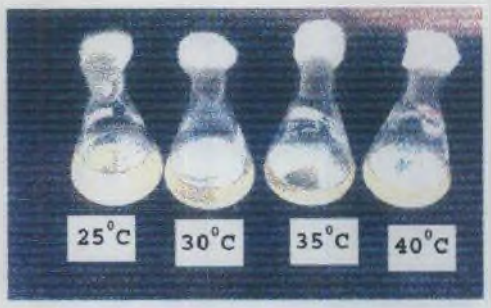


E. *Pleurotus florida* (Holland)

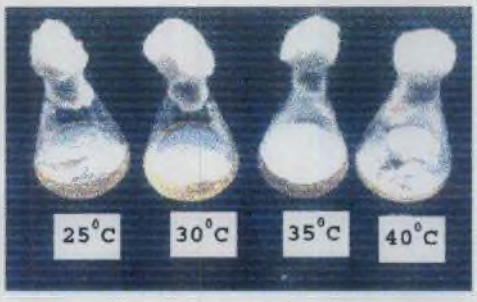
Plate 7. Effect of different temperatures on the growth of lignocellulolytic fungi



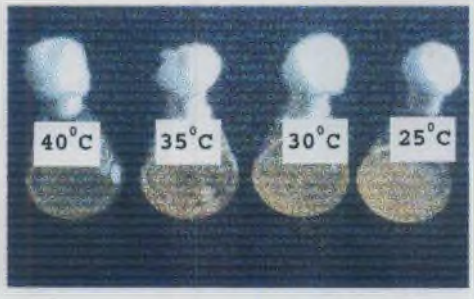
F. *Calocybe indica – 1*



G. *Calocybe indica – 2*



H. *Schizophyllum commune*



I. *Coprinus comatus*

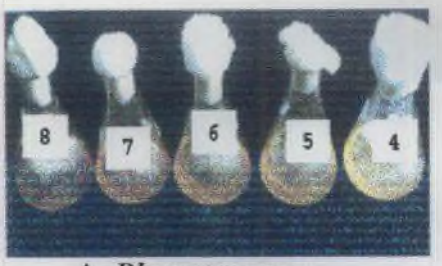
Plate 7. Continued

Table 6 Growth of lignocellulolytic fungi in Potato Dextrose broth at different pH levels after ten days of incubation (dry weight in grams)

Sl. No.	Fungus	Dry weight of mycelium in grams					Mean
		4	5	6	7	8	
1	*Pleurotus eous*	0.23	0.28	0.29	0.27	0.25	0.25
		(0.48)	(0.53)	(0.54)	(0.52)	(0.5)	(0.5)
2	*P. squarrosulus*	0.35	0.39	0.41	0.57	0.4	0.42
		(0.59)	(0.62)	(0.64)	(0.76)	(0.64)	(0.65)
3	*P. sajor-caju*	0.33	0.34	0.33	0.28	0.24	0.30
		(0.57)	(0.58)	(0.58)	(0.53)	(0.49)	(0.55)
4	*P. florida*	0.20	0.23	0.24	0.2	0.17	0.21
		(0.45)	(0.48)	(0.49)	(0.44)	(0.41)	(0.45)
5	*P. florida* (Holland)	0.15	0.1	0.09	0.08	0.08	0.1
		(0.38)	(0.32)	(0.3)	(0.28)	(0.28)	(0.32)
6	*Calocybe indica*-1	0.25	0.42	0.49	0.3	0.27	0.34
		(0.5)	(0.65)	(0.7)	(0.55)	(0.52)	(0.59)
7	*C. indica*-2	0.25	0.45	0.50	0.31	0.26	0.35
		(0.50)	(0.67)	(0.71)	(0.56)	(0.51)	(0.59)
8	*Schizophyllum commune*	0.08	0.13	0.42	0.4	0.3	0.24
		(0.28)	(0.36)	(0.65)	(0.63)	(0.55)	(0.49)
9	*Coprinus comatus*	0.06	0.09	0.2	0.13	0.12	0.12
		(0.25)	(0.31)	(0.44)	(0.37)	(0.35)	(0.34)
	Mean	0.20	0.25	0.31	0.27	0.22	
		(0.44)	(0.50)	(0.56)	(0.52)	(0.47)	

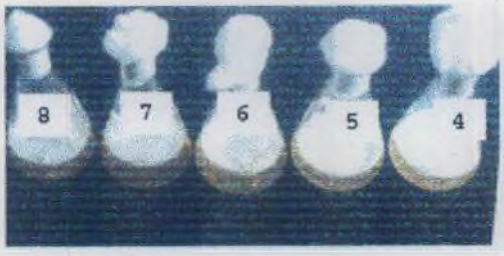
Values in parentheses are square root transformed values

CD (0.05 level) : Fungus 0.078, pH 0.061, Fungus x pH 0.017

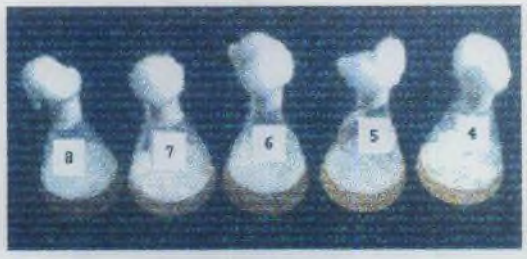


A. *Pleurotus eous*

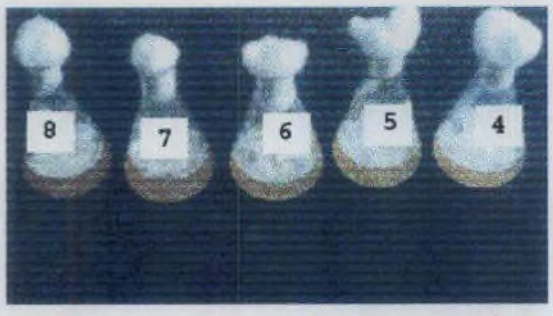
B. *Pleurotus squarrosulus*



C. *Pleurotus sajor-caju*



D. *Pleurotus florida*



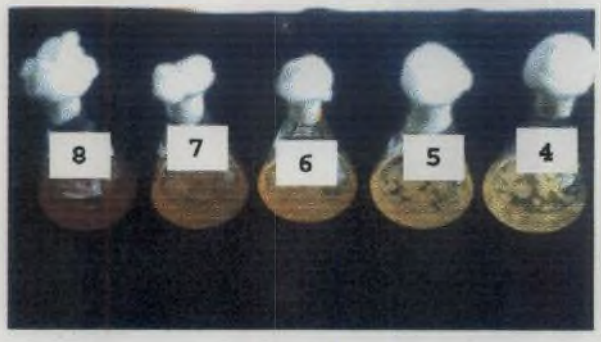
E. *Pleurotus florida* (Holland)

Plate 8. Effect of different P^H on the growth of lignocellulolytic fungi

F. *Calocybe indica – 1*

G. *Calocybe indica – 2*

H. *Schizophyllum commune*



I. *Coprinus comatus*

Plate 8. Continued

4.3 PRE COMPOSTING PHYSICO-CHEMICAL STATUS OF COIRPITH SAMPLES

Physico-chemical properties of retted and non retted coirpith samples showed variations (Table 7). Even among the retted and non retted coirpith samples variations were noticed based on the place of collection.

Both retted and non retted coirpith were acidic in nature. However, retted coirpith were more acidic compared to non retted. The pH of retted coirpith from two locations in Thiruvananthapuram district were 4.29 (Vazhamuttom) and 4.84 (Thiruvallam). The pH of the non retted coirpith from Vellayani was 6.16 compared to 5.93 when the sample was collected from Thenkasi. EC was less in retted coirpith compared to non retted coirpith. There were no variation in the EC of retted coirpith collected from Thiruvananthapuram district. The EC value of non retted coirpith collected from Thenkasi (2.74 dSm^{-1}) was almost six times the EC of retted coirpith from Thiruvananthapuram district (0.44 dSm^{-1}) and two and half times that of non retted coirpith collected from Vellayani (0.64 dSm^{-1}).

Maximum water holding capacity was high in retted coirpith and it was nearly double (1019.00 and 1134.63 per cent) when compared to non retted coirpith collected from Vellayani (612.12 per cent). The MWHC of Thenkasi sample of non retted coirpith was more than that of Vellayani (886.66 per cent).

Organic carbon content was more in retted coirpith. A 4.21 per cent difference in the organic carbon content was noticed in the retted coirpith collected from Vazhamuttom (45.59 cent) and Thiruvallam (49.80 per cent). The organic carbon content of non retted coirpith collected from two locations did not vary considerably (42.50 and 42.90 per cent).

Nutrient status (NPK) of coirpith was very low. The total nitrogen content was marginally more in non retted coirpith (0.60 and 0.56 per cent)

Table 7 Physico-chemical properties of coirpith samples before composting

Chemical composition	Retted coirpith		Non retted coirpith	
	Vazhamuttom	Thiruvallam	Vellayani	Thenkasi
pH	4.29	4.84	6.16	5.93
EC, dSm^{-1}	0.44	0.44	0.64	2.74
Maximum water holding capacity, %	1019.00	1134.63	612.12	886.66
Organic carbon, %	45.59	49.80	42.50	42.90
Total nitrogen, %	0.53	0.52	0.60	0.56
Total phosphorus, %	0.03	0.03	0.04	0.03
Total potassium, %	0.06	0.05	0.26	0.92
Cellulose, %	29.30	30.85	35.97	36.92
Lignin, %	33.99	34.27	38.75	39.40
C : N ratio	86.68 : 1	96.23 : 1	70.57 : 1	75.82: 1

compared to retted (0.53 and 0.52 per cent). Total phosphorus content of retted and non retted coirpith did not differ markedly. The retted coirpith had a P content of 0.03 per cent and in non retted coirpith 0.04 and 0.03 per cent. The potassium content was very low in retted coirpith (0.05 and 0.06 per cent). In non retted coirpith the per cent of potassium in samples collected from Thenkasi was three times more (0.92) compared to that collected from Vellayani (0.26 per cent). The C : N ratio of non retted coirpith was narrow (70.57 : 1 and 75.82 : 1) compared to retted coirpith (86.68 : 1 and 96.23 : 1).

Retted coirpith had a lower cellulose content. The cellulose per cent in retted coirpith ranged from 29.30 (Vazhamuttom) to 30.85 per cent (Thiruvallam), while that of non retted coirpith varied from 35.97 (Vellayani) to 36.92 per cent (Thenkasi). A similar pattern was noticed in the case of lignin content also. Maximum lignin content of 39.40 was noticed in non retted coirpith collected from Thenkasi and a minimum of 33.99 per cent in retted coirpith sample collected from Vazhamuttom.

4.4 POST COMPOSTING PHYSICO-CHEMICAL STATUS OF COIRPITH SAMPLES

4.4.1 Physical Properties of Coirpith Compost

The pH of retted coirpith after the action of lignocellulolytic fungi increased from 4.86 (control) to 5.07 and above depending upon the fungus. The maximum pH of 5.26 was noticed in coirpith composted with *P. florida* (Table 8). On the other hand the pH of the non retted coirpith decreased from 5.75 in control to 5.10 (*Coprinus comatus*) and above. The maximum pH value of 5.43 was noticed in coirpith inoculated with *S. commune*.

EC of retted and non retted coirpith decreased after composting. The uninoculated non retted coirpith collected form Thenkasi had an EC of 2.69 compared to retted coirpith collected from Thiruvallam (0.47 dSm^{-1}).

Table 8 Physical properties of coirpith compost as influenced by lignocellulolytic fungi

Sl. No.	Fungus	Retted coirpith (Thiruvallam)			Non retted coirpith (Thenkasi)		
		pH	EC, dSm^{-1}	Maximum water holding capacity, %	pH	EC, dSm^{-1}	Maximum water holding capacity, %
1	*Pleurotus eous*	5.11	0.41	895.81	5.19	2.14	495.05
2	*P. squarrosulus*	5.11	0.46	798.21	5.38	2.16	571.28
3	*P. sajor-caju*	5.09	0.40	737.77	5.28	2.58	459.04
4	*P. florida*	5.26	0.41	757.81	5.31	2.37	613.76
5	*P. florida* (Holland)	5.11	0.44	759.68	5.24	2.54	605.67
6	*Calocybe indica*-1	5.17	0.40	1038.91	5.36	2.56	550.81
7	*C. indica*-2	5.16	0.40	1009.55	5.37	2.55	528.86
8	*Schizophyllum commune*	5.16	0.43	772.69	5.43	2.55	504.28
9	*Coprinus comatus*	5.07	0.44	984.06	5.10	2.56	583.27
10	Control (raw coirpith)	4.86	0.47	1109.28	5.75	2.69	886.66
	CD (0.05 level)	0.04	0.02	64.32	0.05	0.03	19.70

The maximum reduction in the EC in retted coirpith was only 0.07 (*P. sajor-caju*), *Calocybe indica* 1 and 2), while it was 0.55 dS m^{-1} in non retted coirpith inoculated with *P. eous* (2.14 dSm^{-1}).

In general, maximum water holding capacity was reduced in both retted and non retted coirpith after composting. The minimum reduction of water holding capacity in retted coirpith after composting was noticed in two strains of *C. indica* (1038.91 and 1009.55 per cent). Reduction in the water holding capacity of *P. squarrosulus*, *P. sajor-caju*, *P. florida*, *P. florida* (Holland) and *S. commune* did not differ significantly. In the non retted coirpith maximum reduction was noticed with *P. sajor-caju* (459.04) and minimum in *P. florida* (613.76) and *P. florida* (Holland) (605.67 per cent).

4.4.2 Post Composting Chemical Analysis (*in vitro*)

4.4.2.1 Organic Carbon

In vitro studies have shown that the organic carbon content of retted coirpith (35.80 per cent) was significantly different from the non retted coirpith (33.86 per cent) (Table 9). Among the various lignocellulolytic fungi tested *P. eous* brought about maximum degradation of organic carbon (27.44 per cent) which was significant from all other treatments. Organic carbon content of *Coprinus* treated coirpith was high (37.39 per cent) compared to all other treatments inoculated with fungi. Significant difference existed between organic carbon content of coirpith during different periods of composting i.e., 20 days (38.3 per cent), 30 days (34.8 per cent) and 45 days (31.35 per cent).

The interaction between coirpith and fungi tested had revealed that inoculation of *P. eous* on retted coirpith showed significant difference from all other treatments (24.54 per cent). Influence of period was significant for degradation of both retted and non-retted coirpith.

Table 9 Effect of lignocellulolytic fungi in the per cent organic carbon content of retted and non retted coirpith at different time intervals

Sl. No.	Fungus	Retted coirpith				Non retted coirpith				Mean
		20D	30D	45D	Mean	20D	30D	45D	Mean	
1	*Pleurotus eous*	27.60	23.68	22.35	24.54	33.43	30.29	27.30	30.34	27.44
2	*P. squarrosulus*	42.28	39.83	28.46	36.86	33.15	30.03	27.47	30.22	33.54
3	*P. sajor-caju*	36.55	32.91	30.60	33.36	31.25	28.61	24.81	28.22	30.79
4	*P. florida*	39.00	32.89	30.07	33.99	38.52	35.03	30.56	34.70	34.35
5	*P. florida* (Holland)	37.77	31.97	28.82	32.85	37.88	35.20	30.63	34.57	33.71
6	*Calocybe indica* -1	38.61	34.79	32.07	35.16	39.29	34.73	31.83	35.29	35.22
7	*C. indica* -2	38.91	35.41	31.81	35.38	39.52	34.316	30.13	34.65	35.02
8	*Schizophyllum commune*	42.51	40.08	35.68	39.42	37.97	34.96	29.62	34.19	36.80
9	*Coprinus comatus*	44.22	41.42	36.92	40.85	39.49	32.46	29.86	33.94	37.39
10	Control (raw coirpith)	45.59	45.59	45.59	45.59	42.50	42.50	42.50	42.50	44.05
	Mean	39.31	35.86	32.24	35.80	37.30	33.81	30.47	33.86	

CD (0.05 level)

Coirpith : 0.791, fungus : 1.768, period : 0.598, Coirpith x fungus : 2.5, Coirpith x period : 0.845, coirpith x fungus x period : 2.673

Data on interaction of coirpith, fungus and different periods of composting showed that *P. eous* on retted coirpith at 30 days (23.68 per cent) and 45 days (22.35 per cent) and *P. sajor-caju* on non retted coirpith at 45 days (24.81 per cent) of incubation brought about significant reduction of organic carbon content.

4.4.2.2 Total Nitrogen

The nitrogen content of retted and non retted coirpith samples treated with nine different lignocellulolytic fungi at 20 days, 30 days and 45 days of composting under *in vitro* condition was analysed. It was found that there was significant difference between the nitrogen content of retted (1.17 per cent) and non retted (1.14 per cent) coirpith samples (Table 10).

Among the various fungi the nitrogen content recorded by *C. indica*-2 (1.36), *P. eous* (1.34), *C. indica*-1 (1.32 per cent) was significantly higher compared to all other treatments. While considering the different time intervals for composting it was seen that nitrogen content was significantly different in 20 days (1.08 per cent), 30 days (1.12 per cent) and 45 days (1.15 per cent) after inoculation.

In the case of interaction between coirpith and fungus it was observed that the per cent nitrogen content recorded by *S. commune* (1.4), *C. indica*-2 (1.39) and *P. eous* (1.3.7) on retted coirpith was significantly higher compared to other treatments. Nitrogen content of *Coprinus comatus* treated retted (0.79 per cent) and non retted (0.86 per cent) coirpith was significantly different from other fungus inoculated treatments.

Three factor interactions between coirpith, fungus and different time for composting the per cent nitrogen content recorded by *C. indica*-2 (1.48), *S. commune* (1.48), *P. eous* (1.46) and *C. indica*-1 (1.43) on retted coirpith after 45 days of incubation was significantly higher than any other treatments.

Table 10 Effect of lignocellulolytic fungi in the per cent total nitrogen content of retted and non retted coirpith at different time intervals

Sl. No.	Fungus	Retted coirpith				Non retted coirpith				Mean
		20D	30D	45D	Mean	20D	30D	45D	Mean	
1	*Pleurotus eous*	1.27	1.38	1.46	1.37	1.26	1.29	1.39	1.31	1.34
2	*P. squarrosulus*	1.17	1.28	1.31	1.25	0.94	1.10	1.23	1.09	1.17
3	*P. sajor-caju*	1.21	1.23	1.29	1.24	1.12	1.15	1.18	1.15	1.19
4	*P. florida*	1.18	1.20	1.25	1.20	1.20	1.29	1.30	1.26	1.24
5	*P. florida* (Holland)	1.12	1.15	1.28	1.18	1.22	1.28	1.35	1.28	1.23
6	*Calocybe indica* -1	1.20	1.29	1.43	1.30	1.25	1.32	1.41	1.33	1.32
7	*C. indica* -2	1.30	1.39	1.48	1.39	1.27	1.32	1.39	1.33	1.36
8	*Schizophyllum commune*	1.32	1.40	1.48	1.40	1.03	1.15	1.25	1.14	1.27
9	*Coprinus comatus*	0.70	0.76	0.91	0.79	0.73	0.92	0.94	0.86	0.83
10	Control (coirpith alone)	0.53	0.53	0.53	0.53	0.60	0.60	0.60	0.60	0.57
	Mean	1.10	1.16	1.24	1.17	1.06	1.14	1.20	1.14	

CD (0.05 level)

Coirpith : 0.021, fungus : 0.046, period : 0.015, Coirpith x fungus : 0.065, Coirpith x period : 0.022, coirpith x fungus x period : 0.068

4.4.3.3 C : N ratio

C : N ratio of retted and non retted coirpith samples treated with different species of fungi analysed at 20, 30 and 45 days after inoculation is described in Table 11. The C : N ratio of retted coirpith (86.68 : 1) and non retted coirpith (70.57 : 1) differed significantly.

While considering the degradation potential of fungus it was found that *P. eous* brought down the C : N ratio to the minimum level (20.66 : 1 from 78.63 : 1) in the raw coirpith which was significantly different from all other fungi tested. C : N ratio narrowed down by *Coprinus comatus* was the least (46.83 : 1) and it differed from all other fungi tested.

Effect of different periods on coirpith composting showed significant difference existed between 20 days (38.89 : 1), 30 days (33.59 : 1) and 45 days (29.17 : 1) after inoculation with the fungus.

Two factor interaction of coirpith and different fungi tested showed that *P. eous* on retted coirpith narrowed down the C : N ratio to the maximum (18.08 : 1). C : N ratio of *Coprinus* treated retted (52.85 : 1) and non retted (40.8 : 1) coirpith was least significant when compared to all other treatments. All the treatment combinations showed significant difference with period of composting.

In three factor interaction of coirpith, different fungal species and time interval revealed that *P. eous* at 30 days (17.16 : 1) and 45 days (15.43 : 1) after inoculation in retted and *P. eous* (19.63 : 1) and *P. sajor-caju* (21.12 : 1) after 45 days of inoculation in non retted coirpith recorded significantly lower values of C : N ratio.

4.4.4.4 Total Phosphorus

Significant difference existed between the phosphorus content of retted coirpith (0.08) and non retted coirpith (0.11 per cent). Phosphorus content was greater for non retted coirpith (Table 12).

Table 11 Effect of lignocellulolytic fungi in the C : N ratio of retted and non retted coirpith at different time intervals

Sl. No.	Fungus	Retted coirpith				Non retted coirpith				Mean
		20D	30D	45D	Mean	20D	30D	45D	Mean	
1	*Pleurotus eous*	21.66 : 1	17.16 : 1	15.43 : 1	18.08:1	26.64 : 1	23.44 : 1	19.63 : 1	23.24 : 1	20.66:1
2	*P. squarrosulus*	36.18 : 1	31.15 : 1	21.70 : 1	29.68:1	35.38 : 1	27.24 : 1	22.39 : 1	28.34 : 1	29.01:1
3	*P. sajor-caju*	30.21 : 1	26.84 : 1	23.82 : 1	26.96:1	28.09 : 1	24.96 : 1	21.12 : 1	24.72 : 1	25.84:1
4	*P. florida*	33.23 : 1	27.33 : 1	24.20 : 1	28.25:1	32.21 : 1	27.14 : 1	23.55 : 1	27.63 : 1	27.94:1
5	*P. florida* (Holland)	33.90 : 1	27.86 : 1	22.49 : 1	28.09:1	31.07 : 1	27.53 : 1	22.70 : 1	27.10 : 1	27.59:1
6	*Calocybe indica* -1	32.29 : 1	27.09 : 1	22.47 : 1	27.28:1	31.58 : 1	26.27 : 1	22.55 : 1	26.80 : 1	27.04:1
7	Calocybe indica -2	29.90 : 1	25.50 : 1	21.49 : 1	25.63:1	31.01 : 1	25.94 : 1	21.70 : 1	26.20 : 1	25.92:1
8	*Schizophyllum commune*	32.30 : 1	28.61 : 1	24.22 : 1	28.38:1	36.97 : 1	30.57 : 1	23.82 : 1	30.45 : 1	29.42:1
9	*Coprinus comatus*	63.60 : 1	54.26 : 1	40.70 : 1	52.85:1	54.50 : 1	35.69 : 1	32.19 : 1	40.80 : 1	46.83 : 1
10	Control (coirpith alone)	86.68 : 1	86.68 : 1	86.68 : 1	86.68:1	70.57 : 1	70.57 : 1	70.57 : 1	70.57 : 1	78.63 : 1
	Mean	39.99 : 1	35.25 : 1	30.32 : 1	35.19:1	37.80 : 1	31.94 : 1	28.02 : 1	32.59 : 1	

CD (0.05 level)

Coirpith : 1.614, fungus : 3.609, period : 1.135, Coirpith x fungus : 5.104, Coirpith x period : 1.606, coirpith x fungus x period : 5.078

Table 12 Effect of lignocellulolytic fungi in the per cent Total phosphorus content of retted and non retted coirpith at different time intervals

Sl. No.	Fungus	Retted coirpith				Non retted coirpith				Mean
		20D	30D	45D	Mean	20D	30D	45D	Mean	
1	*Pleurotus eous*	0.08	0.11	0.12	0.10	0.12	0.13	0.16	0.14	0.12
2	*P. squarrosulus*	0.08	0.10	0.10	0.09	0.07	0.12	0.14	0.11	0.10
3	*P. sajor-caju*	0.08	0.09	0.09	0.08	0.06	0.07	0.08	0.07	0.08
4	*P. florida*	0.07	0.08	0.08	0.08	0.08	0.09	0.11	0.09	0.09
5	*P. florida* (Holland)	0.08	0.09	0.09	0.08	0.12	0.16	0.17	0.15	0.12
6	*Calocybe indica* -1	0.07	0.08	0.09	0.08	0.10	0.14	0.16	0.13	0.10
7	*Calocybe indica* -2	0.07	0.08	0.08	0.08	0.11	0.15	0.18	0.15	0.11
8	*Schizophyllum commune*	0.08	0.09	0.09	0.09	0.14	0.16	0.18	0.16	0.12
9	*Coprinus comatus*	0.06	0.07	0.07	0.07	0.04	0.05	0.05	0.05	0.06
10	Control (coirpith alone)	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.03
	Mean	0.06	0.08	0.08	0.08	0.09	0.10	0.13	0.11	

CD (0.05 level)

Coirpith : 0.004, fungus : 0.009, period : 0.003, Coirpith x fungus : 0.013, Coirpith x period : 0.004, coirpith x fungus x period : 0.014

Among the various fungi tested phosphorus content was maximum (0.12 per cent) with *P. eous*, *P. florida* and *S. commune*.

Two factor interaction between coirpith and nine different species of fungi showed that the phosphorus content was on par on non retted coirpith treated with *S. commune* (0.16 per cent), *P. florida* (Holland) (0.15 per cent) and *C. indica*-2 which inturn was significantly better than other treatments including control. The total phosphorus content of retted coirpith was less compared to non retted coirpith.

The effect of various composting periods in the level of phosphorus content revealed that there was significant difference between 20 days (0.08 per cent) and 30 days (0.10 per cent) of composting. Phosphorus content at 30 days was found to be on par with phosphorus content at 45 days (0.1 per cent).

In the three factor interaction between type of coirpith, different fungal species and different periods for composting, phosphorus content was found to be maximum for *S. commune* and *C. indica*-2 inoculated in non retted coirpith after 45 days of incubation (0.18 per cent). No significant difference existed between 20, 30 and 45 days of inoculation.

4.4.4.5 Total Potassium

Significant difference existed between the total potassium content of retted coirpith (0.11) and non retted coirpith (0.32 per cent). The potassium content was greater for non retted coirpith (Table 13). Among the various fungi tested total potassium content was found to be maximum for *Calocybe indica*-2 and *Pleurotus eous* (0.26 per cent). These two were significantly different from other treatments. Effect of periods of incubation on coirpith decomposition revealed that significant difference existed between 20 days (0.20 per cent), 30 days (0.22 per cent) and 45 days (0.23 per cent).

Table 13 Effect of lignocellulolytic fungi in the per cent Total potassium content of retted and non retted coirpith at different time intervals

Sl. No.	Fungus	Retted coirpith				Non retted coirpith				Mean
		20D	30D	45D	Mean	20D	30D	45D	Mean	
1	*Pleurotus eous*	0.12	0.20	0.20	0.17	0.33	0.35	0.38	0.35	0.26
2	*P. squarrosulus*	0.08	0.08	0.13	0.11	0.32	0.34	0.35	0.34	0.22
3	*P. sajor-caju*	0.11	0.11	0.13	0.11	0.31	0.32	0.34	0.33	0.22
4	*P. florida*	0.08	0.10	0.12	0.10	0.31	0.32	0.33	0.32	0.21
5	*P. florida* (Holland)	0.10	0.11	0.15	0.12	0.32	0.34	0.36	0.34	0.23
6	*Calocybe indica* -1	0.11	0.14	0.15	0.13	0.31	0.32	0.35	0.33	0.23
7	*Calocybe indica* -2	0.11	0.15	0.18	0.15	0.36	0.38	0.39	0.38	0.26
8	*Schizophyllum commune*	0.08	0.12	0.13	0.11	0.30	0.31	0.32	0.31	0.21
9	*Coprinus comatus*	0.06	0.07	0.08	0.07	0.28	0.29	0.29	0.28	0.18
10	Control (coirpith alone)	0.06	0.06	0.06	0.06	0.26	0.26	0.26	0.26	0.16
	Mean	0.09	0.11	0.13	0.11	0.31	0.32	0.34	0.32	

CD (0.05 level)

Coirpith : 0.004, fungus : 0.010, period : 0.003, Coirpith x fungus : 0.014, Coirpith x period : 0.004, coirpith x fungus x period : 0.014

In the two factor interaction on coirpith and different periods of incubation showed that all the combinations were significantly different from each other. Maximum potassium content was recorded for non retted coirpith at 45 days of incubation (0.34 per cent) which was significantly different from other treatments. Regarding two factor interaction between different species of fungi and different type of coirpith the potassium content was maximum for *Calocybe indica*-2 inoculated on non retted coirpith (0.38 per cent).

Three factor interactions among different fungal species, types of coirpith and different periods of incubation for composting showed that potassium content was maximum for non retted coirpith inoculated with *Calocybe indica*-2 at 45 days of incubation (0.39 per cent). Among the fungi inoculated least potassium content was recorded by *Coprinus* on retted coirpith at 20 days which was on par with control.

4.4.4.6 Cellulose

The cellulose content of retted coirpith (19.54 per cent) differed significantly from non retted coirpith (24.94 per cent) (Table 14). Among the fungi tested *P. eous* reduced the cellulose content from 32.33 per cent in raw coirpith to 17.23 per cent in composted coirpith which was on par with *P. sajor-caju* (17.83 per cent). Significant difference existed between degradation of cellulose in 20, 30 and 45 days of incubation.

The interaction between coirpith and different fungal species the maximum degradation of cellulose was found in retted coirpith inoculated with *P. eous* (14.72 per cent).

Three factor interactions of coirpith, fungus and different periods of incubation on degradation of cellulose content of coirpith showed that the cellulose content was minimum in retted coirpith after 45 days of inoculation with *Pleurotus eous* (7.39 per cent) which was on par with *Pleurotus sajor-caju* (8.90 per cent).

Table 14 Effect of lignocellulolytic fungi in the per cent cellulose content of retted and non retted coirpith at different time intervals

Sl. No.	Fungus	Retted coirpith				Non retted coirpith				Mean
		20D	30D	45D	Mean	20D	30D	45D	Mean	
1	*Pleurotus eous*	23.75	13.00	7.39	14.72	27.00	17.97	14.25	19.74	17.23
2	*P. squarrosulus*	26.10	17.40	12.60	18.71	29.22	22.43	16.62	22.76	20.74
3	*P. sajor-caju*	23.97	15.09	8.90	15.99	26.88	17.41	14.73	19.67	17.83
4	*P. florida*	26.55	16.70	12.00	18.42	30.86	25.53	17.90	24.73	21.57
5	*P. florida* (Holland)	26.03	17.89	13.97	19.30	30.59	25.44	17.67	24.57	21.93
6	*Calocybe indica* -1	27.07	17.72	14.65	19.81	28.31	27.70	23.47	26.49	23.15
7	Calocybe indica -2	25.78	16.50	12.51	18.26	30.86	27.27	22.58	26.91	22.58
8	*Schizophyllum commune*	24.76	16.45	13.11	18.11	30.52	24.76	19.74	25.00	21.56
9	*Coprinus comatus*	26.75	23.12	18.38	22.75	29.84	22.48	18.45	23.59	23.17
10	Control (coirpith alone)	29.30	29.30	29.30	29.30	35.97	35.97	35.97	35.97	32.33
	Mean	26.01	18.32	14.28	19.54	29.99	24.70	20.14	24.94	

CD (0.05 level)

Coirpith : 0.359, fungus : 0.804, period : 0.348, Coirpith x fungus : 1.137, Coirpith x period : 0.492, coirpith x fungus x period : 1.556

4.4.4.7 Lignin

Significant difference existed between the lignin content of retted (19.48 per cent) and non retted (24.66 per cent) coirpith samples (Table 15). Among the various fungi tested *P. sajor-caju* (17.67 per cent), *S. commune* (17.78 per cent) and *P. eous* (18.15 per cent) were equally effective in degrading lignin and significantly different from other treatments. The data showed that there was significant difference among 20 days (17.15 per cent), 30 days (21.98 per cent) and 45 days (26.87 per cent) after inoculation for lignin degradation.

The interaction between coirpith and fungi showed that *P. sajor-caju* (15.11), *P. eous* (15.94) and *S. commune* (16.09 per cent) inoculated on retted coirpith brought down maximum lignin degradation and significantly differed from others. Lignin content of *Coprinus comatus* (32.98 per cent) inoculated on non retted coirpith was significantly different from other treatments. *Coprinus comatus* was found to be a poor degrader of lignin.

Three factor interaction of coirpith, species of fungi and periods for lignin degradation *P. sajor-caju* (9.66 per cent), *P. eous* (10.33 per cent) and *S. commune* (11.22 per cent) on retted coirpith after 45 days of incubation brought about significantly lower levels of lignin.

4.5 FIELD LEVEL TRIALS ON COIRPITH COMPOSTING

Based on the results from *in vitro* trial out of nine lignocellulolytic fungi, six were selected for field trial. Out of the two strains of *C. indica*, strain 1, *Coprinus comatus*, *P. florida* (Holland) were not used for field trials as they were found to be poor decomposers.

4.5.1 Organic Carbon

From the field level trials, it revealed that there was significant difference existed between the organic carbon content of retted (38.95 per cent) and non retted coirpith (30.74 per cent). Among the various fungi

Table 15 Effect of lignocellulolytic fungi in the per cent lignin content of retted and non retted coirpith at different time intervals

Sl. No.	Fungus	Retted coirpith				Non retted coirpith				Mean
		20D	30D	45D	Mean	20D	30D	45D	Mean	
1	*Pleurotus eous*	20.64	16.85	10.33	15.94	26.57	20.48	14.02	20.36	18.15
2	*P. squarrosulus*	21.70	17.47	11.80	16.99	28.20	21.42	15.57	21.73	19.36
3	*P. sajor-caju*	20.10	15.57	9.66	15.11	26.97	19.93	13.80	20.23	17.67
4	*P. florida*	26.62	19.94	15.63	20.73	30.42	24.37	17.73	24.17	22.45
5	*P. florida* (Holland)	22.38	16.90	12.22	17.17	29.67	22.29	16.54	22.83	19.99
6	*Calocybe indica* -1	22.93	16.86	12.44	17.41	28.20	23.28	16.60	22.69	20.05
7	Calocybe indica -2	22.13	16.35	12.29	16.92	27.39	22.64	15.91	21.98	19.45
8	*Schizophyllum commune*	20.68	16.38	11.22	16.09	25.90	19.31	13.20	19.47	17.78
9	*Coprinus comatus*	28.29	24.37	20.74	24.47	35.89	32.51	30.55	32.98	28.73
10	Control (coirpith alone)	33.99	33.99	33.99	33.99	38.75	38.75	38.75	38.75	36.37
	Mean	23.95	19.47	15.03	19.48	29.80	24.50	19.27	24.66	

CD (0.05 level)

Coirpith : 0.511, fungus : 1.143, period : 0.377, Coirpith x fungus : 1.616, Coirpith x period : 0.533, coirpith x fungus x period : 1.686

tested, the decomposing ability of *P. eous* (31.99 per cent) and *S. commune* (32.31 per cent) were better than other decomposers in reducing the organic carbon content of coirpith. Significant difference existed between the organic carbon content of different treatments after 20 days (39.23 per cent), 30 days (35.29 per cent) and 45 days (29.52 per cent) of incubation (Table 16).

Two factor interaction between coirpith and fungus revealed that the organic carbon content in coirpith inoculated with *P. eous* (27.51 per cent) and *S. commune* (27.87 per cent) were significantly lower than other treatments. Reduction in organic carbon content was less pronounced in retted compared to non retted coirpith.

The results revealed that the interaction between coirpith and days after inoculation were significantly different from each other.

Three factor interaction of coirpith, fungus and days after inoculation revealed that the organic carbon content of non retted coirpith treated with *P. eous* (20.13 per cent) and *S. commune* (21.23 per cent) after 45 days of incubation were significantly lower than all other treatments tried.

In the interaction between fungus and days after inoculation the organic carbon content of *P. eous* (25.53 per cent) and *S. commune* (26.12 per cent) inoculated coirpith after 45 days of incubation were significantly lower than all other treatments tried (Fig.1).

4.5.2 Total Nitrogen

The nitrogen content of retted (1.15 per cent) and non retted (1.14 per cent) coirpith samples were on par with each other. The total nitrogen content was highest for *P. eous* treated coirpith (1.39 per cent). All other treatments differ significantly from this. Significant difference existed between different days of incubation and nitrogen content of coirpith. The

Table 16 Effect of lignocellulolytic fungi in the per cent organic carbon content of retted and non retted coirpith at different time intervals (field level trial)

Sl. No.	Fungus	Retted coirpith				Non retted coirpith				Mean
		20D	30D	45D	Mean	20D	30D	45D	Mean	
1	*Pleurotus eous*	41.62	36.86	30.92	36.47	33.37	29.03	20.13	27.51	31.99
2	*P. squarrosulus*	43.15	38.04	31.51	37.57	36.27	31.27	24.30	30.62	34.09
3	*P. sajor-caju*	42.75	37.92	31.52	37.40	34.21	28.78	23.86	28.95	33.17
4	*P. florida*	44.11	39.28	32.53	38.64	37.27	29.99	24.39	30.55	34.59
5	*Calocybe indica -2*	42.20	38.19	32.29	37.56	34.91	30.37	23.85	29.71	33.63
6	*Schizophyllum commune*	42.34	37.20	31.01	36.85	33.40	28.69	21.23	27.78	32.31
7	Control (coirpith alone)	49.20	48.23	47.06	48.16	41.40	40.24	38.63	40.09	44.13
	Mean	43.62	39.39	33.84	38.95	35.83	31.20	25.20	30.74	

CD (0.05 level)

Coirpith : 0.408, fungus : 0.763, period : 0.308, coirpith x fungus : 1.079, coirpith x period : 0.435, coirpith x fungus x period : 1.151

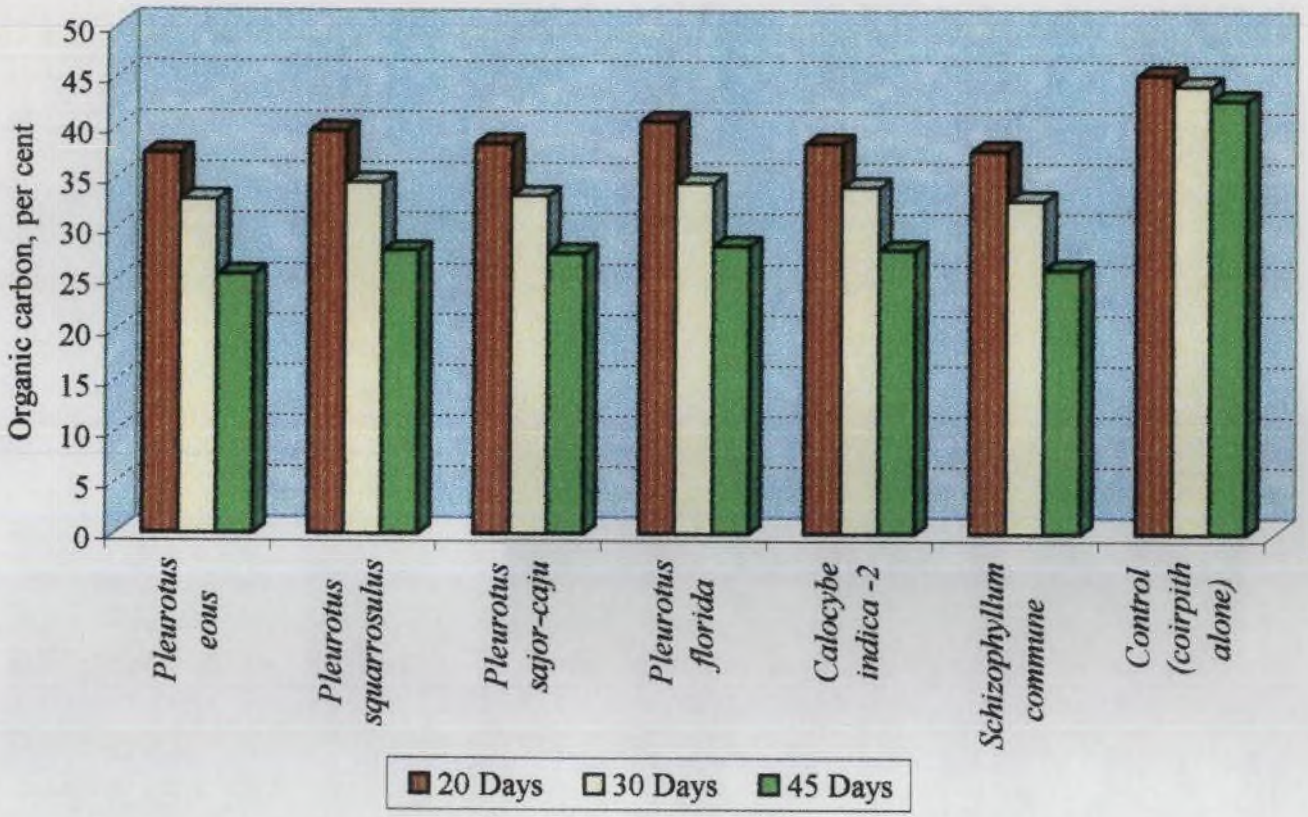


Fig. 1 Effect of duration on organic carbon content of coirpith inoculated with lignocellulolytic fungi

total nitrogen content was 1.03, 1.16 and 1.24 per cent respectively for 20th, 30th and 45th day after inoculation (Table 17).

In the two factor interaction between coirpith and fungi tested the maximum nitrogen content of *P. eous* and *S. commune* inoculated retted (1.40 per cent) and *P. eous* inoculated non retted coirpith (1.38 per cent) was significantly higher than all other treatments. Interaction between coirpith and period of composting revealed that maximum nitrogen content was observed in retted coirpith after 45 days of incubation (1.27 per cent) which was significantly different from all other treatments. Nitrogen content was least for retted coirpith after 20 days of incubation (1.02 per cent) which was significant from non retted coirpith after 20 days of incubation (1.05 per cent).

Interaction of coirpith, fungus and days after inoculation showed that the nitrogen content of *S. commune* (1.57 per cent) and *P. eous* (1.52 per cent) inoculated retted coirpith after 45 days of incubation was significantly higher compared to all other treatment combinations tried.

P. eous inoculated coirpith supported the maximum nitrogen after 45 days of incubation. This was significantly different from all other treatments (Fig. 2).

4.5.3 C : N Ratio

Significant difference existed between the C : N ratio of retted (39.55 : 1) and non retted coirpith (30.1 : 1). Among the fungi tested *P. eous* and *S. commune* narrowed down the C : N ratio from the original value of 77.42 : 1 to 23.38 : 1 and 24.62 : 1 respectively. These fungi were better than all other treatments in narrowing C : N ratio. There was significant difference between different days of incubation on coirpith degradation (Table 18).

Two factor interaction between coirpith and fungi tested revealed that the C : N ratio for non retted coirpith inoculated with *P. eous* (20.32 : 1),

Table 17 Effect of lignocellulolytic fungi in the per cent nitrogen content of retted and non retted coirpith at different time intervals (field level trial)

Sl. No.	Fungus	Retted coirpith				Non retted coirpith				Mean
		20D	30D	45D	Mean	20D	30D	45D	Mean	
1	*Pleurotus eous*	1.28	1.42	1.52	1.40	1.27	1.38	1.49	1.38	1.39
2	*P. squarrosulus*	0.80	0.96	1.15	0.97	1.03	1.15	1.20	1.13	1.05
3	*P. sajor-caju*	1.10	1.26	1.37	1.25	1.12	1.30	1.37	1.26	1.26
4	*P. florida*	1.09	1.22	1.32	1.21	1.08	1.19	1.29	1.19	1.20
5	*Calocybe indica -2*	1.10	1.24	1.40	1.25	1.07	1.23	1.27	1.19	1.22
6	*Schizophyllum commune*	1.22	1.42	1.57	1.40	1.18	1.26	1.31	1.25	1.33
7	Control (coirpith alone)	0.53	0.54	0.56	0.54	0.60	0.62	0.63	0.61	0.58
	Mean	1.02	1.15	1.27	1.15	1.05	1.16	1.22	1.14	

CD (0.05 level) Coirpith : 0.022, fungus : 0.041, period : 0.018, Coirpith x fungus : 0.058, coirpith x period : 0.026, coirpith x fungus x period : 0.068

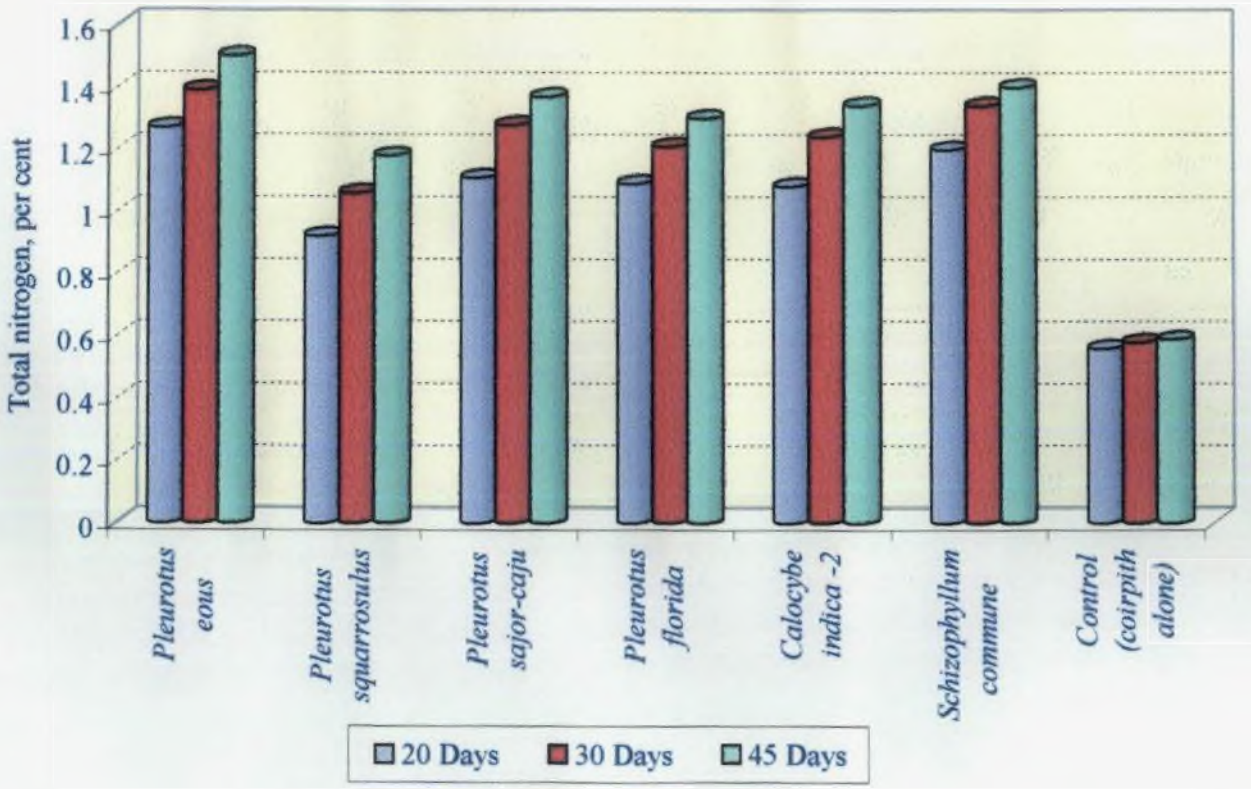


Fig. 2 Effect of duration on total nitrogen content of coirpith inoculated with lignocellulolytic fungi

Table 18 Effect of lignocellulolytic fungi in the C : N ratio of retted and non retted coirpith at different time intervals (Field level trial)

Sl. No.	Fungus	Retted coirpith				Non retted coirpith				Mean
		20D	30D	45D	Mean	20D	30D	45D	Mean	
1	*Pleurotus eous*	32.62:1	26.33:1	20.37:1	26.44:1	26.33:1	21.1:1	13.54:1	20.32:1	23.38:1
2	*P. squarrosulus*	53.96:1	39.59:1	27.46:1	40.34:1	35.37:1	27.21:1	20.19:1	27.59:1	33.96:1
3	*P. sajor-caju*	38.77:1	30.12:1	22.96:1	30.61:1	30.49:1	22.10:1	17.46:1	23.35:1	26.98:1
4	*P. florida*	40.47:1	32.16:1	24.64:1	32.42:1	34.46:1	25.2:1	18.97:1	26.21:1	29.32:1
5	*Calocybe indica -2*	38.37:1	30.74:1	23.02:1	30.71:1	32.77:1	24.76:1	18.76:1	25.43:1	28.07:1
6	*Schizophyllum commune*	34.62:1	26.13:1	19.73:1	26.83:1	28.32:1	22.78:1	16.18:1	22.43:1	24.62:1
7	Control (coirpith alone)	93.02:1	89.92:1	85.54:1	89.50:1	69.41:1	64.95:1	61.66:1	65.34:1	77.42:1
	Mean	47.40:1	39.28:1	31.96:1	39.55:1	36.74:1	29.73:1	28.82:1	30.1:1	

CD (0.05 level) Coirpith : 1.831, fungus : 3.425, period : 1.288, coirpith x fungus : 4.844, coirpith x period : 1.822, coirpith x fungus x period : 4.821

S. commune (22.43 : 1) and *P. sajor-caju* (23.35 :1) were significantly better than the remaining three fungi. The interaction between coirpith and days of incubation was found to be significant.

Three factor interaction between coirpith, fungus and days of inoculation showed that *P. eous* (13.54 : 1), *S. commune* (16.18 : 1) and *P. sajor-caju* (17.46 : 1) inoculated on non retted coirpith after 45 days of incubation narrowed down the C : N ratio significantly better level compared to the other three fungi.

P. eous (16.96 : 1), *S. commune* (17.95 : 1) and *P. sajor-caju* (20.21 : 1) were efficient in narrowing down the C : N ratio after 45 days of incubation (Fig. 3).

4.5.4 Total Phosphorus

Significant difference existed between the phosphorus content of retted (0.05 per cent) and non retted coirpith (0.08 per cent). All the fungi were able to increase the total phosphorus content of the coirpith during composting compared to control. There was no significant difference among the lignocellulolytic fungi tested (Table 19).

In the interaction between coirpith and fungi revealed that the phosphorus content of non-retted coirpith inoculated with all the six lignocellulolytic fungi were significantly higher compared to that in retted coirpith.

Three factor interaction of coirpith, fungus and days after inoculation showed that *C. indica*-2 (0.11 per cent), *P. eous* (0.10 per cent), *S. commune* (0.1 per cent), *P. florida* (0.1 per cent), *P. squarrosulus* (0.1 per cent) after 45 days and *P. eous* (0.1 per cent), *Calocybe indica*-2 (0.1 per cent) after 30 days of incubation on non retted coirpith recorded significantly higher levels of phosphorus compared to all other treatments. The retted coirpith in general recorded low phosphorus value compared to non retted coirpith.

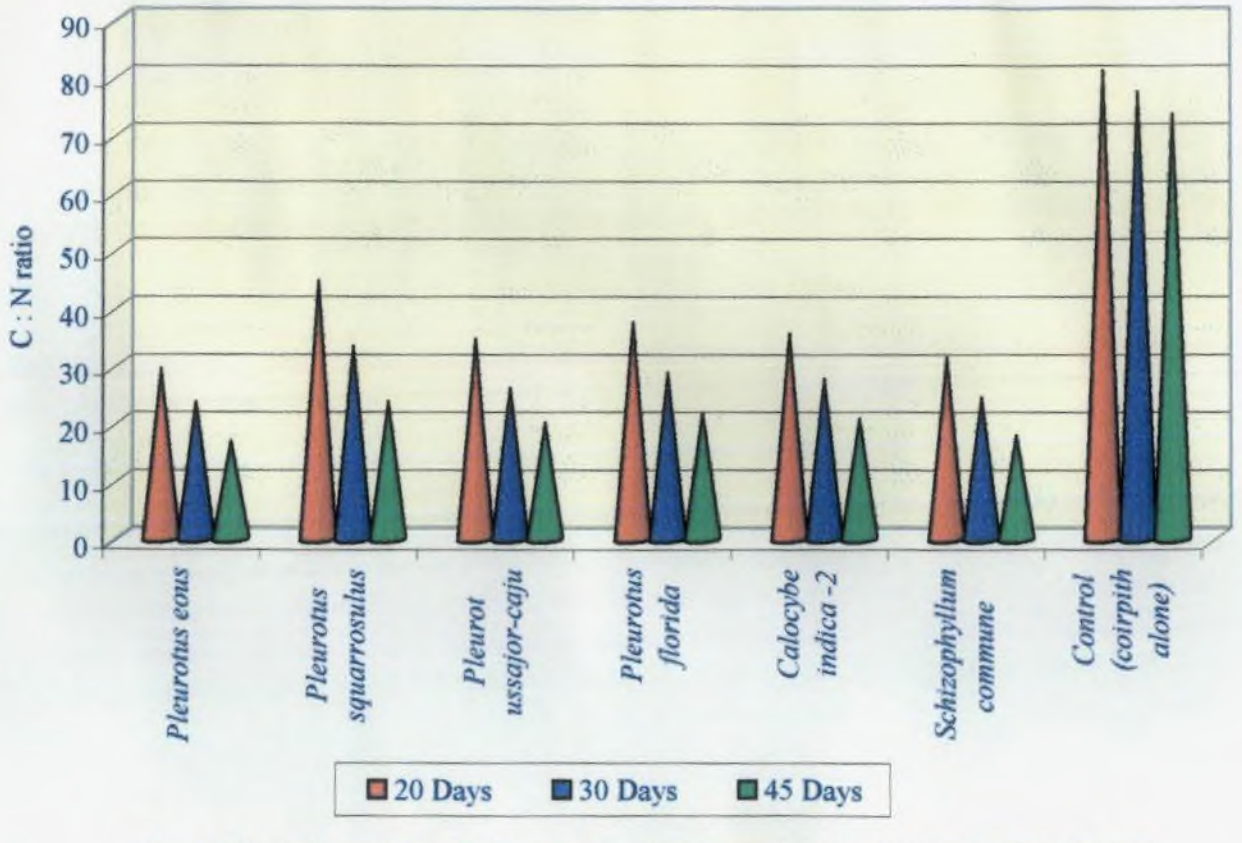


Fig. 3 Effect of duration on C:N ratio of coirpith inoculated with lignocellulolytic fungi

Table 19 Effect of lignocellulolytic fungi in the per cent Total phosphorus content of retted and non retted coirpith at different time intervals (Field level trial)

Sl. No.	Fungus	Retted coirpith				Non retted coirpith				Mean
		20D	30D	45D	Mean	20D	30D	45D	Mean	
1	*Pleurotus eous*	0.05	0.05	0.06	0.05	0.09	0.10	0.10	0.10	0.08
2	*P. squarrosulus*	0.04	0.05	0.06	0.05	0.07	0.09	0.10	0.09	0.07
3	*P. sajor-caju*	0.04	0.05	0.05	0.05	0.07	0.08	0.09	0.08	0.06
4	*P. florida*	0.04	0.05	0.06	0.05	0.07	0.08	0.10	0.08	0.07
5	*Calocybe indica -2*	0.05	0.05	0.06	0.05	0.09	0.10	0.11	0.10	0.08
6	*Schizophyllum commune*	0.04	0.05	0.06	0.05	0.07	0.09	0.10	0.09	0.07
7	Control (coirpith alone)	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04
	Mean	0.04	0.05	0.05	0.05	0.07	0.08	0.09	0.08	

CD (0.05 level) Coirpith : 0.013, fungus : 0.023, period : 0.014, coirpith x fungus : 0.033, coirpith x period : 0.020, coirpith x fungus x period : 0.054

Interaction between fungi and different periods of incubation revealed that *P. eous, C. indica*-2, *P. squarrosulus*, *P. florida*, *S. commune* after 45 days and *P. eous* and *C. indica* even at 30 days of incubation were efficient in increasing the phosphorus content significantly higher levels than that of all other treatment combinations tried (Fig. 4).

4.5.5 Total Potassium

Significant difference existed between the potassium content of retted (0.1 per cent) and non retted coirpith (1.41 per cent). Among the fungi tested it was observed that the potassium content was maximum with *P. eous* (0.88 per cent) which was significantly different from all other treatments. The influence of period of incubation was significant on potassium content (Table 20).

Considering the two factor interaction between type of coirpith and fungus it was found that *P. eous* inoculated on non retted coirpith brought about maximum potassium content (1.63 per cent) which was significant from all other treatments.

For the two factor interactions between fungus and different days after inoculation, it was found that potassium content was maximum for *P. eous* after 45 days of incubation (0.97 per cent) (Fig. 5).

In three factor interaction of coirpith, fungus and days after inoculation, it was observed that potassium content was maximum in non retted coirpith treated with *P. eous* after 45 days of incubation (1.78 per cent) which was on par with *P. sajor-caju* on non retted coirpith after 45 days (1.74 per cent).

4.5.6 Cellulose

Cellulose content of non retted coirpith (24.42 per cent) was higher than that of retted coirpith (22.54 per cent) and significantly different from each other (Table 21).

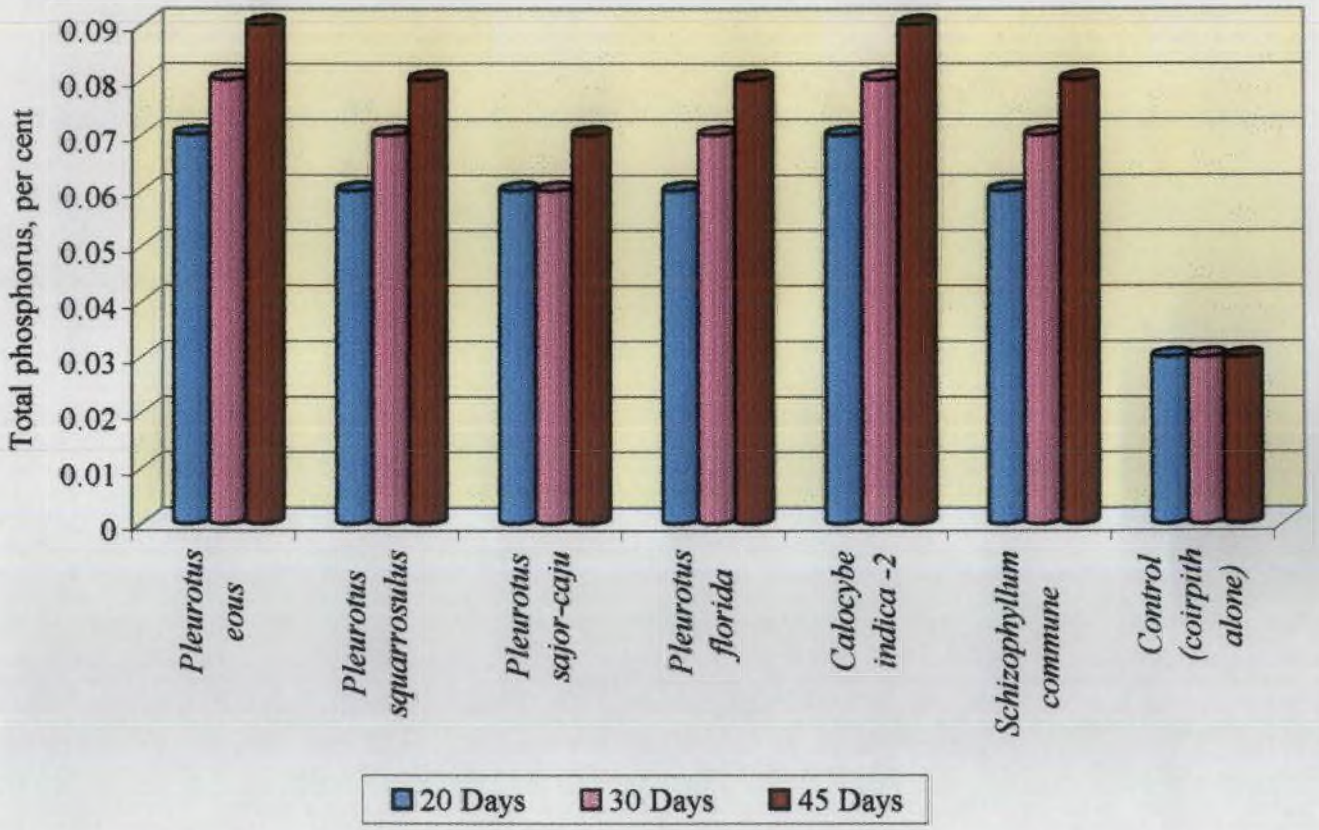


Fig. 4 Effect of duration on total phosphorus content of coirpith inoculated with lignocellulolytic fungi

Table 20 Effect of lignocellulolytic fungi in the per cent Total potassium content of retted and non retted coirpith at different time intervals (Field level trial)

Sl. No.	Fungus	Retted coirpith				Non retted coirpith				Mea n
		20D	30D	45D	Mean	20D	30D	45D	Mean	
1	*Pleurotus eous*	0.11	0.13	0.15	0.13	1.40	1.65	1.78	1.63	0.88
2	*P. squarrosulus*	0.06	0.08	0.10	0.08	1.26	1.42	1.59	1.42	0.75
3	*P. sajor-caju*	0.10	0.12	0.13	0.12	1.40	1.52	1.74	1.55	0.84
4	*P. florida*	0.10	0.11	0.13	0.11	1.32	1.53	1.63	1.49	0.80
5	*Calocybe indica -2*	0.11	0.13	0.14	0.13	1.26	1.30	1.54	1.37	0.75
6	*Schizophyllum commune*	0.09	0.10	0.12	0.11	1.26	1.38	1.51	1.38	0.74
7	Control (coirpith alone)	0.05	0.05	0.05	0.05	0.95	0.98	1.03	1.00	0.52
	Mean	0.09	0.10	0.12	0.10	1.27	1.40	1.55	1.41	

CD (0.05 level) Coirpith : 0.017, fungus : 0.031, period : 0.016, coirpith x fungus : 0.044, coirpith x period : 0.022, coirpith x fungus x period : 0.058

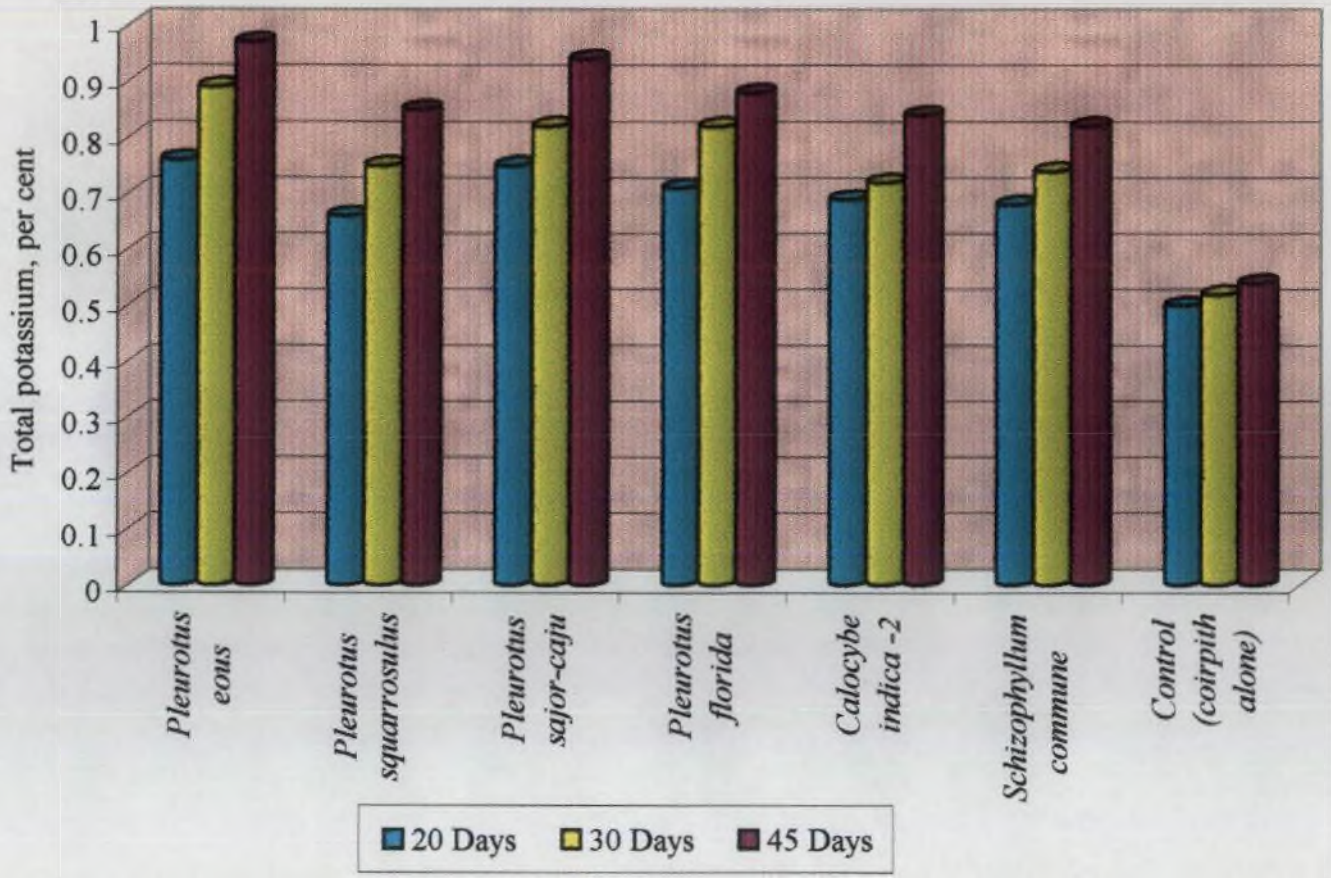


Fig. 5 Effect of duration on total potassium content of coirpith inoculated with lignocellulolytic fungi

Table 21 Effect of lignocellulolytic fungi in the per cent cellulose content of retted and non retted coirpith at different time intervals (Field level trial)

Sl. No.	Fungus	Retted coirpith				Non retted coirpith				Mean
		20D	30D	45D	Mean	20D	30D	45D	Mean	
1	*Pleurotus eous*	25.28	17.98	14.92	19.39	27.47	20.37	16.87	21.57	20.48
2	*P. squarrosulus*	27.73	21.60	16.93	22.09	28.80	21.93	19.20	23.31	22.70
3	*P. sajor-caju*	25.85	18.31	14.05	19.40	26.40	18.87	17.03	20.77	20.09
4	*P. florida*	27.92	21.97	17.33	22.40	29.17	23.47	19.43	24.02	23.21
5	*Calocybe indica* -2	28.30	20.30	18.07	22.22	29.23	23.81	19.01	24.02	23.12
6	*Schizophyllum commune*	27.74	21.51	18.93	22.73	27.26	21.83	17.40	22.16	22.45
7	Control (coirpith alone)	30.39	29.37	28.90	29.56	36.37	35.03	33.77	35.05	32.31
	Mean	27.60	21.58	18.45	22.54	29.24	23.62	20.39	24.42	

CD (level) Coirpith : 0.347, fungus : 0.649, period : 0.336, coirpith x fungus : 0.917, coirpith x period : 0.476, coirpith x fungus x period : 1.259

P. sajor-caju brought down the cellulose content from 32.31 to 20.09 per cent. A similar reduction was noticed in *P. eous* (20.48 per cent) inoculation on coirpith.

The two factor interaction between coirpith and fungus revealed that *P. eous* (19.39 per cent) and *P. sajor-sajor* (19.40 per cent) inoculated retted coirpith reduced the cellulose content to the maximum and significantly different from other treatments.

Three factor interaction among coirpith, fungus and different days of incubation on cellulose degradation, showed that maximum degradation of cellulose was observed for *P. sajor-caju* (14.05 per cent) and P. eous (14.92 per cent) on retted coirpith after 45th day of incubation.

Two factor interaction between fungus and different periods of incubation showed that maximum cellulose reduction was observed in treatments where *P. sajor-caju* (15.54 per cent) and *P. eous* (15.89 per cent) inoculated for 45 days (Fig. 6).

4.5.7 Lignin

The lignin content of retted coirpith (22.71 per cent) and non retted coirpith (25.33 per cent) was statistically significant from each other. Among the fungi tested the maximum lignin degradation was brought about by *P. eous* and *P. sajor-caju*. The lignin content was reduced from 36.84 per cent in raw coirpith to 20.33 and 20.59 per cent by *P. eous* and *P. sajor-caju* respectively, which were on par with each other and significantly different from other treatments (Table 22).

The two factor interaction between coirpith and fungi tested has revealed that *P. eous* (19.52 per cent), *P. sajor-caju* (19.80 per cent) and *P. squarrosulus* (20.47 per cent) on retted coirpith reduced the lignin content to the maximum and significantly different from other treatments.

Significant difference existed between two factor interaction viz., the type of coirpith and days after inoculation on lignin degradation.

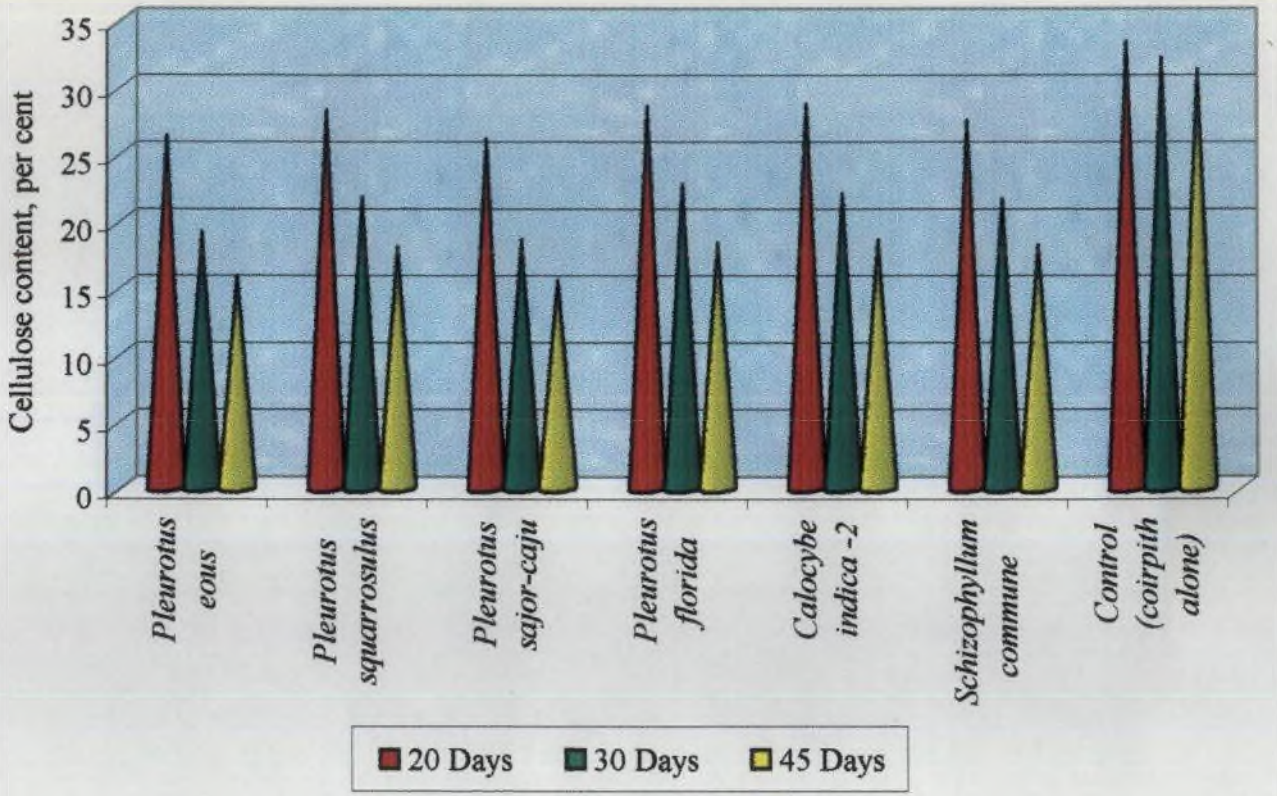


Fig. 6 Effect of duration on cellulose content of coirpith inoculated with lignocellulolytic fungi

Table 22 Effect of lignocellulolytic fungi in the per cent lignin content of retted and non retted coirpith at different time intervals (Field level trial)

Sl. No.	Fungus	Retted coirpith				Non retted coirpith				Mean
		20D	30D	45D	Mean	20D	30D	45D	Mean	
1	*Pleurotus eous*	25.19	18.80	14.56	19.52	26.80	21.45	15.17	21.14	20.33
2	*P. squarrosulus*	25.37	19.63	16.40	20.47	29.53	24.27	17.40	23.73	22.10
3	*P. sajor-caju*	24.75	20.07	14.58	19.80	27.90	21.83	14.40	21.38	20.59
4	*P. florida*	27.13	22.33	17.20	22.22	31.73	26.70	20.93	26.46	24.34
5	*Calocybe indica -2*	26.80	22.53	17.07	22.13	28.47	23.13	18.13	23.24	22.69
6	*Schizophyllum commune*	25.67	20.83	15.13	20.55	27.53	22.03	16.23	21.93	21.24
7	Control (coirpith alone)	34.27	34.27	34.27	34.27	39.40	39.40	39.40	39.40	36.84
	Mean	27.03	22.64	18.46	22.71	30.20	25.55	20.24	25.33	

CD (0.05 level) Coirpith : 0.375, fungus : 0.701, period : 0.394, coirpith x fungus : 0.991, coirpith x period : 0.0.557, coirpith x fungus x period : 1.473

Three factor interaction among coirpith, fungus and days after inoculation revealed that *P. sajor-caju* (14.40 per cent) and *P. eous* (15.17 per cent) on non retted coirpith, *P. eous* (14.56 per cent), *P. sajor-caju* (14.58 per cent), *S. commune* (15.13 per cent) on retted coirpith after 45 days of incubation brought about significantly higher levels of lignin degradation.

Two factor interaction between fungus and days after inoculation the maximum lignin degradation was observed for *P. sajor-caju* treated coirpith after 45 days of incubation (14.49 per cent) which was on par with *P. eous* after 45 days of incubation (14.86 per cent) (Fig. 7).

4.6 REDUCTION IN WEIGHT AND VOLUME

Reduction in the weight and volume of retted and non retted coirpith was noticed as a result of decomposition with lignocellulolytic fungi. Among the fungi tested in retted and non retted coirpith samples *Pleurotus eous* brought about maximum reduction in weight after 45th day of incubation. This was followed by *Schizophyllum commune*. *Coprinus comatus* was less effective in causing weight reduction. Eventhough slight weight reduction was noticed in control after 45th day on incubation it was significantly different from all other treatments (Table 23).

P. eous and *S. commune* brought significant reduction in volume of retted coirpith compared to other treatments. In non retted coirpith *S. commune* brought about maximum volume reduction. This was followed by *P. eous*. *Coprinus comatus* was found to be less effective in causing volume reduction in both retted and non retted coirpith (Table 23).

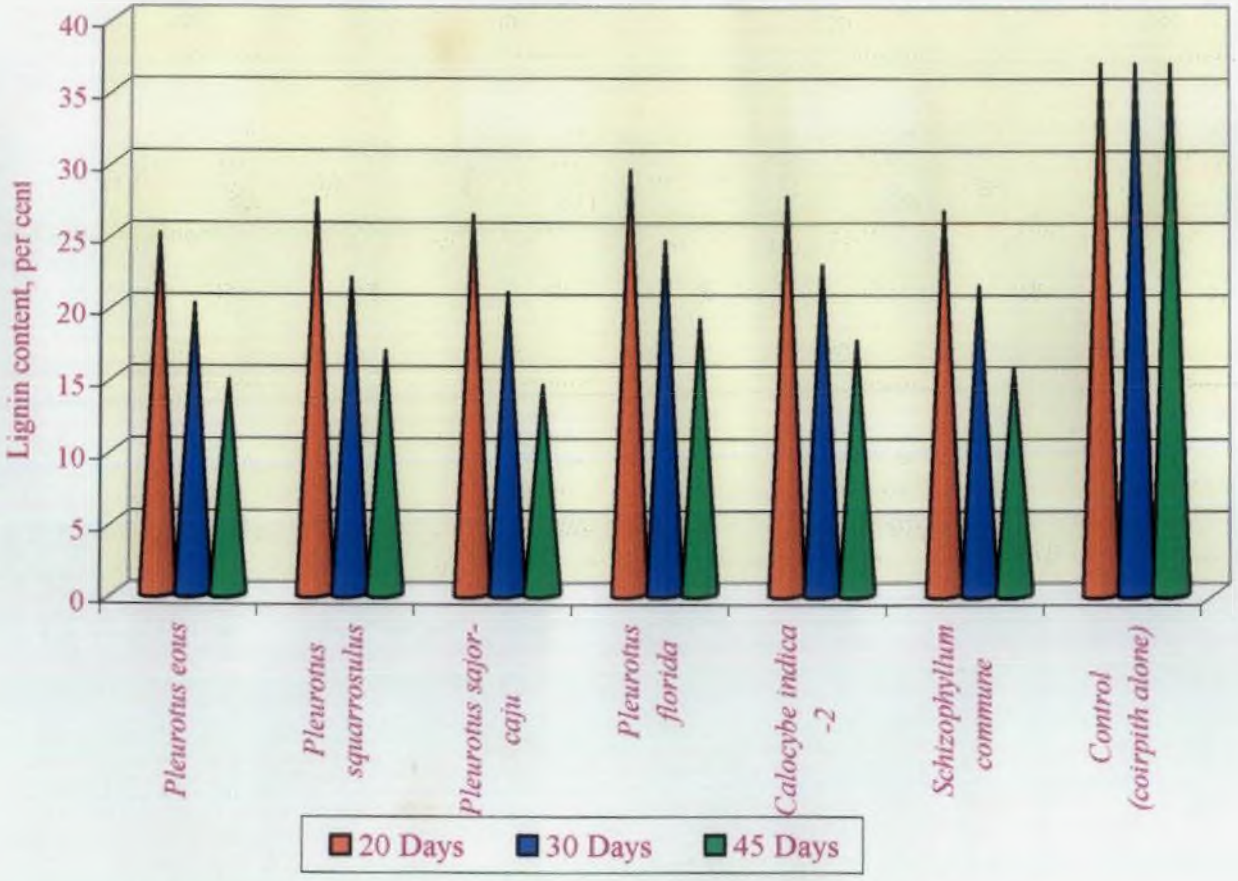


Fig. 7 Effect of duration on lignin content of coirpith inoculated with lignocellulolytic fungi

Table 23 Reduction in weight and volume of coirpith after 45 days of incubation with lignocellulolytic fungi

Sl. No.	Fungus	Per cent reduction in weight		Per cent reduction in volume	
		Retted coirpith	Non retted coirpith	Retted coirpith	Non retted coirpith
1	*Pleurotus eous*	8.98 (3)	9.43 (3.07)	29.52 (5.43)	25.24 (5.02)
2	*P. squarrosulus*	6.06 (2.46)	6.67 (2.58)	24.56 (4.96)	21.88 (4.68)
3	*P. sajor-caju*	7.19 (2.68)	7.83 (2.8)	26.56 (5.15)	22.85 (4.78)
4	*P. florida*	5.63 (2.37)	5.95 (2.44)	25.46 (5.05)	22.91 (4.79)
5	*P. florida* (Holland)	6.96 (2.64)	6.50 (2.55)	25.56 (5.06)	23.33 (4.83)
6	*Calocybe indica* -1	6.95 (2.64)	4.68 (2.16)	26.02 (5.1)	23.36 (4.83)
7	Calocybe indica -2	7.05 (2.66)	5.23 (2.29)	26.72 (5.17)	24.53 (4.95)
8	*Schizophyllum commune*	7.99 (2.83)	8.20 (2.86)	28.71 (5.36)	26.85 (5.18)
9	*Coprinus comatus*	4.37 (2.09)	4.91 (2.22)	22.43 (4.73)	21.19 (4.60)
10	Control (raw coirpith alone)	2.23 (1.49)	2.10 (1.45)	9.24 (3.04)	9.50 (3.08)

CD for weight reduction in retted coirpith : 0.148
non retted coirpith : 0.218

Volume reduction in retted coirpith : 0.14
non retted coirpith : 0.118

DISCUSSION

5. DISCUSSION

Coconut coirpith, the waste from coir industry which is available in significant quantities, is currently considered as a waste product because of its low burning quality and resistance to microbial degradation. The wider C : N ratio of 112 : 1 coupled with low nitrogen content, presence of soluble tannin related phenolic compounds (8-12 per cent), low and difficult biodegradability due to high lignin content are some of the problems associated with direct application of coirpith to the field crops (Fan *et al.*, 1982).

Among the coconut growing states in India, Kerala ranks first in area and production. During 1999 – 2000 Kerala's share in coconut production was 42.17 per cent (Rethinam and Thampan, 2002).

In India 7.5 million tonnes of coirpith is produced annually (Kamaraj, 1994) out of which 11 lakh metric tonne is from Kerala alone. One tonne of coirpith is produced from 10,000 nuts (Nagarajan *et al.*, 1985). According to Arumughan and Damodharan (1993) there are about 84,000 retting and coir extracting units in Kerala producing white fibre and about 650 brown coir units located in Tamil Nadu, Karnataka and Andhra Pradesh. At present the coir industries are facing problems of disposal of this waste. Therefore, an attempt has been made to convert it as compost for crop production.

Fungi belonging to class Hymenomycetes, especially mushroom fungi, which produce cellulolytic enzymes (Kannan and Oblisami, 1990) and lignolytic enzymes (Munoz *et al.*, 1997) are capable of degrading coirpith which is rich in cellulose and lignin.

In the present investigation, comparative efficiency of different lignocellulolytic fungi *viz.*, *Pleurotus eous, P. squarrosulus, P. sajor-caju, P. florida, P. florida* (Holland), two strains of *Calocybe indica,*

Schizophyllum commune and *Coprinus comatus* in degrading retted and non retted coirpith were studied along with its ability in reducing organic carbon, cellulose and lignin, narrowing down the C : N ratio and increasing the nutrient status.

Attempts were made to collect native strains of lignocellulolytic fungi *viz., P. eous, P. squarrosulus, C. indica, S. commune. Coprinus comatus* by conducting intensive survey during the monsoon periods. These native strains were identified and preliminary study was conducted in the laboratory to evaluate their efficiency in degrading coirpith. In addition to these native strains, mushroom cultures procured from AICRP Centre on Mushrooms, College of Agriculture, Vellayani were also included in the study.

Earlier workers have reported the occurrence of different strains of *Pleurotus* spp. from Kerala including those collected in the present investigation (Devi, 1982; Suharban, 1987; Balakrishnan, 1994; Anitha, 1998).

P. eous which was collected from dead stumps of cocoa tree during South West monsoon in the present study was collected earlier by Hooker and identified by Berkeley (1850). The detailed observations made in the present study are found to be in full agreement with the description of the species made by Purkayastha and Chandra (1985); Suharban (1987) and Anitha (1998).

P. squarrosulus previously described as *Lentinus subnudus* and subsequently as *L. squarrosulus* appear caespitose on logs and was collected from mango trees for the present study. The characters of this fungus was similar to those described by Devi (1982); Purkayastha and Chandra (1985); Suharban (1987).

C. indica strains 1 and 2 collected from Thiruvananthapuram district during South West monsoon for the present study were earlier reported

and studied by Purkayastha and Chandra (1985). The characters of these two strains did not differ markedly. However, they showed some variation in their ability to decompose coirpith.

S. commune collected during South West monsoon was included in the study. This mushroom was found to be distributed all over India. Watling and Gregory (1980) reported this fungus from Sanatnagar, Srinagar and Jammu-Tawi. The sporophores of this mushroom was found growing in groups on branches or trunks of trees, dead wood etc. They were coriaceous and attached laterally to the substratum. The morphological characters of this fungus isolated in the present investigation are similar to that reported by Purkayastha and Chandra (1985).

Coprinus comatus reported by Bose and Bose (1940) collected for the present study was from mushroom beds at the College of Agriculture, Vellayani. The detailed observations of *Coprinus comatus* made in the present study are found to be similar to the description of the species made by Purkayastha and Chandra (1985).

Eventhough certain species of lignocellulolytic fungi preferred certain media, in general, all these fungi preferred natural media and their growth in synthetic substrates were very poor. Among the natural media tested, all these fungi grew best in Oatmeal Agar (OA), Potato Dextrose Agar (PDA) and their growth in Yeast Extract Agar (Yeast Extract Agar) and Carrot Agar (CA) were less than that observed in these two media. The growth in YEA and CA was thin and superficial. Many of the earlier workers suggested PDA fortified with yeast extract (Jandaik and Kapoor, 1975; Rangad and Jandaik, 1977), OA (Suharban, 1987; Balakrishnan, 1994) for the growth of *Pleurotus* spp. None of the workers have suggested the use of synthetic media for the growth of lignocellulolytic fungi. The results of the present investigation also clearly indicate that

for growth and fruit-body production lignocellulolytic fungi prefer natural media.

Out of the nine lignocellulolytic fungi studied three preferred a temperature of 30°C, other three grew best at 35°C. *P. eous* and *P. florida* grew best at 25 and 30°C. *P. sajor-caju* gave the best growth at 25°C. None of these strains grew well above 35°C. Inability/poor growth of fungi at high temperature (40°C) was attributed to failure of methionine biosynthesis to keep pace with other processes (Cochrane, 1958). Observations of certain workers that *P. sajor-caju* preferred a temperature of 25°C for its growth is again confirmed by this study. The finding that all the *Pleurotus* spp. failed to grow at a temperature above 35°C is supported by previous studies. However, contradictory to the observation made by Rangad and Jandaik (1977) that *Pleurotus* spp. cannot grow at 35°C, in the present study, *P. squarrosulus* preferred a temperature of 35°C for its better growth. This difference may be due to the strain variation of the fungus studied by earlier workers and the species used in the present study. Confirmity exist in the present study to *Coprinus* spp. with the findings of Yungchang and Yee (1977) and Geetha (1982) that this fungus preferred a temperature of 35°C for its growth. *S. commune* preferred a temperature of 35°C for its growth. The two strains of *C. indica* grew best at 30°C. This is also confirmed by the findings of Chandra and Purkayastha (1977). These indicate that there are variations in the species regarding its ability to grow under different temperatures. This indirectly indicates that care should be taken while selecting species for decomposition of coirpith at different seasons of the year and in different locations having high / low temperature.

Under given conditions, growth of a fungus will be maximum over a certain range of initial pH values of the medium and will fail to grow at high and low extremes. In this study, all the fungi tested preferred neutral to acidic pH. *P. eous* and *P. florida* were able to grow at pH range of 5 and 6.

P. sajor-caju preferred a wide pH range of 4 to 6, while *C. indica* strains 1 and 2, *S. commune* and *Coprinus comatus* grew best at pH 6. *P. squarrosulus* preferred pH 7 for its optimum growth. The ideal pH requirement of *Pleurotus* spp. was reported differently by different workers. 5 to 7 (Chandra and Purkayastha, 1977), 5.5 (Suharban, 1987) and 6 (Balakrishnan, 1994). These findings clearly showed that wide variation exist among the *Pleurotus* spp. in their preference towards pH.

Retted and non retted coirpith collected from different locations were found to be acidic in nature (4.29 – 6.16). The EC of retted coirpith did not differ based on the place of collection, while marked variation was observed in non retted coirpith collected from different locations. The EC of non retted coirpith collected from Thenkasi was almost six times more than that from Vellayani. This may be due to inefficient leaching of the salts present in coirpith collected from Thenkasi. In Kerala, coirpith is exposed to continuous leaching by rain. This results in washing away of sizable quantity of salt present in it. This is one of the reasons for getting a low EC value for non retted coirpith collected from Vellayani. In the case of retted coirpith the EC value is still lower than non retted coirpith from Vellayani. This is because the salt content in the coirpith is removed by retting of husk in fresh water. Maximum water holding capacity of retted coirpith was found to be more compared to non retted coirpith.

Variations existed in the content of organic carbon, total nitrogen, phosphorus, potassium, cellulose, lignin and C : N ratio in raw coirpith samples collected from different places (Vazhamuttom, Thiruvallam, Vellayani and Thenkasi). Similar variations were reported by other workers also (Ravichandran, 1988; Biddappa *et al.*, 1998; Kadalli and Nair, 2000). The reason for such a high variation may depend on the fertility status of the coconut gardens, method of extraction, disposal, time of collection and other environmental factors. Also the coirpith obtained from fully mature and older nuts contains higher amount of cellulose and

lignin compared to younger nuts. A high quantity of phosphorus and potash in non retted coirpith may be attributed to loss of phosphorus in retted coirpith in the form of phosphoric acid and leaching of potash during retting. Similar observations were made by Sampson (1923) and Savithri and Khan (1994).

The physico-chemical properties of retted and non retted coirpith varied considerably as a result of decomposition. This variation was influenced by the lignocellulolytic fungi used for decomposition.

In the case of non retted coirpith a decrease in pH (5.75 to 5.10) was observed after composting. This may be due to the production of weak organic acids during the process of decomposition, whereas, in retted coirpith slight increase in pH was observed (4.86 to 5.26). The retting process may influence the initial period of decomposition and further accelerated decomposition and mineralisation by lignocellulolytic fungi may contribute to the release of basic cations. Apart from that slight production of ammonia during composting period may also raise the pH slightly (Anand, 1998).

EC of retted and non retted coirpith decreased after composting. In the present study the maximum reduction in EC of retted coirpith was 0.07 while non retted coirpith from Thenkasi was 0.55 dSm^{-1}. The reduction in EC may be due to the loss of soluble salts during composting.

The maximum water holding capacity of coirpith after composting was reduced. The reduction in retted coirpith ranged from 6.34 (*C. indica*-1) to 33.49 (*P. sajor-caju*) per cent, while in the non retted coirpith it was 30.77 (*P. florida*) to 48.22 (*P. sajor-caju*) per cent. Several factors can be attributed for this reduction. The fungal decomposition of coirpith reduces the cellulose considerably. Since cellulose can imbibe water several fold its weight degradation of cellulose during composting reduces the water holding capacity. Results of the field level trials revealed that *P. sajor-caju* in retted (51.38 per cent) and *P. eous* and

P. sajor-caju in non retted (50.04 per cent) coirpith brought about maximum degradation of cellulose. Similarly, lignin degradation was significantly higher in retted coirpith inoculated with *P. eous, P. sajor-caju* and *S. commune* (about 57 per cent) and non retted coirpith inoculated with *P. sajor-caju* and *P. eous* (about 62 per cent). There is a positive correlation between lignin and cellulose degradation. Lignin degradation increases the accessibility of native celluloses to cellulolytic enzymes and hence, greater the lignin degradation, more the cellulose degradation (Saxena and Rai, 1992). Similarly a positive correlation also exist between degradation of cellulose and lignin and maximum water holding capacity. Least water holding capacity was observed in treatments where maximum lignin and cellulose degradation had happened. Another factor which causes reduction in water holding capacity is the reduction in organic matter content due to mineralisation. Changes in the organic carbon content is an indirect estimate of organic matter content (Organic carbon x 1.724). Maximum reduction in the organic carbon content in both retted and non retted coirpith was 33 to 34 per cent (*P. sajor-caju* and *P. eous*) and 38 to 47 per cent (*P. eous, S. commune, C. indica*-2 and *P. sajor caju*). This also explains why the water holding capacity of retted and non retted coirpith decomposed by *P. sajor-caju* is low. Anand (1998) and Amlan and Suseeladevi (2001) also opined that the decrease in water holding capacity of coirpith may be due to degradation of cellulose materials and decrease in organic matter content due to mineralisation.

The nutrient status of coirpith especially nitrogen, phosphorus and potassium were increased as a result of decomposition by lignocellulolytic fungi. About 30 per cent reduction in volume of coirpith was observed after composting using the lignocellulolytic fungi, which may result in an increased nutrient concentration in the final product. The presence of these lignocellulolytic fungi and the enzymes produced by them may also contribute to enhance nutrient content in the compost. Apart from these

reasons the nitrogen fixing ability of higher fungi may also contribute to the increased nitrogen content of composted coirpith. The nitrogen fixing ability of *P. sajor-caju* and other higher fungi have been reported by Rangaswamy *et al.* (1975) and Thayumanan (1979, 1980).

C : N ratio of coirpith inoculated with lignocellulolytic fungi decreased after composting. This is because of the oxidation of the organic carbon present in organic matter. Carbon content is reduced thereby lowering the C : N ratio of the material.

The results of this study clearly indicate that composting of retted and non retted coirpith increases the physico-chemical properties of the coirpith and make it an ideal material for incorporation into the soil. The decomposing ability of different lignocellulolytic fungi varied depending upon the place of collection of coirpith, type of coirpith (retted or non retted), period of composting and temperature of the environment. Among the different fungi tried for decomposing coirpith, *P. eous*, *P. sajor-caju* and *S. commune* were found to be better than other species tried. Hence under Kerala conditions these three fungi can be used for effective decomposition of coirpith.

SUMMARY

6. SUMMARY

The study entitled "Comparative efficiency of lignocellulolytic fungi for bioconversion of coirpith" was conducted in Thiruvananthapuram district of Kerala State. Six lignocellulolytic mushrooms *viz.*, *Pleurotus eous, P. squarrosulus,* two strains of *Calocybe indica, Coprinus comatus, Schizophyllum commune* collected from different parts of Thiruvananthapuram and three isolates of *P. sajor-caju, P. florida* and *P. florida* (Holland) obtained from AICRP centre on mushrooms, College of Agriculture, Vellayani were included in the study.

In vitro studies on growth of these mushrooms on different solid media, at different temperature and pH levels revealed that all the nine isolates preferred natural media like Oatmeal Agar and PDA. Their growth on synthetic substrates were very poor.

All the fungi studied preferred a temperature range of 25-35°C for their optimum growth and then drastically reduced at temperature above 35°C.

P. eous and *P. florida* were able to grow at pH 5 and 6, while pH 6 was preferred by two strains of *C. indica, S. commune* and *Coprinus comatus*. Best growth of *P. squarrosulus* was noticed at neutral pH. *P. florida* (Holland) preferred pH 4 for its optimum growth. *P. sajor-caju* grew best at a wide pH range of 4 to 6

In vitro studies were carried out using these nine lignocellulolytic fungi and their ability in degrading organic carbon, cellulose and lignin, narrowing down the C : N ratio and increasing the nutrient status were found out. From these observations six efficient strains *viz., P. eous, P. squarrosulus, P. sajor-caju, P. florida, C. indica-2 and S. commune* were selected and used for field level trials. Physico-chemical analysis of composted samples were also done. Composting increased the pH of

retted coirpith and reduced that of non retted coirpith. A decrease in EC and maximum water holding capacity was noticed after composting. Maximum reduction in organic carbon was noticed in non retted coirpith inoculated with *P. eous* and *S. commune* after 45 days. Maximum nitrogen content was observed when retted coirpith inoculated with *P. eous* and *S. commune* for 45 days. Significantly lower levels of C : N ratio was observed in non retted coirpith after 45th day of incubation with *P. eous*, *P. sajor-caju* and *S. commune*.

The highest cellulose degradation was observed in *P. sajor-caju* and *P. eous* inoculated retted coirpith after 45 days of incubation. Maximum lignin degradation and increase in total potassium was noticed in non retted coirpith inoculated with *P. sajor-caju* and *P. eous* after 45 days of incubation. The total phosphorus content of coirpith compost was found to be maximum in non retted coirpith inoculated with *P. eous*, *C. indica*-2 after 30 days and *S. commune* and *P. squarrosulus* after 45 days of incubation.

P. eous brought about the maximum reduction in weight of retted and non retted coirpith after 45th day of incubation while maximum reduction in volume of retted and non retted coirpith was noticed in coirpith inoculated with *P. eous* and *S. commune* respectively.

Raw retted and non retted coirpith has wide C : N ratio (96.23 : 1 and 75.82 : 1), high content of organic carbon, cellulose, lignin and low nutrient status. Composting with lignocellulolytic fungi narrowed the C : N ratio (19.73 : 1 an 13.54 : 1), reduced the organic carbon, cellulose, lignin and increased the total nitrogen, phosphorus and potassium. The final compost obtained after decomposition was dark brown in colour and of very fine texture.

Based on the experiment *P. eous*, *S. commune* and *P. sajor-caju* were considered as ideal lignocellulolytic fungi capable of decomposing retted and non retted coirpith.

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*Original not seen

APPENDICES

APPENDIX – I

Collection No. ..... Date of collection .....

Collected by .....

1. General
   - Locality
   - Habitat
   - Any other details

2. Pileus

   Colour ..... Diameter .....

   Shape : immature mature .....

   Texture : soft, brittle, fleshy, coriaceous, membranous

   Surface : dry, moist, greasy, smooth, downy, velvety, shaggy, peeling out easily or not.

   Margin : Regular, wavy, smooth, rough, furrowed, incurved or not/striate or not

   Veil : Present/absent Colour
   abundant, scarce,
   appendiculate/membranous

   Chemical
   Reaction : amyloid/non-amyloid/dextrnoid

3. Gills/Pores/Teeth
   - Colour
   - Arrangement : remote/free/adnate/adnexed/sinuate crowded or distant, easily separable from pilear tissue or not.
   - Consistency : Pliable/brittle/waxy/fleshy
   - Size : no/cm.
   - Gill edge : Special features if any

4. Stipe

   Position : Central/eccentric/sessile

   Colour :

   Size : Length ..... diameter .....

Consistency : fleshy/leathery/woody
Surface

Characters : fibrillose/dry/viscid
Pubscent/squamose/glabrous

Annulus : present/absent size ..... single/double
Membranous/filamentous

Position : apical, medial, basal

Volva : present/absent
Shape
Colour

Texture : fleshy/tough/papery

5. Flesh

When wet When dry

Colour in pileus
Colour in stipe
Changes in colour when exposed to air

6. Others

Presence of abnormal liquid/milkyfluid/others
before cutting/after cutting
Any other character

Macro characters

1. Basidia

Size
Shape
No. of sterigmata : 2/4

2. Basidiospores

Colour
Size
Shape : apiculate/arcuate/bullet like/
Cylindrical/echinulate/elliptical/ fusiform/
Globose/ovate/reticulate

Chemical reactions : Cyanophilous / acyanophilous/
amyloid/dextrinoid
non-amyloid

3. Cystidia

Present/absent
Gill edge/
Gill medium/
on pileus/
stipe

Shape :
Size :
Nature : thin walled coloured/hyaline

4. Others

Spore print
Any other details

Specimen identified as :

APPENDIX – II

Composition of the media and reagents used for the study

1. Potato Dextrose Agar

Potato	-	200 g
Dextrose	-	20 g
Agar	-	15 g
Distilled water	-	1000 ml
pH	-	6 to 6.5

2. Carrot Agar

Carrot	-	200 g
Dextrose	-	20 g
Agar	-	15 g
Distilled water	-	1000 ml
pH	-	6 to 6.5

3. Oat Meal Agar

Oats	-	100 g
Agar	-	15 g
Distilled water	-	1000 ml
pH	-	6 to 6.5

4. Yeast Extract Agar

Yeast extract	-	7.5 g
Agar	-	15 g
Distilled water	-	1000 ml
pH	-	6 to 6.5

5. Czapek's Dox Agar

Sucrose	-	30 g
Sodium nitrate	-	2 g
Dipotassium phosphate	-	1 g
Magnesium sulphate	-	0.5 g

Potassium chloride	-	0.5 g
Ferrous sulphate	-	0.01 g
Agar	-	15 g
Distilled water	-	1000 ml

6. Richards' medium

Potassium nitrate	-	10 g
Potassium dihydrogen phosphate	-	5 g
Magnesium sulphate	-	2.5 g
Ferric chloride	-	0.02 g
Sucrose	-	50 g
Agar	-	15 g
Distilled water	-	1000 ml
pH	-	6.6 to 7.2

Reagents and stains

1. Melzer's reagent (Melzer, 1934)

Potassium iodide	-	1.5 g
Iodine	-	0.5 g
Water	-	20 ml
Chloral hydrate	-	22 g

2. Lactophenol cotton blue

Anhydrous lactophenol	-	67 ml
Distilled water	-	20 ml
Cotton blue	-	0.1 g

APPENDIX – III

Caespitose	–	In groups or tufts like grass
Imbricate	–	Partly covering one another like the tiles on a roof
Spathulate	–	Like a spoon in form
Flabelliform	–	Like a fan; in the form of a half circle
Coriaceous	–	Like leather in texture
Decurrent	–	Running down the stipe
Infundibuliform	–	Funnel like
Squamose	–	Having scales
Squarrose	–	Rough with scales
Attenuate	–	Narrowed
Tomentose	–	Having a covering of soft, matted hairs. downy
Concolorous	–	Of one colour
Campanulate	–	Bell like in form
Hygrophanous	–	Having a water soaked appearance when wet
Clavate	–	Club like
Lamellulae	–	A small lamella which runs from the edge of the pileus towards the stipe
Trama	–	The tissue lying between two hymenial layer, usually consisting of densely packed or loosely interwoven hyphae
Appressed	–	Closely flattened down
Amyloid	–	Colour reaction with Melzer's reagent – black or slightly greyish if amyloid, brown to purplish brown when pseudoamyloid, yellowish if non amyloid

COMPARATIVE EFFICIENCY OF LIGNOCELLULOLYTIC FUNGI FOR BIOCONVERSION OF COIRPITH

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8. ABSTRACT

Nine lignocellulolytic fungi, of which six *viz.*, *Pleurotus eous*, *P. squarrosulus*, two strains of *Calocybe indica*, *Schizophyllum commune* and *Coprinus comatus* collected from Thiruvananthapuram district as a part of the study entitled "Comparative efficiency of lignocellulolytic fungi for bioconversion of coirpith" and three from AICRP Centre on Mushrooms, College of Agriculture, Vellayani were used to find out their ability to decompose coirpith.

All these fungi preferred natural media such as Oatmeal Agar and Potato Dextrose Agar, a temperature range of 25 - 35°C and neutral to acidic pH for their optimum growth.

In vitro studies were conducted to find out the efficient strains. Pre and post composting physico-chemical analysis were carried out. Coirpith was found to be acidic in nature. EC of non retted coirpith was very high. Ability of these fungi in degrading organic carbon, cellulose and lignin thereby narrowing down the C : N ratio and increasing the nutrient status were found out.

Field level trials using *P. eous*, *P. squarrosulus*, *P. sajor-caju*, *P. florida*, *C. indica*-2 and *S. commune* revealed that all these fungi could efficiently degrade coirpith. Better degradation of retted and non retted coirpith was observed when they were inoculated with lignocellulolytic fungi for 45 days.

Maximum reduction in organic carbon and C : N ratio was brought about by *P. eous* and *S. commune* in non retted coirpith. Higher concentration of nitrogen was observed in retted coirpith inoculated with the same fungi.

The phosphorus content was maximum in non retted coirpith inoculated with *P. eous*, *C. indica*-2, *S. commune* and *P. squarrosulus*.