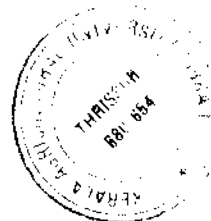
172187



UTILIZATION OF FUNGI FOR COMPOSTING AND MUSHROOM PRODUCTION ON COIRPITH

SHERIN. A. SALAM

Thesis submitted in partial fulfilment of the requirement
for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

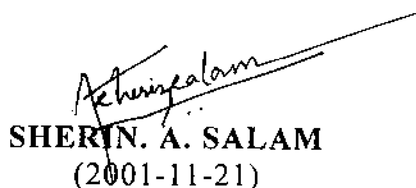
2003

Department of Plant Pathology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM 695522

DECLARATION

I hereby declare that this thesis entitled "**Utilization of fungi for composting and mushroom production on 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.

Vellayani,
17-09-2003.



SHERIN. A. SALAM
(2001-11-21)

CERTIFICATE

Certified that this thesis entitled "**Utilization of fungi for composting and mushroom production on coirpith**" is a record of research work done independently by Ms. Sherin. A. Salam (2001-11-21) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Vellayani,
17-09-2003.

Dr. (Mrs.) D. GEETHA
(Chairperson, Advisory Committee)
Associate Professor,
Instructional Farm,
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522.

Approved by

Chairperson :

Dr. (Mrs.) D. GEETHA
Associate Professor,
Instructional Farm,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

Members:

Dr. C.K. PEETHAMBARAN
Professor and Head,
Department of Plant Pathology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

Dr. M. SUHARBAN
Associate Professor,
Instructional Farm,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

Dr. K. HARIKRISHNAN NAIR
Associate Professor and Head,
Instructional Farm,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

External Examiner : 01/11/03

Dr. K. SEETHARAMAN, Ph.D.,
Professor & ~~Head~~
Department of Plant Pathology
Agrl. College & Res. Institute
MADURAI - 625 104

To

My

Umma and Vappicha

For all that I am or hope to be . . . .

ACKNOWLEDGEMENT

I would like to express my deep sense of gratitude and indebtedness to:

Dr. (Mrs.) D. Geetha, Associate Professor, Instructional Farm, Chairperson, Advisory Committee for her expert and affectionate guidance, friendly approach, constant encouragement, sustained interest, ever willing help, constructive criticism, valuable suggestions, unflinging support and constant supervision throughout the study, research and preparation of thesis which enabled me to complete this work successfully.

Dr. C.K. Peethambaran, Professor and Head, Department of Plant Pathology, for the sincere, keen, ever willing interest and enthusiastic approach shown in my work and spending his valuable time inspite of the busy schedule by initial scrutiny and evaluation of the thesis, giving valuable suggestions with utmost patience.

Dr. M. Suharban, Associate Professor, Instructional Farm and member of Advisory Committee for her valuable suggestions, co-operation, constant encouragement and enthusiastic approach throughout the study.

Dr. K. Hariksirhnan Nair, Professor and Head, Instructional Farm and member of Advisory Committee for his keen interest, constant encouragement and co-operation throughout the period of study.

Dr. S.K. Nair, Professor, Department of Plant Pathology, for his valuable suggestions, constant criticism and timely help throughout the course of research.

Dr. C. Gokulapalan, for the friendly approach, critical suggestions, constant encouragement throughout the course of investigation and unforgettable help rendered in taking photographs amidst his busy schedule.

Dr. Lulu Das (Associate Professor), Dr. K.S. Meenakumari (Assistant Professor), Dr. K. Umamaheswaran (Assistant Professor), Department of Plant Pathology for their constant support, encouragement and help during the course of study.

All the teaching and non-teaching members of Department of Plant Pathology for their co-operation throughout the study.

Dr. V.K. Venugopal, Professor and Head, Dr. K.R. Sudarmaidevi and Dr. Manorama Thampatti, Department of Soil Science and Agricultural Chemistry for their ever willing help and constant encouragement during chemical analysis.

Mr. C.E. Ajithkumar, Programmer, Department of Agricultural Statistics for his help in analysing the data.

Dr. Peter Roberts, Mushroom Taxonomist, Royal Botanic Garden, England for identifying the mushrooms.

Ajith for his moral support, timely advice, constant encouragement, valuable suggestions and unforgettable help without which my dream for post graduation might not be fruitful.

Priya, Puli, Deepthi, Hima, Sangeetha, Bikri, Lekha, Ancy, Senthil, Rajitha, Reeja, Subramanian, Jeena, Bini, K., Dhanya chechi, Priya chechi, Heera chechi, Sreekala chechi, Resmi, Rinichechi and all other junior and senior friends and Appoos for their wholehearted support and valuable help for my thesis work.

Biju of ARDRA for the neat and timely preparation of thesis.

Vappicha, Umma, Sanikka, Shermi, Sanju, Suroor, Kollathumma and all other relatives for their unbound love, support and constant encouragement without which I would not have pursued this work successfully.

STED for giving financial assistance and fellowship for my thesis work.

Kerala Agricultural University for giving necessary facilities for the conduct of research work.

Above all I bow my head before God Almighty who gave me the courage, patience, mental strength and persistence to finish my work in time.

Sherin A. Salam

(2001-11-21)

CONTENTS

	Page No.
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	3
3. MATERIALS AND METHODS	25
4. RESULTS	33
5. DISCUSSION	57
6. SUMMARY	66
7. REFERENCES	68
APPENDICES	
ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1	Effect of retted, non-retted and composted coirpith on yield of oyster and milky mushroom	29
2	Fungi isolated from coirpith	33
3	Mushrooms obtained during survey	35
4	Mushroom flora used in the present study	35
5	Growth of lignocellulolytic fungi on retted coirpith	38
6	Effect of lignocellulolytic fungi on the organic carbon, total nitrogen and C:N ratio of retted coirpith	40
7	Effect of lignocellulolytic fungi on the cellulose and lignin content of retted coirpith	42
8	Efficacy of retted coirpith on oyster mushroom production	44
9	Efficacy of non-retted coirpith on oyster mushroom production	46
10	Efficacy of composted coirpith on oyster mushroom production	48
11	Correlation between retted and non-retted coirpith on oyster mushroom production	49
12	Efficacy of retted coirpith on milky mushroom production	51
13	Efficacy of non-retted coirpith on milky mushroom production	53
14	Efficacy of composted coirpith on milky mushroom production	54
15	Correlation between retted and non-retted coirpith on milky mushroom production	55

LIST OF FIGURES

Figure No.	Title	Between pages
1	Growth of lignocellulolytic fungi on retted coirpith	38-39
2	Effect of lignocellulolytic fungi on organic carbon content	40-41
3	Effect of lignocellulolytic fungi on total nitrogen content	40-41
4	Effect of lignocellulolytic fungi on C:N ratio	40-41
5	Effect of lignocellulolytic fungi on cellulose and lignin content	42-43
6	Efficacy of retted coirpith on oyster mushroom production	44-45
7	Efficacy of non-retted coirpith on oyster mushroom production	46-47
8	Efficacy of composted coirpith on oyster mushroom production	47-48
9	Correlation between retted and non retted coirpith on oyster mushroom production	49-50
10	Efficacy of retted coirpith on milky mushroom production	51-52
11	Efficacy of non-retted coirpith on milky mushroom production	53-54
12	Efficacy of composted coirpith on milky mushroom production	53-54
13	Correlation between retted and non retted coirpith on milky mushroom production	55-56

LIST OF PLATES

Plate No.	Title	Between pages
1	Cultivation trials on Oyster mushroom (*P. florida*)	31-32
2	Cultivation trials on Milky mushroom (*C. indica*)	31-32
3	*Pycnoporus sanguineus* on coconut stump	34-35
4	*G. applanatum* on coconut stump	34-35
5	*Pleurotus tuber-regium* on mango tree stump	34-35
6	Spawn of *P. tuber-regium*	37-38
7	Sporophore of *P. tuber-regium* on paddy straw beds	37-38
8a	Growth of *T. harzianum* on RCP	39-40
8b	Growth of *P. sanguineus* on RCP	39-40
8c	Growth of *G. applanatum* on RCP	39-40
8d	Growth of *C. indica* on RCP	39-40
9	Spawn of *P. florida*	43-44
10	Spawn of *C. indica*	43-44
11	Yield of *P. florida* on RCP + SMS (1:1) compared to control	45-46
12	Yield of *P. florida* on NRCP + SMS (1:1) compared to control	45-46
13	Yield of *P. florida* on NRCP + PS (1:3) compared to control	47-48
14	Yield of *P. florida* on NRCP + SMS (1:3) compared to control	47-48
15	Yield of *P. florida* on CCP + PS (1:3) compared to control	47-48
16	Yield of *C. indica* on RCP + PS (1:3) compared to control	50-51
17	Yield of *C. indica* on NRCP + PS (1:3) compared to control	50-51
18	Yield of *C. indica* on CCP + neem cake (four per cent) compared to control	50-51

LIST OF APPENDICES

Sl. No.	Title	Appendix No.
1	Key to the identification of mushrooms	I
2	Composition of the media used for the study	II
3	Glossary	III

LIST OF ABBREVIATIONS

%	per cent
μg	Micro gram
μm	Micro metre
^{0}C	Degree Celsius
AICMIP	All India Coordinated Mushroom Improvement Project
AICRP	All India Coordinated Research Project
C : N ratio	Carbon : Nitrogen ratio
CCP	Composted coirpith
CD	Critical difference
cm	Centimetre
et al.	And others
Fig.	Figure
g	Gram
h	Hour
kg	Kilogram
l	Litre
mg	Milligram
ml	Millilitre
mm	Millimetre
N	Nitrogen
NC	Neem cake
NRCP	Non-retted coirpith
ppm	Parts per million
PS	Paddy Straw
RCP	Retted coirpith
SMS	Spent mushroom substrate
sp.	Species
t	Tonnes
TNAU	Tamil Nadu Agricultural University
viz.	Namely

INTRODUCTION

1. INTRODUCTION

About 357 million tonnes of biodegradable agrowastes are being generated every year in India (Tewari and Pandey, 2002). This include different straws, sugarcane bagasse, cotton waste, jute, coirpith, cocoa pods, different oil cakes, groundnut pod shell, tapioca starch wastes, banana wastes and other market wastes (Jandaik, 2002). Out of these, 170 million tonnes are left out for burning or cause environmental pollution, which can be avoided to some extent by bioremediation. Bioremediation is the biological process of degradation of contaminated substrates using specific microorganisms.

In India about 7.5 million tonnes of coirpith is being produced annually (Kamaraj, 1994), out of which major contribution is from Kerala. The nutrient value of coirpith is very low and as such it cannot be used as manure. Further it decomposes only very slowly in soil because of its very high C : N ratio and high tannins and phenolic compounds. Studies (Owseph, 1999; Reeja, 2002) have indicated the possibility of converting coirpith into useful biomass through bioremediation. Coirpith can also be used as a medium for mushroom production thereby reducing the cost of cultivation of mushroom.

Mushrooms represent an efficient group of microorganisms gifted with the unique ability to degrade cellulose, hemicellulose and lignin for producing its fruiting bodies. There are more than 2000 edible species of mushroom of which 300 species belonging to 70 genera are reported from India. However, only a few have been brought under cultivation on a commercial scale. In south India, oyster mushroom and milky mushroom are the two commonly cultivated types.

For cultivation of mushrooms paddy straw, the most abundant agrowaste in India is being used as the substrate. But in Kerala unlike in

REVIEW OF LITERATURE

other parts of India paddy straw is costly resulting in increased cost of cultivation. Inorder to make mushroom cultivation profitable in Kerala, it is necessary to use a substrate which is cheap and readily available. Coirpith is one such substrate. Considering the above aspects, the present study was undertaken with the following objectives.

1. Isolation of lignocellulolytic fungi from native coirpith
2. Collection of wood inhabiting mushroom flora and isolation into pure culture
3. Screening of the isolated cultures for selecting the most efficient fungi for degradation
4. Standardization of techniques for mushroom production on coir pith

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 SURVEY

2.1.1 *Pleurotus* sp.

Nature, with varied agro climatic conditions favour growth of a number of mushrooms – edible as well as poisonous .The genus *Pleurotus* was first established by Fries in 1821. Berkeley in his survey of Indian sub-continent (1850, 1852, 1854) reported nine species of *Pleurotus viz.*, *P. anserinus* Berk on dead wood from Jallapahar, Darjeeling, *P. dryinus* (Pers.) Fr. on standing tree from Kashmir, *P. eous* (Berk.) Sacc. and *P. hapalosclerus* Berk. on tree trunks from Darjeeling, *P. ninguidus* Berk. on dead timber from Sikkim, *P. petaloides* (Bull.) Fr. on dead wood from Nepal, *P. placentodes* Berk. from Birchwood, Sikkim; *P. salignus* (Pers.) Fr. from Sikkim and *P. verrucaris* Berk. on dead wood from Darjeeling. The pioneering study by Berkeley was followed by Cooke (1888) who collected and identified *P. platypus* Cooke and Massee from tree trunks from Nepal. *P. sajor-caju* (Fr.) Singer was reported from India at the beginning of 20th century (1904–1919) by Llyod (Butler and Bisby, 1931) and by Bose (1920). This mushroom was reported from Kerala by Natarajan (1978).

According to Pegler (1976) and Singer (1986) the genus *Pleurotus* is known to contain 50 species. Of these, nearly 25 species were known to occur in India-Nepal areas (Pegler, 1976; Bilgrami *et al.*, 1979 and Sarbhoy *et al.*, 1986). Among these, ten species such as *P. anserinus* (Berk.) Sacc., *P. eous* (Berk.) Sacc; *P. flabellatus* (Berk. & Br.) Sacc., *P. fossulatus* (Cooke) Sacc., *P. aff. gemmellaii* (Inzeg) Sacc., *P. membraneous* Massee, *P. ninguidus* (Berk.) Sacc., *P. ostreatus* (Jacq. Fr.) Kummer; *P. placentodes* (Berk.) Sacc. and *P. platypus* (Cooke & Massee) Sacc. were included in his monograph (Pegler,1976) and described them properly.

Bhavani Devi (1982) recorded five species of *Pleurotus* from Kerala. They were *P. cornucopiae* (Paulet. Pers) Rolland, *P. ostreatus* (Jacq. Fr.) Kummer., *P. platypus* (Cooke & Massee) Sacc., *P. spathulatus* Pent. and *P. squarrosulus* Mont. A new white mushroom *P. citrinopileatus* was collected from lower Palani hills of Tamil Nadu and the cultivation technique was standardised by Sivaprakasam *et al.* (1986). Abraham (1991) recorded seven species of Pleurotus from different places in Kashmir and these were *P. cornucopiae*, *P. dryinus*, *P. fossulatus*, *P. flabellatus*, *P. membraneous* and *P. platypus*. In the same year, Marimuthu *et al.* (1991) reported *P. platypus* on a dead silk cotton tree from Tamil Nadu. Dessai *et al.* (1991) collected pink mushroom *P. eous*, which were found growing in clusters on dead wood of hyga tree in Tumbkhane forest, Karnataka. *P. tuber-regium* (Fr.) Singer was first reported by Fries in 1821 and placed under the genus *Lentinus* but Singer (1986) recombined it into *Pleurotus* .The most complete literature of *P. tuber-regium* was from Nigeria where sclerotia and basidiomata were eaten and attempts were made to commercialise the fungus. Fresh mushrooms and sclerotia are valuable in the traditional medical practice in Nigeria (Isikhuemhen and Okhuoya, 1998). It is pan- tropical in distribution and is found growing on decaying logs in the forest (Okhuoya *et al.*; 1998). Tubers of *P. tuber-regium* were produced in the laboratory when the fungus was cultured in moist straws of wheat, sugarcane leaves, sawdust, wheat bran and rice bran. The resulting tubers were viable and produce fruit bodies when buried in soil or kept in polythene bags in high humid conditions (Doshi and Sharma, 2001).

Suharban *et al.* (2002) reported the collection, identification and description of twenty species of Pleurotus from Kerala among which *P. citrinopileatus*, *P. eous*, *P. flabellatus*, *P. opuntiae* and *P. platypus* were cultivated artificially. Kushwaha *et al.* (2002) collected more than 15 species of wild fleshy fungi from forests of Uttaranchal, India which included *Pleurotus*, *Auricularia*, *Lycoperdon*, *Ganoderma* and *Amanita*.

2.1.2 *Calocybe indica* (P.&C.)

C. indica was first reported from India by Purkayastha and Chandra in1974. In rainy seasons, wild forms of *C. indica* were sold in Calcutta and their edibility was confirmed by Purkayastha and Chandra (1974)and Purkayastha and Nayak (1979). Natural occurrence of *C. indica* in plains of Tamil Nadu and Rajasthan has also been reported (Doshi *et al.*, 1989). The characters of *C. indica* were described by Purkayastha and Chandra (1985) as solitary growth of sporophore in soil, robust size and centrally stipulate, white or pale coloured with base not hollow, without annulus and volva and flesh white. Krishnamoorthy *et al.* (1997) identified a potential strain of *C. indica* occurring on sugarcane field near Coimbatore.

Krishnamoorthy and Nakkeeran (2002) conducted a survey to study the biodiversity and habitat of occurrence of *C. indica* belonging to Tricholomataceae family. Seven strains were collected from different locations. Out of seven isolates, two were found at the base of coconut tree, two occurred in paddy field bund and one each at the base of banyan tree, arecanut tree and in banana field. Anandh and Prakasam (2003) reported the occurrence of *C. indica* and *C. gambosa* (Fr.) Singh during a survey conducted in the Western Ghats. The sporophores of *C. indica* were robust, sometimes smaller, usually centrally stipulate and white coloured. This species was reported to be very close to *C. gambosa* differing in the slightly larger, broadly ellipsoid basidiospores and more robust and solitary sporocarps.

2.1.3 *Lentinus* spp.

L. edodes, shiitake mushrooms are native to Japan, China and other Asian countries and typically grow on fallen broad leaved trees. It is the second most important mushroom in the world and is specially treasured for its medicinal properties. Its cultivation was first undertaken around 1000-1100 AD (Chang and Miles, 1989). Manjula (1983) developed a key

to the Indian species including the commonly occurring species like *L. squarrosulus*, *L. subdulcis* and *L. connatus*. Most of *Lentinus* spp. are lignolytic in nature and about 14 species have been reported all over the world (Singer,1986). Geetha (1994) reported from oilpalm bunch wastes, the occurrence of *L. squarrosulus* and *L. subdulcis* from Anchal in Kollam district of Kerala state and standardised cultivation technology of *L. squarrosulus*. Upadhyay and Verma (2000) collected *L. squarrosulus* from Bhutan and standardised technology for cultivation on cereal straw and reported a biological efficiency of 48-52 per cent after cultivating it in chemically sterilized paddy straw. Kaur and Lakhanpal (2001) standardised the cultivation of *L. edodes* on indigenous substrates in India.

2.1.4 *Ganoderma* spp.

G. applanatum was first described by Findlay in 1977 and later by Pacioni in 1985, which was found growing on a wide variety of broad leaved trees and fallen trunks of the less durable timbers. The bracket shaped fructification reached a size of 40 centimetres or more. These are hard with lumpy crust and reddish brown spores. The pore surface turns brown when scratched. *G. lucidum* was easily recognized by its rounded, polished lateral stalk which, may be much longer than the rather small kidney shaped cap. It is generally found at the ground level growing out from decayed stumps of hard wood trees.

2.1.5 *Pycnoporus cinnabarinus*

P. cinnabarinus was first described by Pacioni in 1985 as bracket shaped, sessile, deep orange–red carpophore, about 3-6 centimetre, at first slightly pubescent, then glabrous, fairly rugose with faint zonations towards margin. This mushroom can be easily identified by its bright cinnabar red colour.

2.2 CULTIVATION

2.2.1 Cultivation of Oyster Mushroom

2.2.1.1 Substrates

Cultivation of *Pleurotus* spp. on natural habitat like tree stumps and logs was first described at the beginning of twentieth century (Falck, 1917). The foundation for the commercial production of *Pleurotus* on different substrates was laid by several workers (Kalberer and Vogel, 1974; Zadrazil, 1974 and Kurtzman, 1979). In India, the poly bag technology for the production of oyster mushroom on paddy straw was standardised by Baskaran *et al.* (1978).

Chandramohanan and Moorthy (1991) used both freshly fallen areca leaves and leaf sheath alone as substrates for cultivation of *P. sajor-caju* and obtained an average yield of 344 g and 468 g respectively per four kg wet weight of the substrates. Kochu Babu and Nair (1991) recorded oil palm mesocarp as an ideal substrate for *Pleurotus* cultivation and obtained a biological efficiency of 58.4 per cent. Kothandaraman *et al.* (1991) reported the feasibilities of growing oyster mushroom (*P. florida*) on rubber wood sawdust and observed a biological efficiency of 88.80 per cent compared to 50.80 per cent in paddy straw. According to Mathew *et al.* (1991) rubber wastes obtained after processing could be used as a substrate for growing oyster mushroom giving a biological efficiency of 44 to 45 per cent. Pandey and Tewari (1991) obtained very poor yield with distorted and small sporophores when tea waste alone was used for growing *Pleurotus,* while they got better growth when tea waste and coffee pulp in combination with paddy straw was used as substrate. They could enhance the growth further by spraying tea waste extract at various concentrations. Coffee pulp when mixed with 25, 50 and 75 per cent paddy straw, recorded significantly higher yield compared to paddy straw alone.

Rao (1991) tried various locally available substrates both alone and in combination with paddy straw and found that karad hay gave high production rate followed by paddy straw, sugarcane bagasse and banana plant residue.

Rawal *et al.* (1991) used chopped maize straw, which was found superior to jowar straw, sugarcane bagasse and sawdust. According to Singh and Dwivedi (1991) silk cotton wood chips and banana pseudostem were suitable substrates for mushroom cultivation. Sharma and Jandaik (1991) reported the yield response of *P. ostreatus* and *P. florida* on spent straw and spent compost. They could obtain the maximum biological efficiency of 78.22 and 75.55 per cent respectively in the combination of wheat straw and spent straw at 3:1 ratio. Suharban and Nair (1991) studied the cultivation of oyster mushroom on wooden logs of 16 trees of Kerala and found that mango tree log yielded the maximum followed by cashew tree. Singh and Singh (1991) reported that a combination of soybean and wheat straw (1:1 ratio) supported better growth compared to paddy and wheat straw alone. According to Kadari (1994) *P. squarrosulus* cultivation can be done on rice straw, maize straw and sorghum waste. Mathai and Suharban (1994) studied the cultivation of oyster mushroom on weed substrate *Eliocharis* and paddy straw and found that rate of spawn run and yield were higher in the case of weed substrate.

Bhatia and Suhag(1997) reported cotton waste and gram straw to be better than paddy straw in terms of production of fruiting bodies by oyster mushroom. The utilization of wild grass in combination with paddy straw as a potential substrate for oyster mushroom production compared to using the substrates alone was reported by Das *et al.* (1997) and Jacob and Abraham (1998). Thomas *et al.* (1997) studied the oyster mushroom production on leaf stalk and bunch waste of coconut, which were found to be superior to leaflets and coirpith and produced significant biological efficiency of 58.9 per cent and 56.9 per cent respectively. Similar

observations were also made by Thomas and Rajagopal (2003) and they reported that mushroom yield was increased significantly (upto 63 per cent) when fermented coirpith was used. Owseph *et al.* (2003) reported the maximum yield of *Pleurotus* spp. on paddy straw followed by non-retted coirpith. While studying the comparative efficacy of different varieties of banana pseudostem, Suharban *et al.* (1997) reported that pseudostem of red banana supported the highest yield of 1966 g/kg in the case of oyster mushroom.

Chandrasekhar and Savalgi (2003) studied the feasibility of growing oyster mushroom (*P. sajor-caju* (Fr.) Singer) on sugarcane byproducts. He obtained a biological efficiency of 73 per cent from sugarcane trash and milled bagasse combination followed by milled bagasse alone (72 per cent) compared to paddy straw (88 per cent).

Gogoi and Adhikary (2002) evaluated the bio-efficacy of non-conventional substrates for oyster mushroom (*P. citrinopileatus*) production and obtained the highest yield in turmeric leaves (93.75 per cent biological efficiency), eventhough it was less superior to paddy straw (95.83 per cent). Lowest biological efficiency was recorded with tea leaves (58 per cent). Singh (2002) studied yield performance of *P. florida* on various sizes of cereal residues and found that paddy straw out yielded wheat straw and white powdered form of substrate producing uniform and more flushes than full sized and 3-4" sized straw. According to Vadivel *et al.* (2002) *P. sajor-caju* has the potential in recycling agrowaste materials and reported its growth on a variety of agrowastes. They obtained maximum yield in paddy straw followed by paddy husk, green gram hay and black gram hay.

2.2.1.2 Supplements

According to Seth (1976) supplementation of organic amendments such as wheat bran, cotton meal, brewer's grain, wood dust and chicken

manure on paddy straw at 14 per cent level enhanced the yield of mushroom by 6 – 8 per cent. Bano and Rajarathnam (1982) reported the highest yield of *P. sajor-caju* on protein supplemented straw. All supplements tried *viz.*, horsegram powder, cotton seed powder, yeast mud, groundnut cake and rice bran enhanced yield of mushrooms, but addition of cotton seed powder to paddy straw recorded a maximum yield of 1.48 kg of fruit bodies per kg of substrate. Gunasegaran and Graham (1987) found rice bran as a better supplement for phoenix mushroom production than soybean meal. The supplementation resulted in 57.2 per cent bioefficiency as compared to 23.4 per cent in the control. Suharban (1987) studied the suitability of organic supplements for fruit body production and obtained a maximum yield when bengal gram powder was used as supplement. Bahram (1989) reported increased yield of *P. sporeless* and *P. eryngii* by supplementation with one per cent wheat bran, 3.8 per cent dried gram flour and 5.6 per cent soybean flour. Jandaik (1989) reported 18.2 and 28.6 per cent increased yield in *P. sajor-caju* when wheat bran and brewer's grain were used as supplements. Bahukhandi and Chamola (1991) reported maximum yield from *Pleurotus* spp. when the substrates were supplemented with 6-8 per cent wheat and rice bran. Dubey and Das (1997) studied the effect of incorporation of six organic supplements with paddy straw at five per cent level and found that pegion pea supplemented substrate produced the highest yield and biological efficiency followed by dhal powder and karanj cake.

Kumuthakalavalli and Daniel (1991) studied the effect of different pulses as protein supplement on the sporophore yield of oyster mushroom. They obtained the highest yield by supplementation with horse gram. Kattan *et al.* (1991) reported the highest yield and net protein content of *P. florida* and *P. sajor-caju* at 0.45 per cent nitrogen supplementation with either soybean flour or cotton seed cake. Kumar *et al.* (1997) studied the effect of supplementation of paddy straw substrate *@* 100 g per kg

substrate with besan and wheat chokar either alone or in combination on the yield of *P. sajor-caju* and found maximum yield after supplementation of the substrate with both giving a biological efficiency of 155 per cent. Mane *et al.* (1997) reported that five per cent supplementation of vermicompost on paddy straw substrate produced significantly better spawn run, yield, biological efficiency and protein conversion efficiency of *P. sajor-caju.* Patra *et al.* (1997) reported that paddy straw supplemented with 25 per cent neem leaves or one per cent neem cake produced an yield of 713 g and 768 g respectively.

Banik *et al.* (1994) studied the effect of residual slurry from biogas when supplemented with rice straw for the production of *P. sajor-caju* and observed significantly higher yield and protein content in fruit bodies. Geetha (1994) reported that supplementation of mushroom beds with four per cent rice bran, two per cent each of cellulose and starch produced significantly higher yield when compared to other supplements. Saroj Kumar and Singh (2002) investigated the effect of different supplements such as wheat bran, rice bran, gram powder, neem cake and chicken manure on the yield of *Volvariella diplasia.*

2.2.2 Cultivation of Milky Mushroom (Summer Mushroom)

Calocybe indica (milky mushroom) is an attractive species with milky white, large sporophores belonging to the family Tricholomataceae of the order Agaricales. *C. indica* was cultivated for the first time by Purkayastha and Chandra (1974). Its characters were first described by Purkayastha and Chandra (1985).

2.2.2.1 Substrates

Purkayastha and Nayak (1981) observed increased yield when nitrogenous substrates were used for cultivation of *Calocybe.* Trivedi *et al.* (1991) reported the cultivation of *C. indica* on different agricultural

wastes. Wheat straw in combination with maize meal was found to be the best substrate for milky mushroom production giving a biological efficiency of 45 per cent. Joseph *et al.* (1991) reported a biological efficiency of 53.3 per cent when rubber wood sawdust was used as a substrate for the cultivation of summer mushroom. Balakrishnan and Lulu Das (2001) developed a low cost technique for cultivation of *C. indica* using spent substrate of oyster mushroom.

Studies conducted by Bhavana and Thomas (2002) revealed that fermentation of coirpith is an effective pretreatment to enhance the yield of milky mushroom. They reported an average fresh mushroom yield of 486.75 g per bag with a biological efficiency of 81.16 per cent and individual mushroom weight of 99.5 g. Krishnamoorthy (2003) reported the cultivation of milky mushroom on a wide range of cellulosic substrate *viz.*, paddy straw, maize stalks, sorghum stalk, pamarosa grass, vetiver grass, sugarcane bagasse, soybean hay and groundnut haulm and found paddy straw as the best substrate for commercial production.

2.2.2.2 Casing

Casing is an absolute requirement for proper fructification of *C. indica.* Tewari and Pandey (1994) reported the use of coir dust as a better casing material because of its higher water holding capacity and porosity. According to Doshi and Sharma (1995) cowdung patties are good casing materials. Krishnamoorthy and Muthusamy (1997) reported clay loam garden soil of pH 8.4 to be a better casing material in *Calocybe* cultivation. Balakrishnan and Lulu Das (2001) obtained the highest yield when cattle dung, sand and soil in 1 : 1 : 1 proportion was used as the casing material. Krishnamoorthy *et al.* (2002a) found that clay loam soil of pH 8.4 having 50 per cent moisture was the best casing material for higher yields of milky mushroom. Use of coirpith with two per cent calcium carbonate as casing material was the best to produce maximum yield of milky

mushroom (Geetha *et al.*, 2002). The optimal casing thickness for higher yields of milky mushroom was reported to be 1-2 centimetre (Sharma *et al.*, 1994 and Krishnamoorthy *et al.*, 2002a).

2.2.2.3 Supplements

Purkayastha *et al.* (1981) reported increased yield of *Calocybe* by utilizing maize meal supplemented paddy straw as substrate. Purkayastha and Chandra (1985) used rice straw and wheat straw in combination with maize meal for the cultivation of *Calocybe*. This combination along with dehydrated lucerne was found to be an ideal substrate for growing this mushroom (Trivedi *et al.*, 1991). Sharma *et al.* (1994) reported an increase in the biological efficiency by supplementing wheat straw with rice husk, maize meal, wheat bran, coconut husk and inorganic salts such as $FeSO_4$ or $ZnSO_4$. Balakrishnan and Lulu Das (2001) used spent substrate of oyster mushroom supplemented with 20 per cent rice bran for cultivation of milky mushroom and produced yield comparatively better than that of paddy straw.

2.3 BIODEGRADATION

Biodegradation is a complex essentially anaerobic fermentation process where a chain of microflora are involved. Mushroom fungi are nature's gift which act on a variety of plant polymers (cellulose, hemicellulose, lignin etc.) by production of enzymes which degrade them. Wood *et al.* (1988) reported that mushroom degrade all major components of lignocellulosics such as lignin, cellulose and hemicellulose and have an advantage over other fungi in degrading the native lignocellulosics without much pre-treatment. The degradation of complex lignocellulosics wastes by microorganism largely depends on the synergistic action of extra cellular enzymes like lignases, cellulases and hemicellulases. These enzymes are characteristically produced by higher fungi particularly the wood degrading basidiomycetes (Thakur *et al.*, 2002).

2.3.1 *Pleurotus* spp.

Jandaik and Kapoor (1975) reported that *P. sajor-caju* could degrade banana pseudostem, milled cobs and paddy straw. Bano *et al.* (1979) recorded higher levels of degradation of rice straw, when *P. flabellatus* was grown on it. Among the various white rot basidiomycetes, *Pleurotus* spp. was reported to be the most efficient colonizers and degraders of lignocellulosics (Rajarathnam *et al.*, 1987; Chang and Miles, 1989). Theradimani and Marimuthu (1991) reported that *P. platypus* was efficient in reducing C : N ratio, lignin and cellulose content when inoculated into coirpith. Similar observation were also recorded with *P. sajor-caju* by Rai and Saxena (1991) and obtained a direct correlation between the amount of enzyme production and fruit body formation. Degradation of lignocellulosic biomass of water hyacinth by *P. ostreatus* and *P. sajor-caju* was reported by Ghosh and Nandi (1995) and *P. djamor* and *P. ostreatus* by Sharma *et al.* (1999).

Geetha and Sivaprakasam (1998a) elaborated the enzyme production potential of *P. citrinopileatus* and *P. djamor* in the biodegradation of lignocellulosic substrate. Paddy straw followed by a combination of paddy straw and paper waste showed higher rate of cellulose reduction indicating that substrate rich in cellulose was preferred by the fungi. *P. djamor* recorded higher rate of cellulose degradation while *P. citrinopileatus* recorded higher lignin degradation. Sharma *et al.* (1999) reported high cellulase activity in *P. ostreatus* WC 598 compared to *P. djamor* R 22 indicating a correlation between biological efficiency and cellulase activity.

2.3.2 *C. indica*

Ramamoorthy *et al.* (1999) reported that *C. indica* was least effective in coirpith degradation. Only 6.89 per cent reduction of cellulose over control was found on coirpith degraded by this fungus.

C. indica produces cellulases, laccases and polyphenol oxidases during their growth on substrate (Krishnamoorthy *et al.*, 2002b). Anandh and Prakasam (2002) studied the enzymology and substrate degradation pattern of wild edible mushroom such as *Tricholoma lobayense* (SI and WCL), *T. giganteum* (KKBM and WMM), *C. indica* (SMM and APK2) and *C. gambosa* (PBS and VMM) and showed that dry matter, cellulose and lignin were degraded by the mushroom accessions at a faster rate during the initial colonization of the substrates followed by a steady decrease in C : N ratio.

Krishnamoorthy *et al.* (2002b) reported higher levels of exo and endo-cellulases production by *C. indica* on paddy straw, sorghum stalks and maize stalks whereas laccase production was higher in blackgram hay and soybean hay and high polyphenol oxidase activity was found on sawdust, groundnut haulms, etc. showing a positive correlation between production of enzyme and yield. Reeja *et al.* (2002) studied the pattern of degradation of dry matter, cellulose and lignin content of coirpith inoculated with *C. indica.*

2.3.3 Biodegradation by other Wood Inhabiting Fungi

Oki *et al.* (1982) reported the biodegradation of soft wood lignin and guaiacyl glycerol-beta guaiacyl ether by means of extra cellular enzyme in the case of *Lentinus edodes.* Later, Blanchette (1984) demonstrated that selective delignification is more prevalent among white rot fungi. In the case of biodegradation of lignocelluloses such as cotton-gin trash, the fungus *Pycnoporus cinnabarinus* did not rapidly remove lignin compared to other lignocellulose components as reported by Sutherland (1984). Ulmer *et al.* (1984) observed that lignocellulolytic enzyme of *Phanerochaete chrysosporium* were activated in the presence of lignin. Arora and Sandhu (1985) in their study observed basidiomycetes to be better laccase producers and decomposers of wood / lignin than other

fungal groups and degraded sawdust causing a total weight loss of seven to eight per cent and a lignin loss of upto 10 per cent in 30 days. Geiger *et al.* (1986) in their study observed that in the case of biodegradation of rubber wood, *Rigidoporus lignosus* has a better tendency to degrade lignin while *Phellinus noxius* preferentially degraded the polysaccharide fraction.

2.3.4 Biodegradation by Native Microflora

Masroor and Biharilal (2002) reported the role of different actinomycetes, fungi and bacteria in biodegradation of agricultural wastes. Among fungi, *Aspergillus flavus*, *A. terreus*, *A. niger*, *Rhizopus stolonifer*, *Chaetomium olivaceum*, *Penicillium chrysogenum*, *P. oxalicum*, *Trichothecium roseum* and *Trichoderma viride* were found to be effective degraders. Savithri and Khan (1994) isolated different organisms *viz.*, *Aspergillus* sp., *Streptomyces* sp., *Penicillium* sp. and *Trichoderma* sp. from native coirpith. Among them *Trichoderma* sp. and *Aspergillus* sp. were found to be potent degraders as they reduced the C : N ratio to its lowest values. Ramamoorthy *et al.* (2000) observed *A. niger* as an effective biodegrader of coirpith among the native microflora studied, followed by *A. flavus*, *Penicillium* sp., *Fusarium* sp. etc. Shirkot *et al.* (2001) could isolate cellulolytic fungi such as *Trichoderma* sp., *Cladobotrym verticillatum* and *Papulospora byssina* from mushroom compost which were able to degrade cellulose for their growth by producing a complete set of cellulase enzymes.

2.4 COIRPITH

The pith material forming non-fibrous tissues of the husk is generally referred to as coirpith or as cocopeat (Bhowmic and Debnath, 1985). Annual production of coirpith in India is about 7.5 million tonnes (Kamaraj, 1994), out of which 11 lakh metric tonnes is from Kerala alone (Reeja *et al.*, 2002) and a major portion of these goes unutilized.

Raw coirpith (RCP) is a recalcitrant lignocellulosic waste and contains 8 – 12 per cent soluble tannins like phenolics (Fan *et al.*, 1982) that inhibit plant growth and microbial activity by immobilizing nitrogen during polymerization (Nagarajan *et al.*, 1990). Savithri and Khan (1994) reported that the composition of coirpith varies depending upon various factors such as fertility status of coconut garden, method of extraction, disposal, time of collection and environmental factors. Composting of coirpith helps in detoxifying phenolic compounds, reducing the bulkiness of the material and converting the plant nutrient to a form more readily available to plant (Ramamoorthy *et al.*, 2000).

2.4.1 Biodegradation of Coirpith

Thayumanavan (1982) reported that extracellular enzymes of fungus play a major role in the degradation of structural elements such as cellulose, hemicellulose, lignin and pectin in natural substrates. The enzyme system of *Pleurotus* spp. involved in degradation of lignocellulosic substrates are endogluconase, β-glucosidase, xylanase, laminarinase, laccase and polyphenol oxidase (Madan and Bisaria, 1983 and Rajarathnam *et al.*, 1987).

Coirpith is a recalcitrant agro residue containing high amount of lignin and cellulose resisting decomposition by microorganism under natural conditions. Organisms belonging to basidiomycetous fungi like mushrooms are capable of degrading coirpith. Some species of *Pleurotus* had the capability of producing laccase which degraded part of cellulose and lignin present in coir dust (Reddy, 1985 and Nagarajan *et al.*, 1985). *Pleurotus* spp. are the most versatile mushroom capable of colonizing and degrading a variety of lignocellulosic waste materials (Chang and Miles, 1989). 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, *P. 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) and Owseph (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 Veena Savalgi *et al.* (1994).

The degradation potential of *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* sp. *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 the species were capable of degrading lignin, cellulose and hemicellulose components of straw.

Owseph *et al.* (2001) assessed the lignocellulose degradation by oyster mushroom and found that *P. sajor-caju* was the most effective degrader of cellulose and lignin. Reeja (2002) reported that *P. eous* and *P. sajor-caju* were effective degraders of cellulose and lignin in coirpith.

Ramamoorthy *et al.* (1999) reported that *C. indica* was the least effective fungus when compared to *Pleurotus* sp. in degradation of cellulose and lignin component of coirpith. Enzyme related biodegradative potential of *C. indica* was studied by Krishnamoorthy *et al.* (2002b).

2.4.1.1 C : N Ratio

The C : N ratio plays an important role in the decomposition of any organic matter. Composting narrowed down the C : N ratio and reduced

cellulose and lignin content. If the material has wider C : N ratio, then it will decompose very slowly and immobilization of nutrients may take place.

Microorganism required carbon for growth and N for protein synthesis. On an average, they utilize 30 parts of carbon per one part of N. Therefore C/N ratio of 30:1 was desirable for composting. If it was above 35, then the process become inefficient and require more time for completion. If it was below 26, the excess N was converted to NH_3 and wasted into atmosphere (Poincelot, 1974). Cappaert *et al.* (1976) recorded a C : N ratio of 25 to 35 per cent to be optimum for composted organic matter. By proper adjustment of C/N ratio, agricultural wastes could be profitably recycled with greater degree of enrichment.

Nagarajan *et al.* (1985) reported that coirpith having a C : N ratio of 24 : 1 could be used as good source of organic matter for field crops. They also found that organic C content was reduced from 29 per cent to 24.5 per cent while the N content increased from 0.26 per cent to 1.06 per cent during decomposition.

Theradimani and Marimuthu (1992) reported *P. platypus* as the most efficient degrader of coirpith. It could narrow down C : N ratio from 104 : 1 to 18 : 1. Nallathambi and Marimuthu (1993) reported that *P. platypus* was very efficient in decreasing organic carbon and increasing N content of the substrate after 15 days of inoculation. Organic carbon content decreased from 45 per cent to 36.6 per cent and N content increased from 0.32 to 1.19 per cent.

According to Alexander (1977), a C : N ratio of less than 20 : 1 served as an indicator of the maturity and stability of organic substrate. 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 N content of coirpith increased from

0.29 per cent to 1.92 percent and organic C content decreased from 29.88 to 25.24 per cent.

According to Ramamoorthy *et al.* (1999) *P. djamor* decreased the organic C content to the maximum *i.e.*, 23.14 per cent and increased the N content to maximum level *i.e.*, 1.15 per cent. The C : N was ratio decreased to 20 : 1. Kadalli and Nair (2000) reported that while composting with *Pleurotus* spp. the C : N ratio of raw coir dust was decreased to 34.19 per cent from 112.9 per cent. Ramamoorthy *et al.* (2000) studied the efficacy of native microflora *viz.*, *Aspergillus niger*, *Penicillium* sp., *Fusarium* sp., *Streptomyces* spp. and *Bacillus* sp. in degrading coconut coirpith and found that *A. niger* brought about maximum degradation. It reduced C : N ratio from 109 : 1 to 39:1. Reeja (2002) reported that the maximum reduction in organic carbon and C : N ratio was brought about by *P. eous* whereas increase in nitrogen was brought about by *C. indica*-2 in both retted and non-retted coirpith.

2.4.1.2 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. Cellulose content of the substrate was reduced following the growth of *Pleurotus* spp. (Zadrazil, 1978; Rajarathnam *et al.*, 1979 and Moorthy, 1981). This is due to the enzyme cellulase which plays a major role in the degradation and recycling of cellulose (Beguin and Anbert, 1985). Degradation of coirpith with *Pleurotus* spp. reduced the cellulose content to 10.1 percent from 26.5 per cent (Nagarajan *et al.*, 1985). Among the different species of *Pleurotus* sp., maximum yield and cellulolytic enzyme production was noticed in *P. florida* (Joseph *et al.*, 1991). Rai and Saxena (1991) reported a direct correlation between enzyme production and fruit body formation in the case of *P. sajor-caju.*

Theradimani and Marimuthu (1991) reported the efficacy of *P. platypus* in reducing the cellulose content from 27 to 11.2 per cent, when inoculated into coconut coirpith. Theradimani and Marimuthu (1992) found maximum reduction of cellulose at 35 days of inoculation of coirpith by *Pleurotus* spp. *P. platypus* brought about 58.6 per cent decrease of cellulose over control. Reduction of cellulose content of the substrate following the inoculation with *Pleurotus* spp. was reported by Geetha (1994). Later Geetha and Sivaprakasam (1997) reported that substrates rich in cellulose are preferred by the fungus and a higher rate of cellulose degradation was recorded by *P. djamor* when inoculated into different substrates indicating that *P. djamor* elaborated maximum amount of cellulase and laccase enzymes.

Owseph (1999) reported that the cellulose content of retted and non-retted coirpith decreased to 11.26 and 22.50 per cent from 25.50 per cent and 36.30 per cent respectively after 30 days of incubation. Maximum per cent of cellulose reduction was recorded in retted coirpith on 30th day of incubation with *P. sajor-caju*. Ramamoorthy *et al.* (1999) reported that *P. djamor* degraded the cellulose of coirpith to maximum level by producing endocellulases (c1) and exocellulases (cx) enzyme activity which lead to the reduction of cellulose content of 62.2 per cent over control. In the same study *C. indica* was found to be least effective in reducing cellulose which was about 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. Owseph *et al.* (2001) found that *P. sajor-caju* was the most effective degrader of cellulose. Enzyme related biodegradation potential and reduction of cellulose content by *C. indica* was reported by Krishnamoorthy *et al.* (2002b). Reeja (2002) found that *P. eous* and *P. sajor-caju* were very good decomposers of cellulose in the case of retted coirpith.

2.4.1.3 Lignin Degradation

It is estimated that the planet currently contains 3 x 10^{11} metric tonnes of lignin with an annual biosynthetic rate of approximately 2 x 10^{11} tonnes (Argyropoulos and Menachem, 1997). Lignin is a very complex structure formed by the oxidative polymerization of coumaril, coniferyl and synapyl alchohol (Kirk and Farrell, 1987 and Boominathan and Reddy, 1992). Lignocellulosic materials comprise about 95 per cent of earth's land based biomass, about 25 per cent of which is lignin (Janshekar and Fiechter, 1985). In general, tremendous amount of lignin produced annually from photosynthesis is balanced by the amount of lignin that is decomposed by microorganism. Lignin degradation is important on global recycling of carbon because of the great abundance of lignin in the biosphere and also it is an important factor determining the degradation of cellulose and other polysaccharides (Kirk and Farrell, 1987).

Complete degradation of lignin may be the result of co-operative action of various fungi, bacteria and other microflora in the soil (Janshekar and Fiechter, 1983). Fungi still remain the most studied organism of all the other inhabitants of the soil. Based on the type of decay, the fungi were classified as white rot, brown rot and soft rot. It is generally recognized that the lignin are first attacked by basidiomycetes and are substantially degraded by ascomycetes and fungi imperfecti. The wood decaying fungi of white rot type are mainly responsible for degradation of the lignocellulosics in forest litter (Kawase, 1962; Kirk and Farrell, 1987).

Thayumanavan (1982) reported that the enzymes frequently associated with lignin degradation in nature were phenol oxidases (tyrosinase and laccase). Enzymology of lignin degradation in coir dust by *P. sajor-caju* was studied by Reddy (1985). He observed that *P. sajor-caju* had the capacity to produce laccase and degrade part of cellulose and

lignin present in coirpith. The commonly cultivated or wild mushroom fungi can utilize lignocellulose as carbon source. Thus they too have a potential in the bioconversion of lignin. According to Hiroi (1981) *P. ostreatus* could degrade wood lignin upto 34.5 per cent. Nagarajan *et al.* (1985) reported 84 per cent reduction in lignin content after composting raw coirpith with *Pleurotus* spp.

Beg *et al.* (1986) reported that *P. sajor-caju* could reduce husk lignin to the extent of 40.9 per cent. Boominathan and Reddy (1992) reported that lignin degradation was occurred by secondary metabolism when the essential nutrients are exhausted and growth ceases. Lignin mineralization was optimal at neutral to slightly acidic pH whereas lignocellulose degradation with lignin solublization and acid precipitable polymeric lignin production was promoted at alkaline pH with *Streptomyces viridosporus* (Pometto and Crawford, 1986).

According to Leal-lara *et al.* (1989) genetically improved strain of *P. ostreatus* could degrade 46 per cent lignin and 30 per cent hexose as compared to the potential strains which could degrade 44 per cent of both cellulose and lignin after 50 days of incubation.

Theradimani and Marimuthu (1991) studied the efficacy of *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) observed that *P. citrinopileatus* degraded lignin more effectively compared to *P. djamor*. Owseph (1999) obtained maximum per cent lignin degradation by *P. sajor-caju* in retted coirpith on 30th day of solid state fermentation. Ramamoorthy *et al.* (1999) tested the efficacy of *P. djamor*, *P. citrinopileatus*, *P. eous* and *C. indica* on degradation of lignin present on 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. Krishnamoorthy *et al.* (2002b) reported the narrowing of C : L ratio in the substrates tested after studying the enzyme related biodegradative potential of *C. indica*. Reeja (2002) repòrted the maximum lignin degradation on non-retted coirpith by *P. sajor-caju* and *P. eous*.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 ISOLATION OF LIGNOCELLULOLYTIC FUNGI FROM NATIVE COIRPITH

3.1.1 Collection of Coirpith Samples

The materials leftover after extraction of coir fibre from the coconut husk, *viz.*, 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 area of Thiruvananthapuram district of Kerala state, while the non-retted coirpith was collected from Thiruvithamcode district of Tamil Nadu and composted coirpith was obtained from Instructional Farm, College of Agriculture, Vellayani, Thiruvananthapuram district of Kerala State.

Lignocellulolytic fungi from native coirpith (retted) were isolated by adopting serial dilution plate technique (Johnson and Curl, 1972).

One gram of coirpith sample (retted coirpith) was taken in a 250 ml conical flask containing 100 ml of sterile water. The contents of the flask were shaken for 20 minutes using a rotary shaker. One ml of this was taken using a sterile pipette and transferred into a series of test tubes containing 9 ml of sterile water to get dilutions of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. Inorder to assess the number of fungi, one ml from 10^{-4} dilution was transferred into sterile petridishes and Rosebengal agar was added. It was allowed it to solidify and incubated under room temperature for 24 hours or fungal colonies to appear.

3.2 COLLECTION AND ISOLATION OF WOOD INHABITING MUSHROOM FLORA

A survey was conducted in different parts of Thiruvananthapuram districts *viz.*, Nedumangadu, Palode, Anchal, Venganoor and Pechipara of

Tamil Nadu and mushrooms were collected from decayed plant parts and tree stumps. The morphological and taxonomic characters of these mushrooms were studied.

Mushrooms were isolated by adopting tissue culture method. Medium sized healthy mushroom sporocarp was surface sterilized by wiping it with cotton dipped in 70 per cent alcohol. The mushroom was split open longitudinally and a small bit of the tissue from the exposed surface was scooped out with the help of a sterile forceps or an inoculation needle and transferred to Potato Dextrose Agar (PDA) slants under aseptic conditions and incubated under room temperature (28 ± 4°C) for four days. The growth obtained from this was then purified by the hyphal tip method and maintained on PDA slants by periodic subculturing.

3.3 SCREENING OF THE ISOLATED CULTURES FOR SELECTING THE MOST EFFICIENT FUNGI FOR DEGRADATION

Retted coirpith (300g) was taken in polypropylene bags of size 7 x 15", plugged with cotton after inserting a PVC ring of 1½" diameter and steam sterilized by autoclaving at 1.05 kg cm^{-2} for one hour. The sterilized coirpith was inoculated by transferring mycelial bits under asceptic conditions. The inoculated bags were kept under room temperature and observations were recorded regarding:

1. Nature of mycelial growth on coirpith
2. Time taken for complete colonization of coirpith
3. Carbon content of coirpith
4. Nitrogen content of coirpith
5. Cellulose content of coirpith
6. Lignin content of coirpith

3.3.1 Assay of 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 which 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 after 30 minutes 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.

3.3.2 Assay of Total Nitrogen

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

This method involved two steps :

i) Digestion of sample to convert organic form of nitrogen to ammonia

ii) Determination of ammonia in digest (distillation)

Five hundred mg of 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 using distilled water. From this 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 contents of the flask was titrated against 0.01N hydrochloric acid.

3.3.3 Assay of 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 the blank.

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

3.3.4 Assay of 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. This filtrate (Kalson 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 STANDARDISATION OF TECHNIQUES FOR MUSHROOM PRODUCTION ON COIRPITH

3.4.1 Spawn Preparation

Paddy grain spawn of *Pleurotus florida* and *Calocybe Indica* were prepared by adopting the method described by Sinden (1932, 1934).

Paddy grains were boiled in water till the husk splits. After draining excess water, it was mixed with $CaCO_3$ at the rate of 50 g per kg of paddy grain to prevent adhesion of grains and for optimizing the pH for spawn run. Glucose drip bottles of 750 ml capacity or polypropylene cover were filled with the grains to two third of its capacity, plugged with cotton and autoclaved at 1.05 kg/cm^2 for two hours. Inoculation of the grains with pure culture of *P. florida* and *C. indica* were carried out and incubated at room temperature 28 ± 4°C. The nature of growth and time taken for completing mycelial colonization of the grains were recorded. The spawn thus prepared was utilized for laying out mushroom beds.

3.4.2 Preparation of Mushroom Beds

Three different substrates were tried for mushroom production *viz.*, retted coirpith, non-retted coirpith, coirpith compost.

Table 1 Effect of retted, non-retted and composted coirpith on yield of oyster and milky mushroom

Substrate	Oyster mushroom yield (g)	Milky mushroom yield (g)
Retted coirpith	58.25	38.25
Non-retted coirpith	42.55	58.45
Composted coirpith	-	-

Preliminary studies on the suitability of retted, non-retted and composted coirpith on mushroom production was conducted and the results indicated the unsuitability of the same as observed by poor mycelial growth and sporophore production. Hence these were not included in the list of treatments and paddy straw was maintained as control. The different treatment combinations included were :

Treatment No.	Treatment combinations
T_1	Retted coirpith + Paddy straw (3 : 1)
T_2	Retted coirpith + Paddy straw (1 : 1)
T_3	Retted coirpith + Paddy straw (1 : 3)
T_4	Retted coirpith + Spent mushroom substrate (3 : 1)
T_5	Retted coirpith + Spent mushroom substrate (1 : 1)
T_6	Retted coirpith + Spent mushroom substrate (1 : 3)
T_7	Retted coirpith + Cellulose 2 %
T_8	Retted coirpith + Starch 2 %
T_9	Retted coirpith + Neem cake 4 %
T_1	Non-retted coirpith + Paddy straw (3 : 1)
T_2	Non-retted coirpith + Paddy straw (1 : 1)
T_3	Non-retted coirpith + Paddy straw (1 : 3)
T_4	Non-retted coirpith + Spent mushroom substrate (3 : 1)
T_5	Non-retted coirpith + Spent mushroom substrate (1 : 1)
T_6	Non-retted coirpith + Spent mushroom substrate (1 : 3)
T_7	Non-retted coirpith + Cellulose 2 %
T_8	Non-retted coirpith + Starch 2 %

T_9	Non-retted coirpith + Neem cake 4 %
T_1	Coirpith compost + Paddy straw (3 : 1)
T_2	Coirpith compost + Paddy straw (1 : 1)
T_3	Coirpith compost + Paddy straw (1 : 3)
T_4	Coirpith compost + Spent mushroom substrate (3 : 1)
T_5	Coirpith compost + Spent mushroom substrate (1 : 1)
T_6	Coirpith compost + Spent mushroom substrate (1 : 3)
T_7	Coirpith compost + Cellulose 2 %
T_8	Coirpith compost + Starch 2 %
T_9	Coirpith compost + Neem cake 4 %

Mushroom beds were prepared following the compact polybag method described by Baskaran *et al.* (1978). Retted and non retted coirpith were soaked in a solution of 0.1 per cent quick lime (calcium oxide) for seven days, after that the substrates were taken out and sterilized chemically by treating with a mixture of 500 ppm formalin and 75 ppm bavistin (Carbendazim 50% WP) for 18 hours (Gokulapalan *et al.*, 1989). The excess water was drained out and beds were laid out following poly bag method. Paddy straw and spent mushroom substrate were sterilized by following the chemical method of sterilization as explained above.

The different supplements *viz.*, neem cake, starch and cellulose were sterilized by autoclaving at 1.05 kg cm^{-2} for one hour and mixed with substrate (as per the given ratio) after cooling.

Polythene tubes of 100 guage thickness and 60 x 30 cm in size were used to prepare the beds. Two holes of one cm diameter were made at the centre of the tube for permitting air circulation. For preparing one bed 500 g of substrate (when dried) and 150 g spawn were used. First layer of

Plate 1 Cultivation trials on Oyster mushroom (*P. florida*)

Plate 2 Cultivation trials on Milky mushroom (*C. indica*)

substrate was placed at the bottom of the bag to a height of about five cm and spawned with paddy grain spawn, again the second layer of substrate to a height of five cm was placed and spawned. Similarly, the third and fourth layers were spawned. Finally at the top of the fourth layer, the rest of spawn was spread. The bags were then tied to form compact mass. The bags were then kept for spawn run (Plate 1).

In the case of *C. indica*, casing was done after spawn run for the proper emergence of fruiting bodies. The casing mixture used in the present study was 1 : 1 : 1 ratio of sand, soil and dried powdered cowdung. The casing mixture was sterilized by autoclaving for one hour and applied at the top of the beds to a thickness of two centimeters after cooling (Plate 2). The following observations were recorded.

1) Nature of mycelial growth in mushroom beds

2) Time taken for mushroom production in beds

3) Number of sporophore per bed

4) Total yield per bed

RESULTS

4. RESULTS

4.1 ISOLATION OF LIGNOCELLULOLYTIC FUNGI FROM NATIVE COIRPITH

Following lignocellulolytic fungi were obtained after performing serial dilution plate technique.

Table 2 Fungi isolated from coirpith

Sl. No.	Fungi
1	*Aspergillus niger* van Tieghem
2	*A. ochraceous* Wilhelm
3	*Rhizopus stolonifer* (Ehrenb. ex. Link) Lind
4	*Trichoderma harzianum* Rifai

4.1.1 *A. niger*

Black powdery colonies of the fungus appeared on PDA plates. It covered a 9 cm petridish within 4-5 days at 28 ± 2°C. Conidiophores were produced from long, broad, thick walled, mostly brownish sometimes branched foot cells. Conidia are large, with radiating heads, mostly globose, irregularly rough, 4.0 – 5.0 μ m dia. Conidiophores bear metulae and phialides carrying black conidiospores at their tips.

4.1.2 *A. ochraceous*

Colonies appeared brown in colour on PDA plates consisting of conidiophores and conidial heads with aerial mycelium. Conidiophores vary in length, commonly several millimeters, rough or pitted bearing

large radiate conidial heads. Vesicles globose, 60-75 µm dia, phialides in two series, primary commonly 15-30 µm long, secondary 7-10 x 1.5-2 µm. Conidia globose to elliptical, smooth or delicately spinulose, yellow, 3.5 – 5 µm, orange to vinaceous or purple, sclerotia commonly present.

4.1.3 *R. stolonifer*

Colonies very fast growing and often over 2 cm high, grey-brown. stolons hyaline to brown, abundantly branched, rhizoids and whorls of sporangiophores produced terminally. Sporangiophores pale to dark brown, usually straight. It bears columella which is subglobose to oval, pale brown. Sporangiospores subglobose, biconical to oval 7-12 x 6-8.5 µm.

4.1.4 *T. harzianum*

Colonies are white at first, turning dull green in colour at sporulation and grows rapidly. Young cultures compactly tufted fluffy and white with prominent aerial mycelium showing good amount of diurnal variation. Sporulate less often under darkness. Hyphae are hyaline and septate. Chlamydospores measure about 4.26 x 4.84 µm. Length of conidiophores varies, highly branched, phialides mainly in group of three, wide apart, length approximately 40 µm. Phialides with length 3.85 µm, phialospores grouped at the tip of phialides, globose, pale green with an average size of 2.2 – 2.78 µm.

4.2 COLLECTION OF WOOD INHABITING MUSHROOM FLORA AND ISOLATION INTO PURE CULTURE

The results of survey conducted in different parts of Thiruvananthapuram district and border areas of Tamil Nadu are presented in Table 3. The collected mushrooms were described, identified and brought them into pure culture.

Plate 3 *Pycnoporus sanguineus* on coconut stump

Plate 4 *G. applanatum* on coconut stump

Plate 5 *Pleurotus tuber-regium* on mango tree stump

Table 3 Mushrooms obtained during survey

Sl. No.	Name of mushroom species collected	Substrate	Location and period of collection
1	*Pycnoporus sanguineus* (Fr.) Murrill Plate 3	Dried coconut (*Cocos nucifera* L.) stump	College of Agriculture, Vellayani, June,2002
2	*Ganoderma applanatum* (Persoon) Patouillard Plate 4	Dried coconut (*Cocos nucifera* L.) stump	College of Agriculture, Vellayani , June,2002
3	*Pleurotus tuber-regium* (Fr.) Singer Plate 5	Dried mango tree (*Mangifera indica)*	University College, Thiruvananthapuram, July, 2002

Table 4 Mushroom flora used in the present study

Sl. No.	Name of species	Source
1	*Pleurotus florida*	Pure culture obtained from Instructional Farm, College of Agriculture, Vellayani
2	*Calocybe indica*	
3	*Lentinus edodes* (Berk.) Sing	Pure culture obtained from AICMIP, TNAU, Coimbatore
4	*P. tuber-regium*	Obtained during survey
5	*P. sanguineus*	
6	*G. applanatum*	

4.2.1 Morphological Characters of Collected Mushrooms

4.2.1.1 P. sanguineus

Collected from College of Agriculture, Vellayani

Habitat : On dried branch and trunks

Carpophores 3-6 cm or more, bracket shaped, sessile, deep orange-red tending to darken, at first slightly pubescent then glabrous, fairly rugose with faint zonation towards margin. Tubes 1-3 mm long, blood red, pores small round, pubescent. Flesh red, leathery, first spongy then suberose. Odour and flavour negligible. Spores white cylindrical, smooth 5-6 x 2 – 2.5 μm.

The mushroom was identified as *P. sanguineus*. The identification was confirmed by Dr. Peter Roberts, Mushroom Taxonomist, Royal Botanical Garden, London.

4.2.1.2 G. applanatum

Collected from College of Agriculture, Vellayani

Habitat : Parasite, persisting after death of the host as saprophyte

Carpophore 10-40 cm, flat, bracket shaped, often imbricate, sessile sometimes first white soon covered by a smooth, honey crust becoming reddish-brown and knobbly or grooved, margin sharp, white or greyish. Tubes rust – coloured, stratified 1-4 μm long, pores white, turning brown when touched, small, round or irregular. Flesh cinnamon brown and felt-like. Spores rusty brown, elliptical or ovate and finely warty 6.5 – 8.5 x 5 – 6.5 μm.

This fungus is easily identified by its flat brackets, somewhat crusty surface and white readily discolouring surface.

4.2.1.3 P. tuber-regium

Collected from University College, Thiruvananthapuram.

Habitat : On dried stumps of *Mangifera indica*

The sporophores are medium to big sized, creamy white coloured, slight yellow in the centre. Soft at immature stage and medium leathery at maturity.

Pileus : 12 to 15 cm diameter, infundibuliform, surface smooth, creamy white, margin entire.

Gills : Decurrent, creamy white

Stipe : 2 to 5.6 cm long, 1.2 to 2.6 cm in diameter, central, cylindrical, creamy white, base slightly flattened and surface is smooth.

Veil, volva and annulus are absent.

Spores : Oval in shape, creamy yellow coloured

Spore print : White

Edibility : Edible

Based on the above characters the mushroom was identified as *P. tuber-regium* and the identification was confirmed by Dr. Peter Roberts, Mushroom Taxonomist, Royal Botanic Garden, London. This fungus is reported for the first time and is a new record for India. Hence trials were conducted to find out its suitability for cultivation.

4.2.1.3.1 Cultivation trials of *P. tuber-regium*

4.2.1.3.1.1 Spawn Production

Complete coverage of mycelium was observed in paddy grains after 15 days of inoculation. The mycelial growth was thick, fluffy and numerous pin heads were noticed after 23-35 days of inoculation (Plate 6).

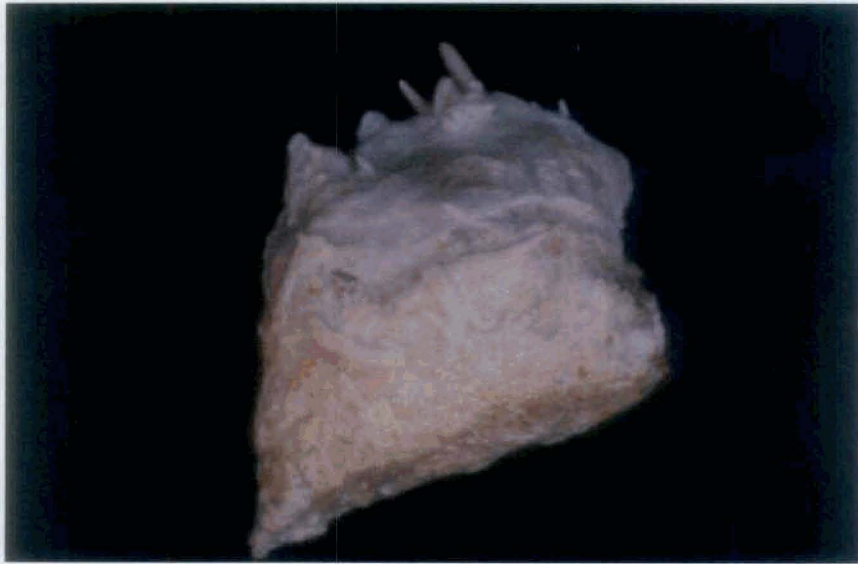


Plate 6 Spawn of *P. tuber-regium*

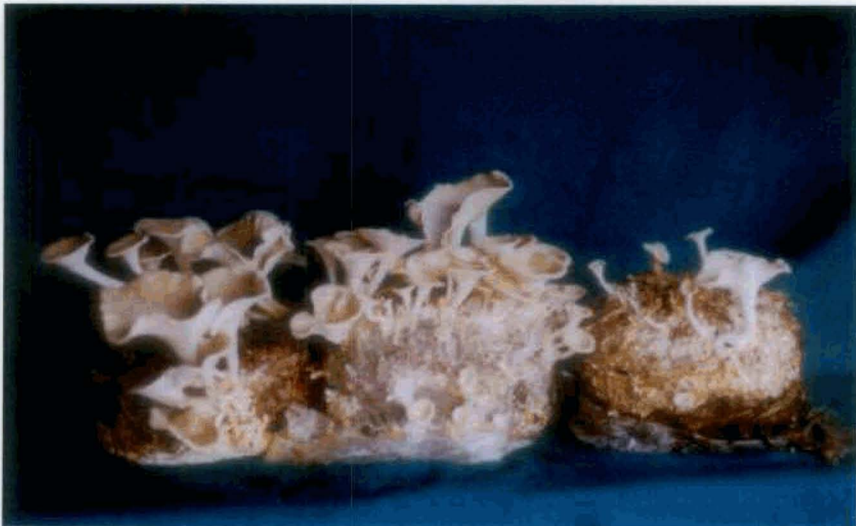


Plate 7 Sporophore of *P. tuber-regium* on paddy straw beds

Table 5 Growth of lignocellulolytic fungi on retted coirpith

Sl. No.	Fungi isolated	Time taken for colonization on retted coirpith (days)	Nature of mycelial growth*
1	*Aspergillus niger*	20	++++
2	*A. ochraceous*	21	+++
3	*Rhizopus stolonifer*	18	++
4	*Trichoderma harzianum*	11.5	++++
5	*Pleurotus florida*	19.75	++++
6	*P. tuber-regium*	16	++++
7	*Calocybe indica*	19	++++
8	*Lentinus edodes*	15.25	+++
9	*Ganoderma applanatum*	12.5	++++
10	*Pycnoporus sanguineus*	22.5	++++

SE 0.933
CD (0.05) 2.695

*
++++ Thick cottony mycelial growth
+++ Moderate cottony mycelial growth
++ Thin growth of mycelium
+ Very thin mycelial growth

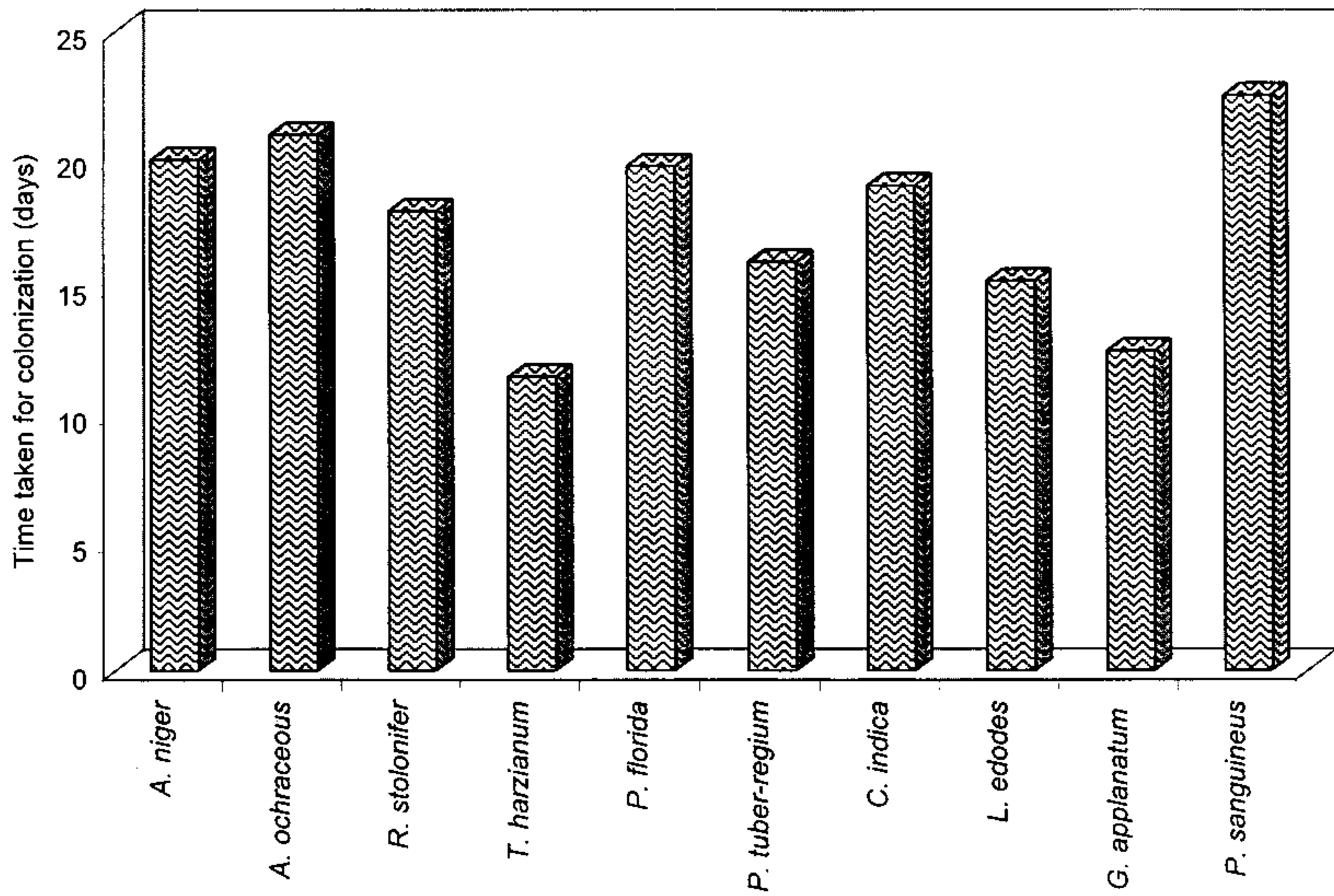


Fig. 1 Growth of lignocellulolytic fungi on retted coirpith

Cultivation trials on paddy straw indicated that spawn run was completed in a period of 13 days after bed preparation and sporophores appeared after 18-20 days (Plate 7). An average yield of 461 g was recorded per kg paddy straw, giving a biological efficiency of 46 per cent. The results indicated that *P. tuber-regium* is a promising species that can be cultivated profitably in Kerala.

4.3 SCREENING OF THE ISOLATED CULTURES FOR SELECTING THE MOST EFFICIENT FUNGI FOR DEGRADATION

4.3.1 Nature of mycelial growth

The results presented in Table 5 indicated that the mycelial growth was thick and fluffy for *T. harzianum* and *A. niger* and moderate for *A. ochraceous*. The mycelial growth was feeble in the case of *R stolonifer*.

Among the mushroom fungi *P. sanguineus, G. applanatum, C. indica, P. florida and P. tuber-regium* produced thick cottony growth while *L. edodes* delivered a moderate growth on the substrate (Plate 8).

4.3.2 Time Taken for Complete Colonization in Retted Coirpith

Among the native mciroflora tested *T. harzianum* was found to be the fastest colonizer of the substrate taking only 11.5 days for completely covering the substrate. This was found to be significantly superior over others followed by *R. stolonifer* (18 days). *A. ochraceous* and *A. niger* were found to be poor colonizers taking maximum time of 20 and 21 days respectively (Table 5 and Fig. 1).

G. applanatum was found to be significantly faster among the mushroom flora tested taking 12.5 days for complete coverage followed by *L. edodes* (15.25 days) and *P. tuber-regium* (16 days) which were found to be on par with each other. *P. sanguineus* was found to be the

Plate 8 a Growth of *T. harzianum* on RCP

Plate 8 b Growth of *P. sanguineus* on RCP

Plate 8 c Growth of *G. applanatum* on RCP

Plate 8 d Growth of *C. indica* on RCP

Table 6 Effect of lignocellulolytic fungi on the organic carbon, total nitrogen and C:N ratio of retted coirpith

Fungi	Organic carbon %	Percentage reduction over control	Total Nitrogen (%)	Percentage increase over control	C:N ratio
Aspergillus niger	23.36	32.25	0.91	196.09	25. 90:1
A. ochraceous	21.38	38.01	0.80	161.27	26.78:1
Rhizopus stolonifer	24.34	29.43	0.96	212.58	25.35:1
Trichoderma harzianum	17.09	50.41	1.66	441.50	10.36:1
Pleurotus florida	24.36	29.37	1.01	257.92	24.48:1
P. tuber-regium	19.62	43.10	0.86	181.08	22.91:1
Calocybe indica	28.08	18.57	1.00	227.01	28.00:1
Lentinus edodes	23.35	32.27	1.23	300.94	18.96:1
Ganoderma applanatum	15.43	55.25	0.72	133.99	21.54:1
Pycnoporus sanguineus	23.37	32.22	0.98	219.82	23.76:1
Control	34.49	0	0.31	0	112.21:1
SE	0.283	0.868	0.004	21.70	1.307
CD (0.05)	0.816	2.507	0.106	62.664	3.761

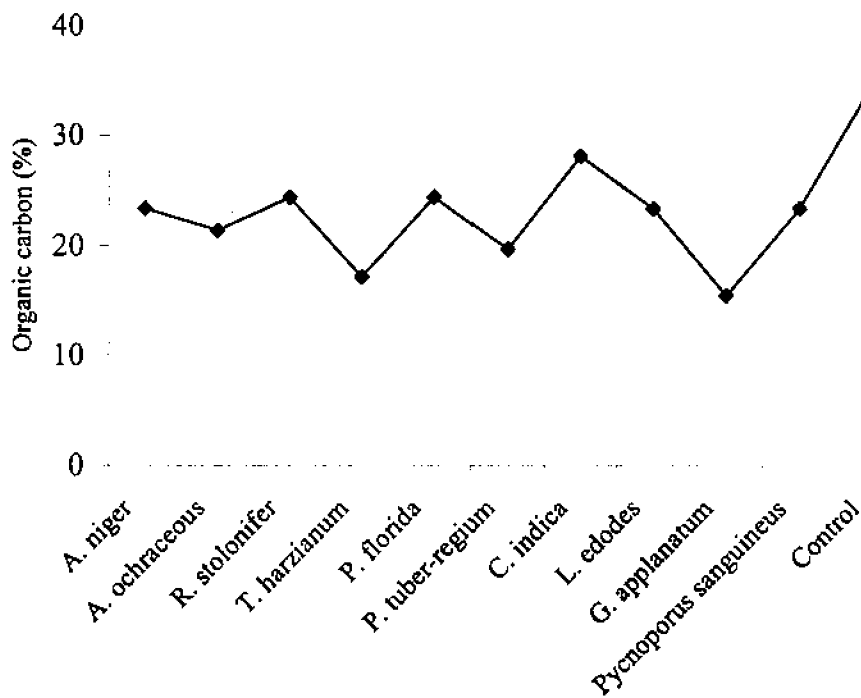


Fig. 2 Effect of lignocellulolytic fungi on organic carbon content

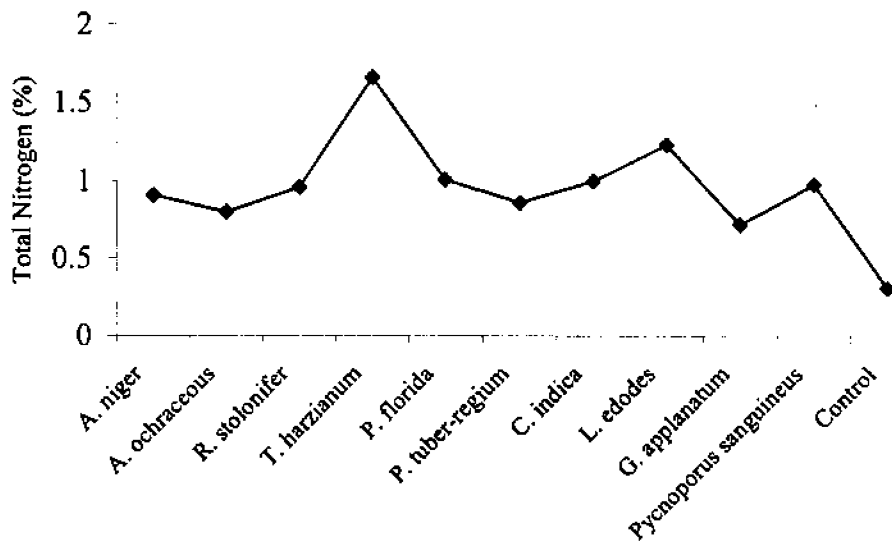


Fig. 3 Effect of lignocellulolytic fungi on total nitrogen content

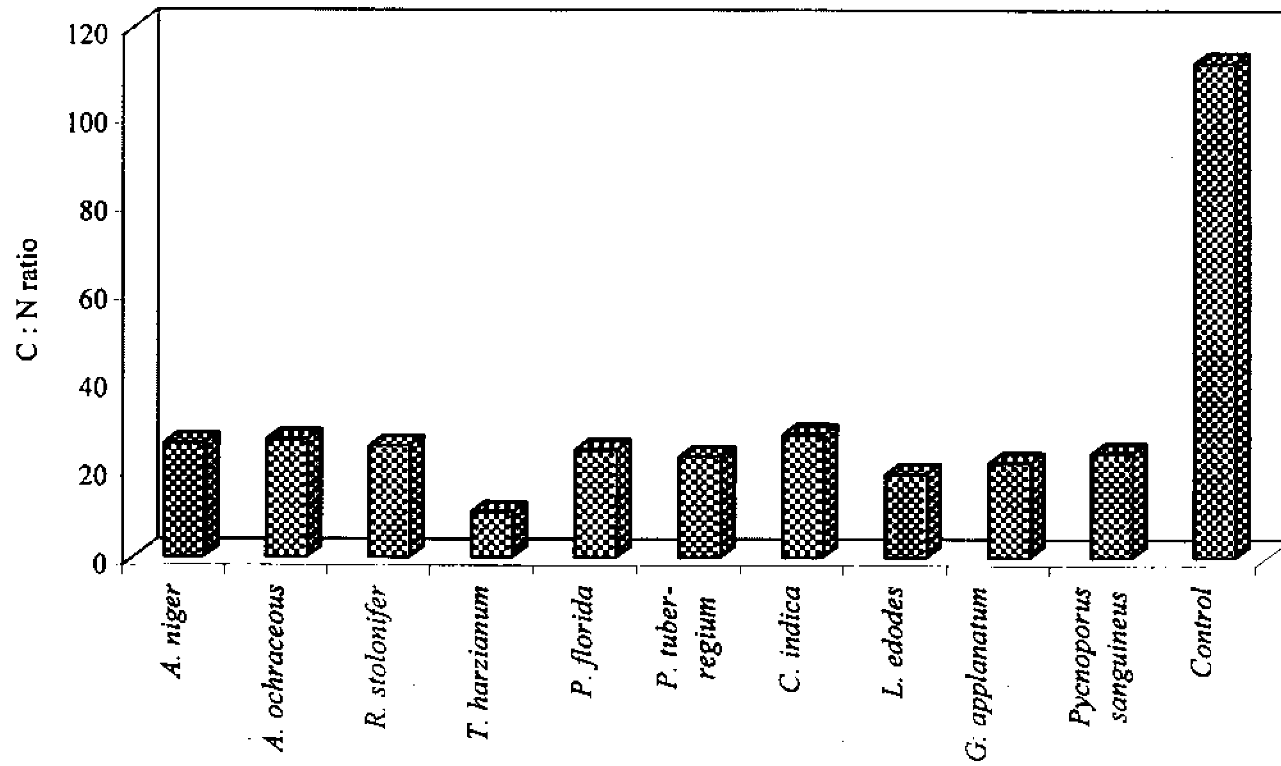


Fig. 4 Effect of lignocellulolytic fungi on C:N ratio

least efficient colonizer which took the maximum time of 22.5 days for covering the substrate.

4.3.3 Organic Carbon

The results on the *in vitro* studies conducted on the degradation of coirpith using different fungi were presented in Table 6 and Fig. 2.

G. applanatum caused the maximum per cent reduction in organic carbon content (55.25 per cent), reducing carbon content from 34.49 to 15.43 per cent followed by *T. harzianum* (17.09 per cent) and *P. tuber-regium* (19.62 per cent) which caused 50.41 and 43.09 per cent reduction over control. *L. edodes*, *A. niger* and *P. sanguineus* were found to be on par with each other causing 32.27, 32.25 and 32.22 per cent reduction over control. *C. indica* was found to be the least efficient degrader which produced only 18.57 per cent reduction of organic carbon compared to control.

4.3.4 Total Nitrogen

All the fungi tested caused increase in N content as a result of degradation. *T. harzianum* caused the maximum increase in N content *i.e.*, 1.66 per cent compared to 0.31 per cent in control, giving rise to 441.50 per cent increase, followed by *L. edodes* which increased nitrogen by 300.94 per cent. *P. florida*, *C. indica*, *R. stolonifer* and *A. niger* were found to be on par with each other causing 1.01, 1.00, 0.96 and 0.91 per cent increase in nitrogen content. *G. applanatum* caused the least increase in nitrogen content *i.e.*, 133.99 per cent increase over control (Table 6 and Fig. 3).

4.3.4.1 C : N Ratio

C : N ratio of retted coirpith was found to be 112.21 : 1 when analysed. After the degradation of coirpith samples with different fungal species, the C : N ratio was diminished. *T. harzianum* produced significantly

Table 7 Effect of lignocellulolytic fungi on the cellulose and lignin content of retted coirpith

Fungi	Cellulose (%)	Percentage reduction over control	Lignin (%)	Percentage reduction over control
Aspergillus niger	17.60	39.78	15.64	51.71
A. ochraceous	21.47	26.48	22.32	31.18
Rhizopus stolonifer	21.41	26.72	26.87	17.03
Trichoderma harzianum	12.65	56.70	12.76	60.68
Pleurotus florida	17.84	38.85	17.46	46.04
P. tuber-regium	21.20	27.28	23.86	26.51
Calocybe indica	19.55	33.00	21.16	34.69
Lentinus edodes	14.21	51.41	16.19	50.06
Ganoderma applanatum	22.72	22.18	24.01	25.87
Pycnoporus sanguineus	25.11	14.04	26.67	17.71
Control	29.21	0	32.43	0
SE	0.640	2.435	0.571	1.948
CD (0.05)	1.841	7.032	1.643	5.625

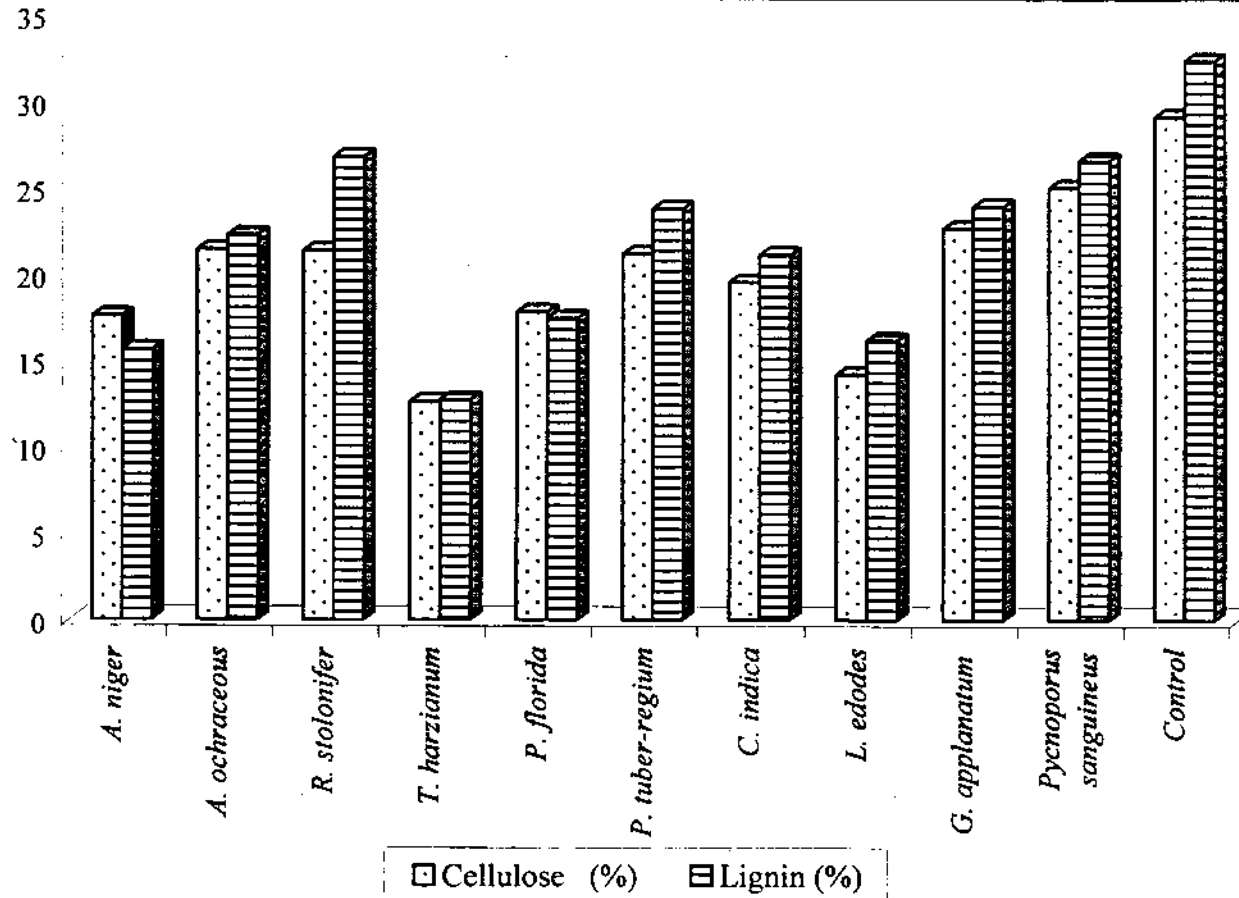


Fig. 5 Effect of lignocellulolytic fungi on cellulose and lignin content

higher reduction of C : N ratio to 10.36 : 1 followed by *L. edodes* (18.96 : 1), *G. applanatum* (21.54 : 1) and *P. tuber-regium* (22.91) while *C. indica* produced significantly lower reduction in C : N ratio (Table 6 and Fig. 4).

4.3.5 Cellulose Content

Cellulose content of retted coirpith was found to be 29.21 per cent. Cellulose content of retted coirpith samples after degradation with different species of fungi were analysed and the results (Table 7 and Fig. 5) showed that the maximum cellulose reduction was brought about by *T. harzianum* (12.65 per cent) indicating 56.70 per cent reduction over control which was found to be highly significant compared to other fungal species except *L. edodes* (14.21 per cent) with 51.41 per cent reduction of cellulose over control followed by *A. niger* (17.60 per cent) and *P. florida* (17.84 per cent) with 39.78 and 38.85 per cent cellulose reduction. *P. sanguineus* was found to be the least efficient degrader of cellulose which caused only 14.04 per cent reduction.

4.3.6 Lignin Content

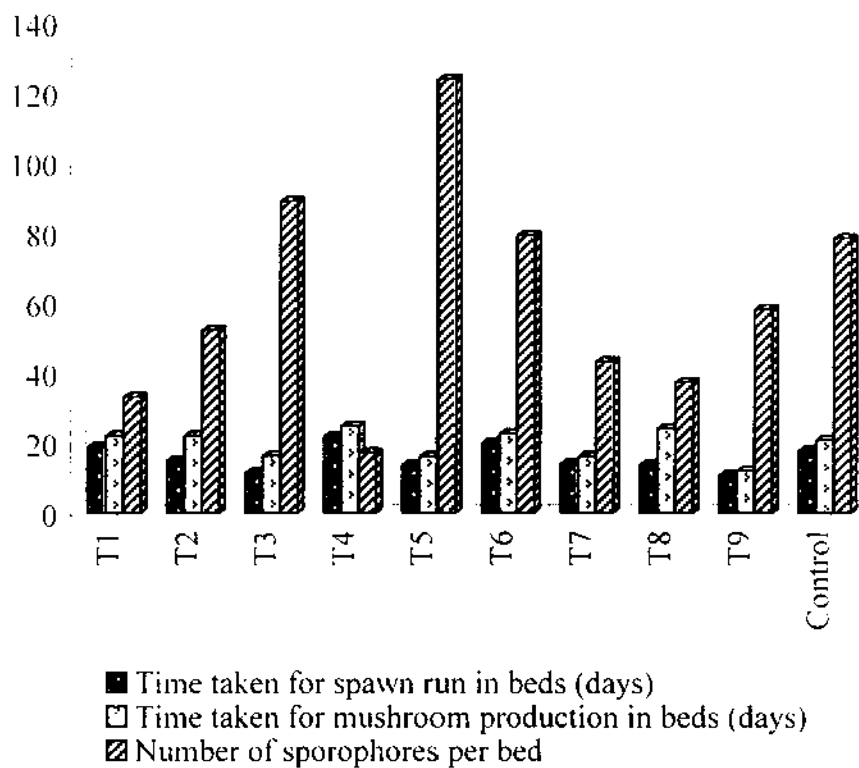
Lignin content of retted coirpith was found to be 32.43 per cent. The lignin content of coirpith samples after degradation with different fungal species presented in Table 7 and Fig. 5 showed that significantly higher lignin reduction was brought about by *T. harzianum* (12.76 per cent) indicating 60.68 per cent reduction over control followed by *A. niger* and *L. edodes* which caused 51.71 and 50.06 per cent reduction of lignin. *C. indica* and *A. ochraceous* were found to be on par with each other causing 21.16 per cent and 22.32 per cent reduction of lignin followed by *P. tuber-regium* (23.86 per cent) and *G. applanatum* (24.01 per cent). *P. sanguineus* and *R. stolonifer* were found to be the least efficient degraders with 26.67 and 26.87 per cent reduction of lignin.

Plate 9 Spawn of *P. florida*

Plate 10 Spawn of *C. indica*

Table 8 Efficacy of retted coirpith on oyster mushroom production

Treatments	Time taken for spawn run in beds (days)	Time taken for mushroom production in beds (days)	Number of sporophores per bed	Total yield / bed (g)
T_1	19.00	22.00	33.13	129.00
T_2	15.00	22.00	52.19	272.98
T_3	11.50	16.25	88.94	440.68
T_4	21.75	24.75	17.13	165.84
T_5	13.75	16.00	123.88	627.50
T_6	20.00	22.50	79.21	401.43
T_7	14.00	16.00	43.19	180.29
T_8	13.75	24.00	37.25	150.80
T_9	10.75	12.00	58.06	286.73
Control	17.75	20.75	78.38	400.78
SE	0.80	0.69	1.85	6.27
CD (0.05)	2.25	1.95	5.22	17.70



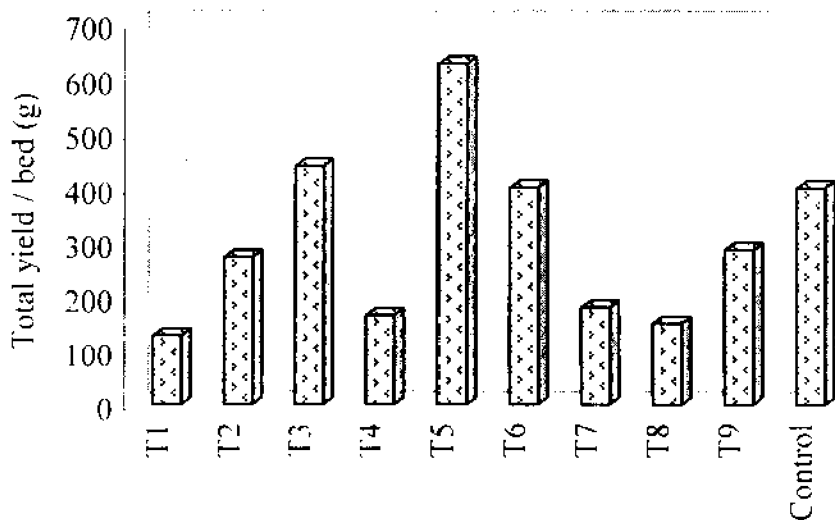


Fig. 6 Efficacy of retted coirpith on oyster mushroom production

4.4 STANDARDISATION OF TECHNIQUES FOR MUSHROOM PRODUCTION ON COIRPITH

4.4.1 Spawn Preparation

Grain spawn of *P. florida* and *C. indica* were prepared on paddy grains (Plate 9 & 10) and 15 days old pure white, good quality spawn were used for laying beds.

4.4.2 Production of *P. florida*

4.4.2.1 Retted Coirpith

Among the different treatments tried for oyster mushroom production, retted coirpith amended with four per cent neem cake (T_9) and fifty per cent paddy straw (T_3) recorded the fastest spawn run which were 10.75 and 11.5 days respectively. These treatments were followed by T_5 (RCP + SMS, 1:1), T_8 and T_7 (two per cent each of starch and cellulose amended beds) and T_2 (RCP + PS, 1:1) which were found to be on par with each other and significantly superior to control (Table 8 and Fig. 6).

Faster mushroom production was obtained in the T_9 followed T_5, T_7 and T_3 were found to be on par with each other and found significantly superior over control.

The maximum yield and sporophore number were seen in a 1 : 1 mixture of retted coirpith and spent mushroom substrate (627.50 g and 123.88 respectively) (Plate 11) which was significantly superior to control (400.78 g and 78.38). A 1:3 combination of retted coirpith and spent mushroom substrate (T_6) which produced an yield of 401.43 g and sporophore number of 79.21 was found to be on par with the control. The least yield as well as sporophore number were recorded when retted coir pith and paddy straw was mixed in the ratio 3 : 1(129 g and 33.13).

As the concentration of paddy straw in retted coirpith increased from 25 to 75 per cent showed a corresponding betterment of all yield

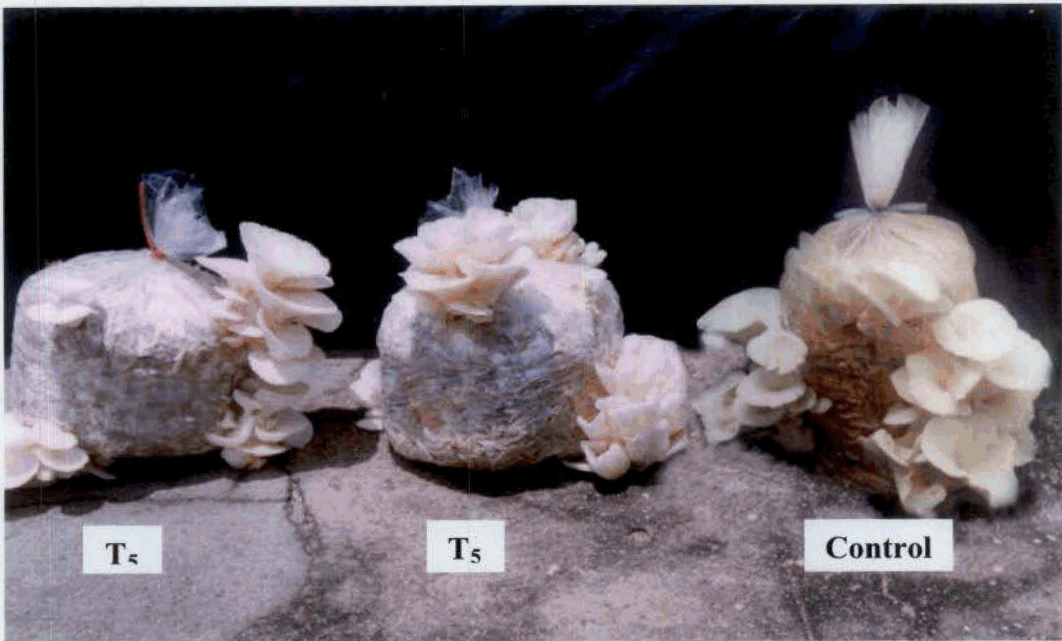


Plate 11 Yield of *P. florida* on RCP + SMS (1:1) compared to control

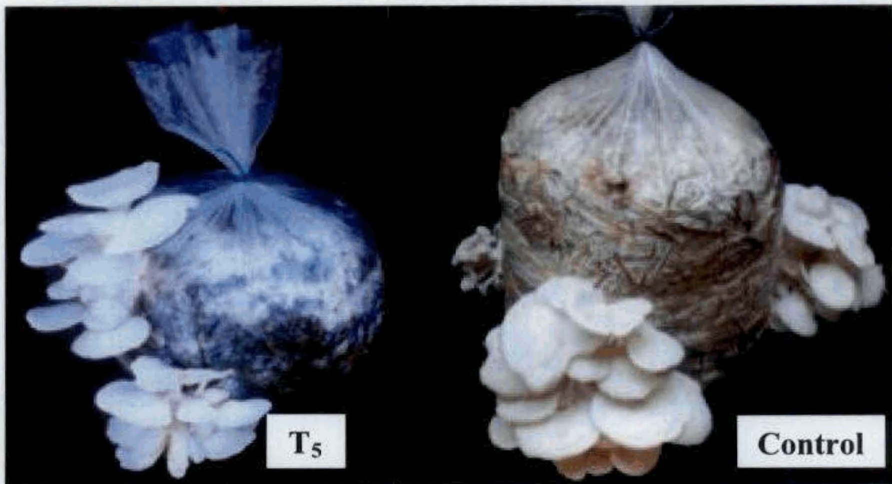


Plate 12 Yield of *P. florida* on NRCP + SMS (1:1) compared to control

Table 9 Efficacy of non-retted coirpith on oyster mushroom production

Treatments	Time taken for spawn run in beds (days)	Time taken for mushroom production in beds (days)	Number of sporophores per bed	Total yield / bed (g)
T_1	19.25	22.00	45.19	225.00
T_2	13.25	24.00	49.56	245.69
T_3	7.00	20.00	55.19	262.50
T_4	24.25	28.75	15.00	59.70
T_5	10.75	19.75	50.50	274.22
T_6	20.50	23.75	49.56	261.45
T_7	25.00	31.25	10.00	41.86
T_8	24.75	30.50	8.81	40.88
T_9	24.50	27.00	24.63	76.77
Control	17.75	20.75	78.38	400.78
SE	0.80	0.69	1.85	6.27
CD (0.05)	2.25	1.95	5.22	17.70

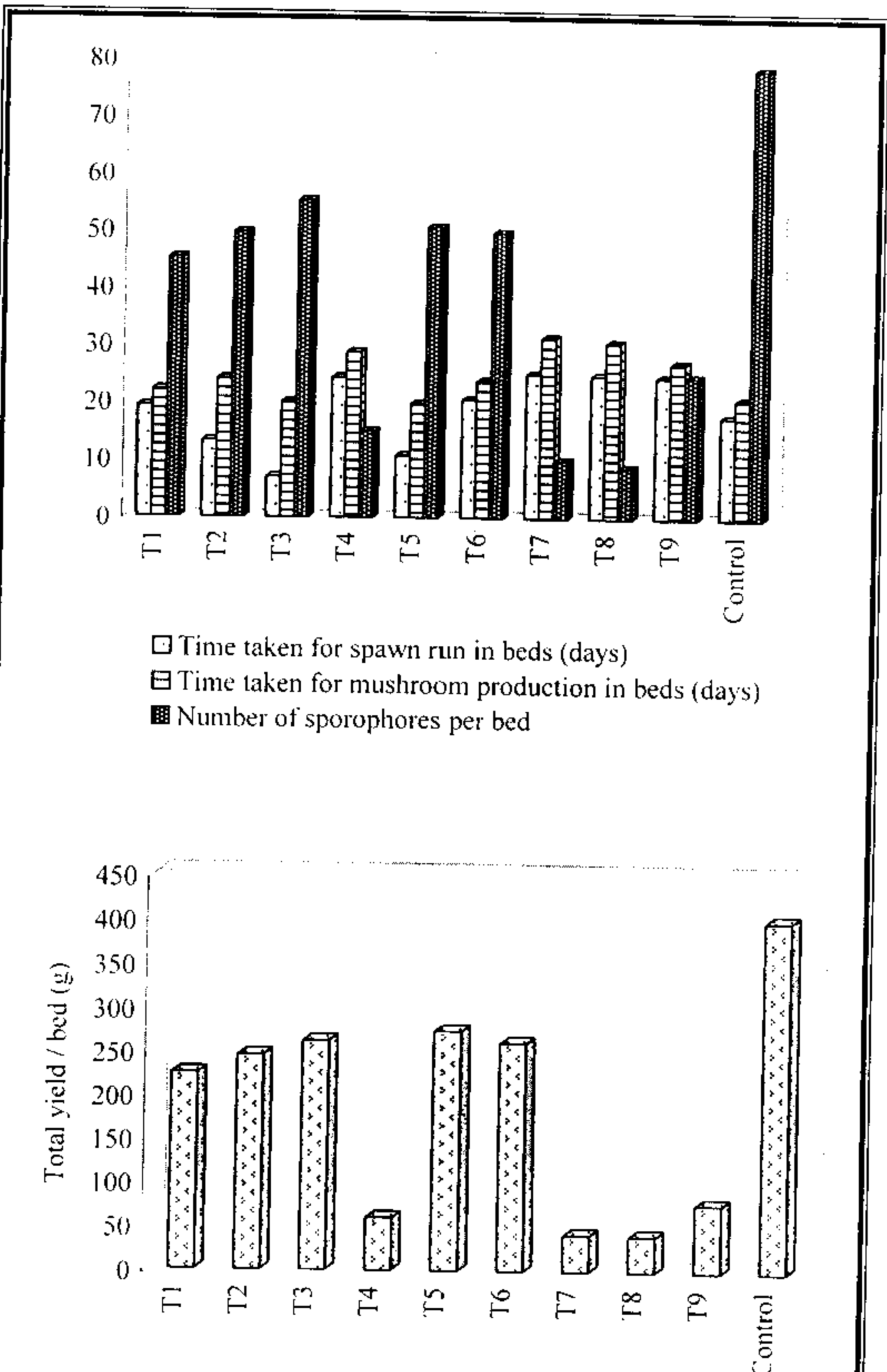


Fig. 7 Efficacy of non-retted coirpith on oyster mushroom production

parameters. In the case of spent mushroom substrate, increase in yield was observed as a concentration increased from 25 to 50 per cent and thereafter the yield decreased.

4.4.2.2 Non-Retted Coirpith

A 1 : 3 mixture of non retted coirpith and paddy straw (T_3) took the minimum time of seven days for spawn run. This was followed by T_5 (NRCP+SMS, 1:1) and T_2(NRCP+PS,1:1) taking 10.75 and 13.25 days respectively. These treatments were found significantly superior to control (17.75 days).

. The time taken for mushroom production is noticed in treatments T_5 and T_3 was on par with the control beds. A two per cent starch and cellulose amended beds took the maximum time for mushroom production (30.5 and 31.25 days respectively) (Table 9 and Fig. 7).

Yield realized in T_5, T_3 and T_6 (274.22, 262.5 and 261.80g respectively) (Plate 12, 13 and 14) were on par and was inferior to the control. The yield obtained in non-retted coir pith amended with starch and cellulose were as low as one tenth of the control.

4.4.2.3 Composted Coirpith

Composted coirpith was not found to be a good medium for production of oyster mushroom, when composted coirpith was amended with starch and cellulose it failed to support the growth of the mushroom (Table 10 and Fig. 8). Compared to 17.75 days in control all the treatments using composted coirpith took more than 24 days to complete spawn run. Similarly the time required for sporophore production in these treatments was more than six to eleven days compared to control. The maximum yield in these treatments was recorded in the bed prepared by mixing composted coirpith and paddy straw in the ratio 1 : 3 (69.26) (Plate 15).

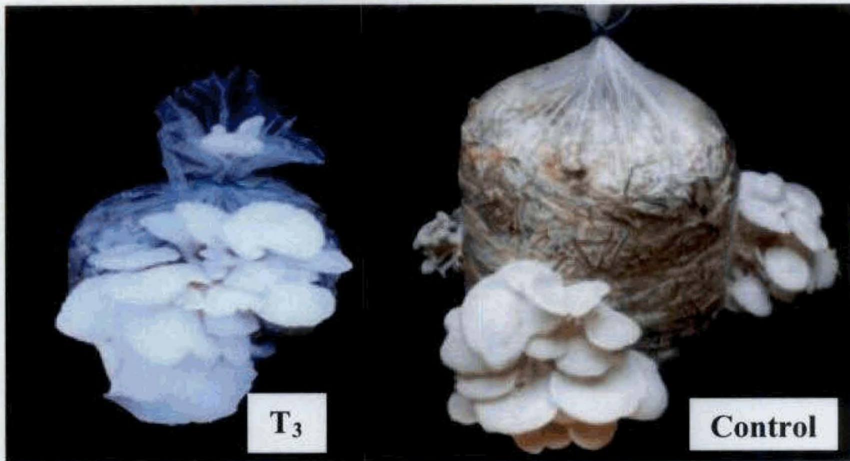


Plate 13 Yield of *P. florida* on NRCP + PS (1:3) compared to control

Plate 14 Yield of *P. florida* on NRCP + SMS (1:3) compared to control

Plate 15 Yield of *P. florida* on CCP + PS (1:3) compared to control

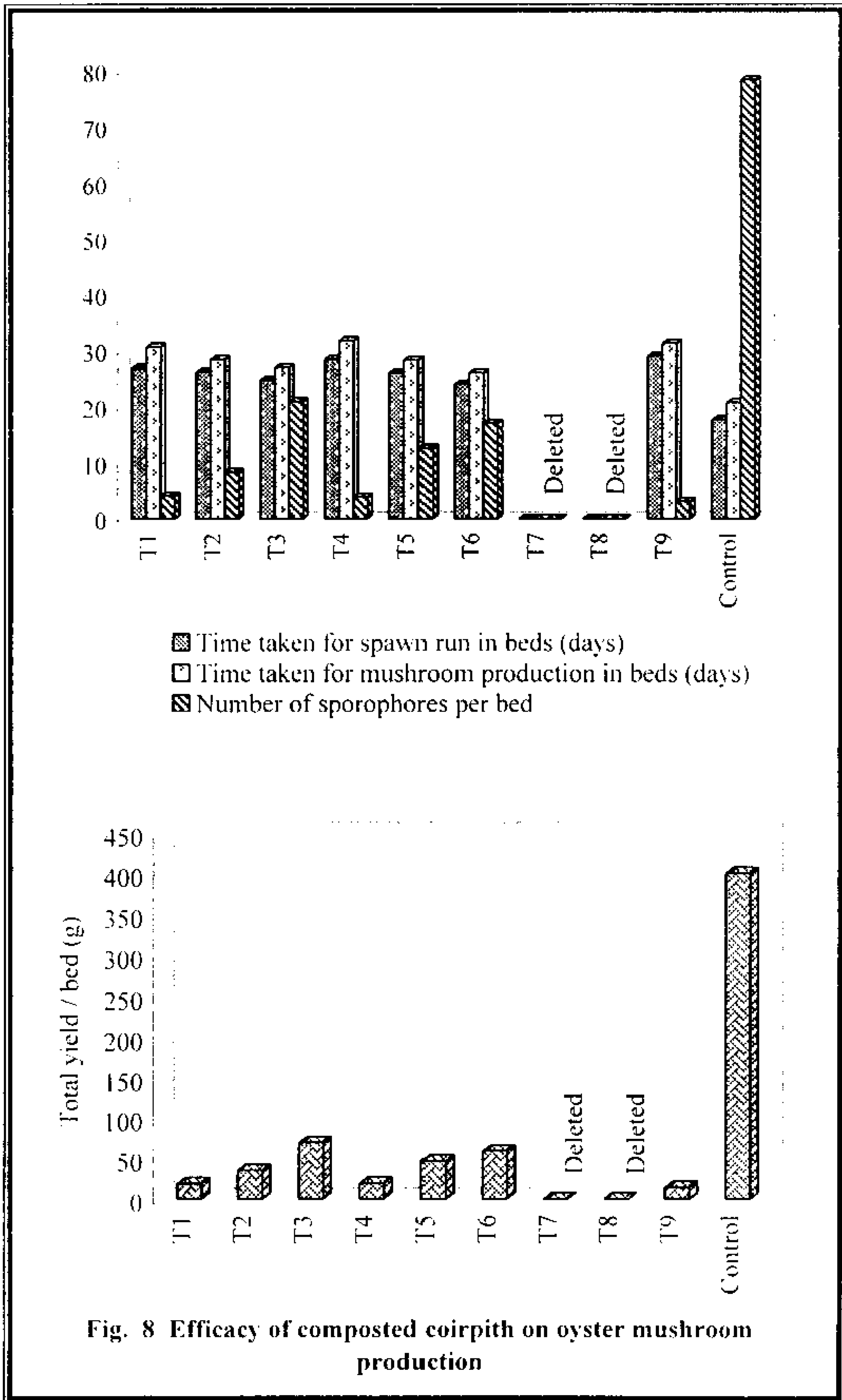


Fig. 8 Efficacy of composted coirpith on oyster mushroom production

Table 10 Efficacy of composted coirpith on oyster mushroom production

Treatments	Time taken for spawn run in beds (days)	Time taken for mushroom production in beds (days)	Number of sporophores per bed	Total yield / bed (g)
T_1	27.00	30.75	4.13	19.16
T_2	26.25	28.50	8.44	35.65
T_3	24.75	27.00	20.94	69.26
T_4	28.50	31.75	3.94	19.50
T_5	26.00	28.25	12.63	46.66
T_6	24.00	26.00	17.06	58.78
T_7	-	-	-	-
T_8	-	-	-	-
T_9	29.00	31.25	3.13	14.69
Control	17.75	20.75	78.38	400.78
SE	0.80	0.69	1.85	6.27
CD (0.05)	2.25	1.95	5.22	17.70

Table 11 Correlation between retted and non-retted coirpith on oyster mushroom production

Particulars	RCP	NRCP	SE	CD
Time taken for spawn run in bed (days)	15.50	18.81	0.27	0.75
Time taken for mushroom production in beds (days)	19.50	25.22	0.23	0.65
Number of sporophores per bed	59.22	34.27	0.62	1.74
Total yield per bed (g)	283.91	165.34	2.09	5.90

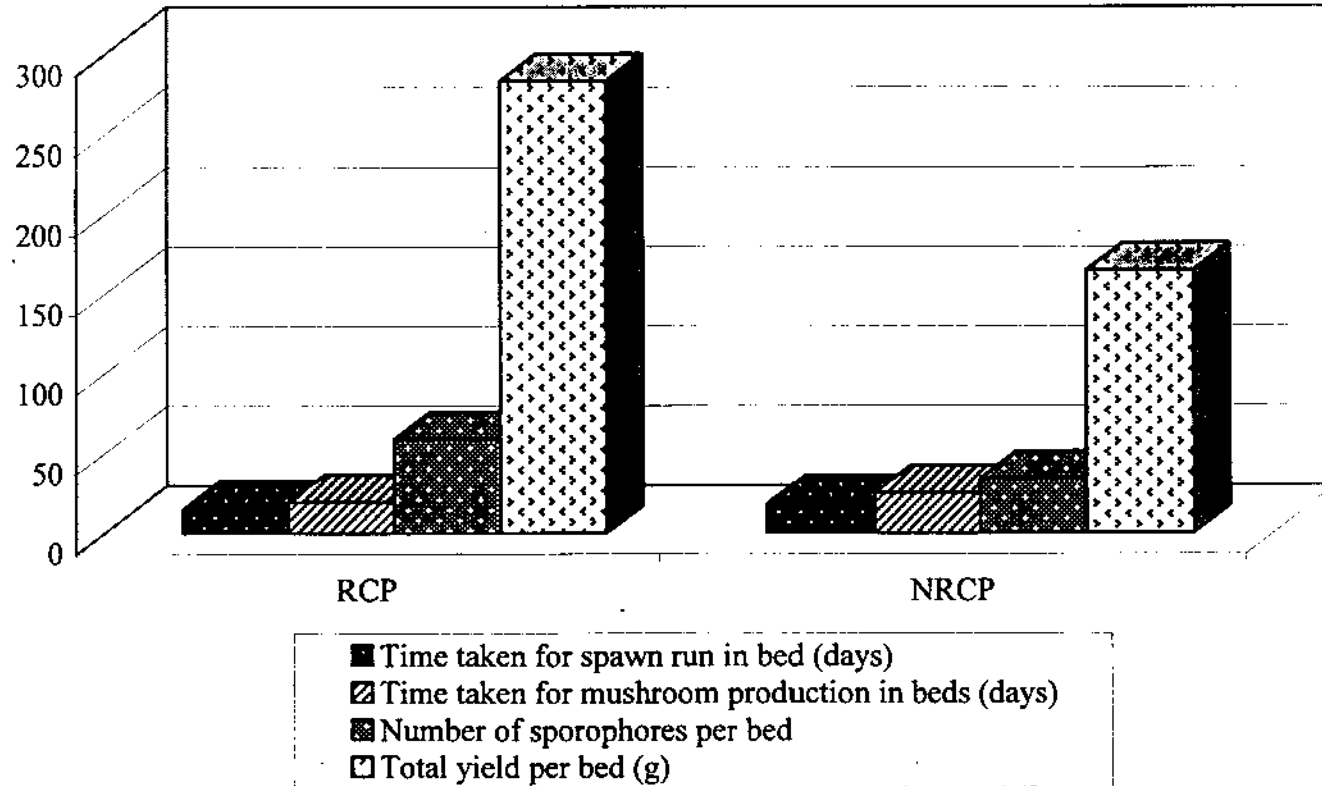


Fig. 9 Correlation between retted and non retted coirpith on oyster mushroom production

4.4.2.4 Comparative Efficacy of non-retted coirpith and retted coirpith on Oyster Mushroom Production

Results presented in Table 11 and Fig. 9 on the interaction between retted coirpith and non-retted coirpith indicated that retted coirpith was significantly superior to non-retted coirpith in all the yield parameters tested in the production of oyster mushroom. It boosted oyster mushroom production by promoting earliness in spawn run and mushroom production, which were 15.5 days, 19.5 days respectively. Similarly yield was also more in beds prepared out of retted coirpith.

4.4.3 Production of *C. indica*

4.4.3.1 Retted Coirpith

The time taken for spawn run in beds prepared from varying combinations of retted coirpith and other amendments varied from 13.50 to 20.75 days (Table 12 Fig. 10). Except treatments T_3 where it took 13.50 days to completely cover the substrate and T_1 which took 20.75 days, all other treatments were on par with the control where paddy straw was used as the substrate.

The time taken for mushroom production in retted coirpith varied from 25.50 (T_3) to 32.75 (T_1). These treatments also took minimum and maximum time to cover the mushroom bed. There was significant difference in the time taken for mushroom production when the retted coirpith was amended with different concentrations of spent mushroom substrate.

Compared to 75 per cent biological efficiency observed in control, the biological efficiency of retted coirpith ranged from 14.40 per cent [RCP + NC (4%)] to 60 per cent [RCP + PS (1:3)]. Out of the nine treatments only two (T_3 and T_5) gave a biological efficiency of more than 50 per cent. All these treatments were inferior to the control (Plate 16).

172187

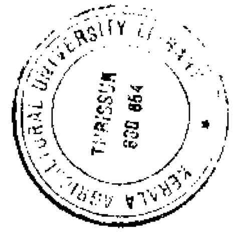


Table 12 Efficacy of retted coirpith on milky mushroom production

Treatments	Time taken for spawn run in beds (days)	Time taken for mushroom production in beds (days)	Number of sporophores per bed	Total yield / bed (g)
T_1	20.75	32.75	2.06	148.53
T_2	18.50	28.50	3.38	165.75
T_3	13.50	25.50	3.86	301.44
T_4	18.00	31.00	2.25	132.45
T_5	15.00	29.00	3.00	285.33
T_6	15.50	28.50	2.56	154.75
T_7	15.50	31.75	1.75	116.06
T_8	17.25	32.75	1.75	90.75
T_9	17.25	29.25	1.88	72.50
Control	17.75	27.75	3.31	350.25
SE	0.89	0.95	0.17	8.77
CD (0.05)	2.52	2.69	0.47	24.75

Plate 16 Yield of *C. indica* on RCP + PS (1:3) compared to control

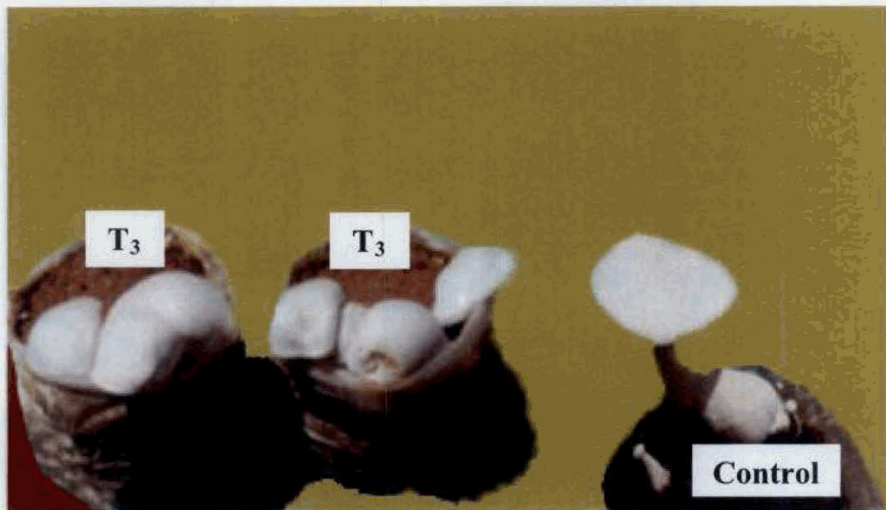


Plate 17 Yield of *C. indica* on NRCP + PS (1:3) compared to control

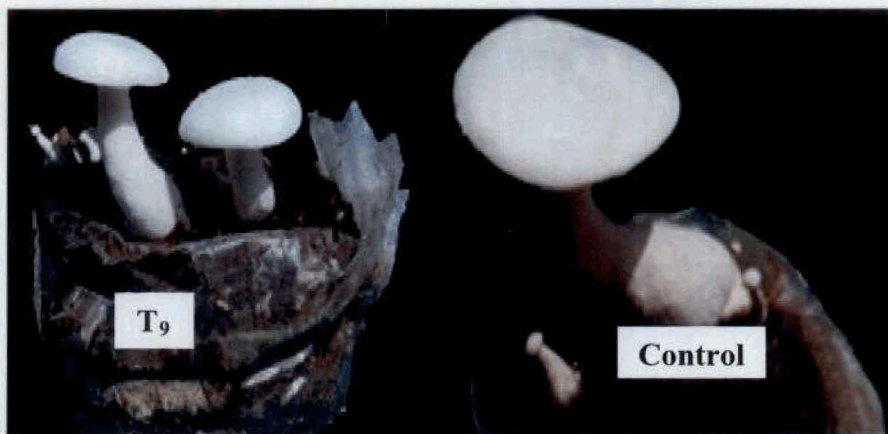
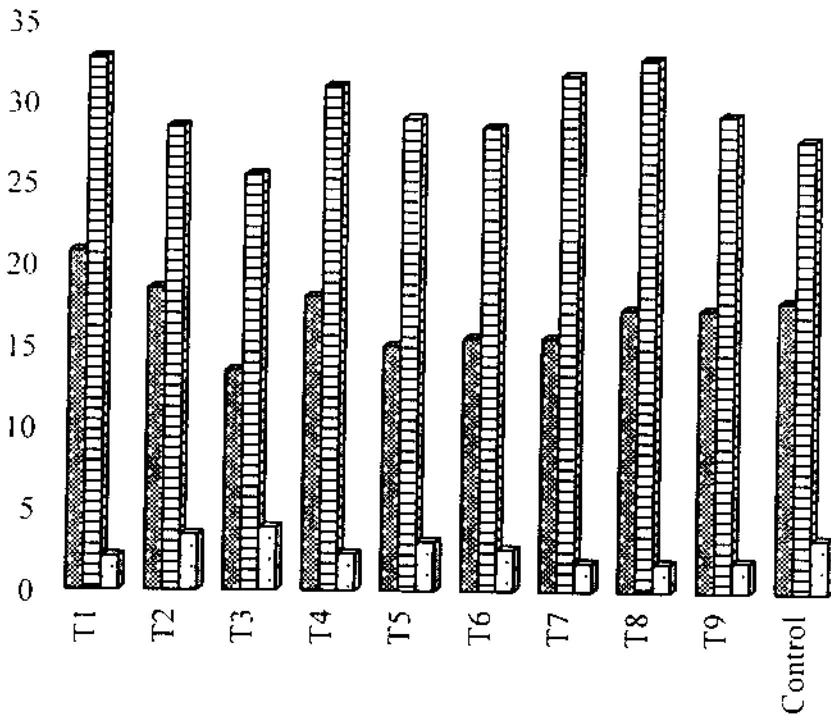


Plate 18 Yield of *C. indica* on CCP + neem cake (four per cent) compared to control



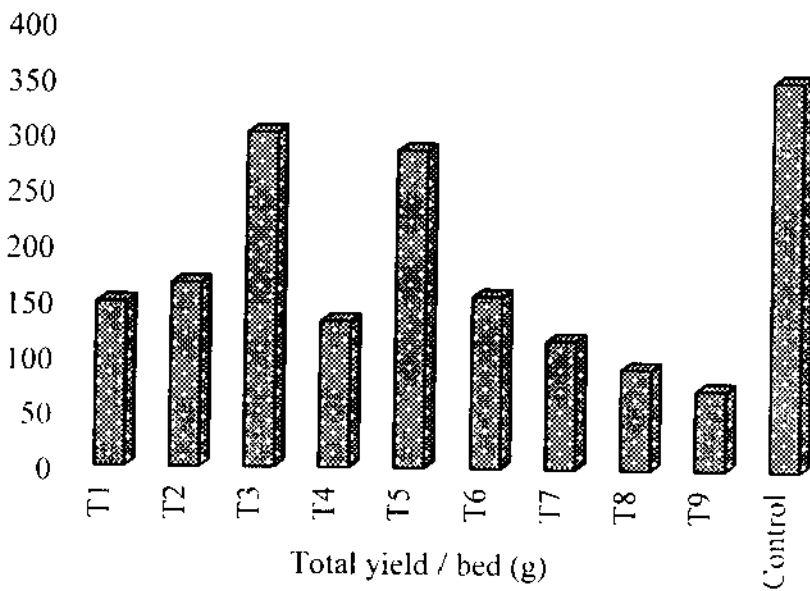


Fig. 10 Efficacy of retted coirpith on milky mushroom production

4.4.3.2 Non Retted Coirpith

Most of the treatments were significantly superior to control beds in spawn run. Treatments T_5 (NRCP + SMS, 1 : 1), T_7 (two per cent cellulose amended beds) and T_8 (two per cent starch amended beds) promoted faster spawn run with 11.25, 11.5, 12 days respectively which were found on par with each other. These were followed by T_3 (NRCP + PS, 1 : 3). All these treatments were found significantly superior over control in spawn run (17.75 days) (Table 13 and Fig.11).

The fastest mushroom production was observed in T_3, T_2 (NRCP + PS, 1 : 1), T_5 and T_7 taking 27, 27.5, 29 and 29.5 days respectively which were found to be on par with each other as well as with the control (27.75 days).

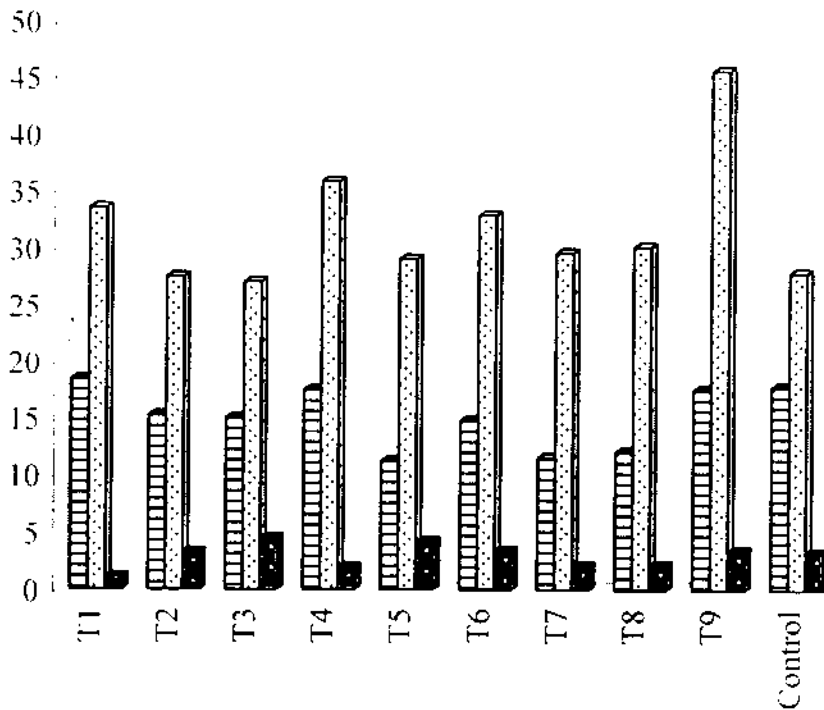
Maximum number of sporophore and yield of 4.5 and 497.5 g was realized in the case of T_3 (Plate 17) which was found significantly superior to control with 3.31 and 350.25 g, respectively. This was followed by T_5 with 4.19 and 342.31 g which was on par with control. In the case of T_2, yield of 297.5 g and sporophore number of 3.31 were observed. The least yield of 61.25 g and sporophore number of 1.13 were observed in the case of T_1 (NRCP + PS, 3 : 1) which was found to be on par with T_8 with 85.75 g and 2.19 number, respectively. Treatments T_3, T_5 and T_2 showed a biological efficiency of more than 50 per cent.

4.4.3.3 Composted Coirpith

Results as per Table 14 and Fig. 12 indicated that composted coirpith was a poor source for milky mushroom production. None of the treatments promoted significantly any of the yield parameters compared to control. A four per cent neem cake amended composted coirpith (T_9) and T_3 (CCP + PS, 1 : 3) took comparatively less time in spawn run *i.e.*, 23.5 and 25.75 days respectively which were found to be on par with each

Table 13 Efficacy of non-retted coirpith on milky mushroom production

Treatments	Time taken for spawn run in beds (days)	Time taken for mushroom production in beds (days)	Number of sporophores per bed	Total yield / bed (g)
T_1	18.50	33.50	1.13	61.25
T_2	15.25	27.50	3.31	297.50
T_3	15.0	27.0	4.50	497.50
T_4	17.50	35.75	2.00	104.69
T_5	11.25	29.00	4.19	342.31
T_6	14.75	32.75	3.44	186.50
T_7	11.50	29.50	2.13	126.56
T_8	12.00	30.00	2.19	85.75
T_9	17.50	45.50	3.44	158.00
Control	17.75	27.75	3.313	350.25
SE	0.89	0.95	0.17	8.77
CD (0.05)	2.52	2.69	0.47	24.75



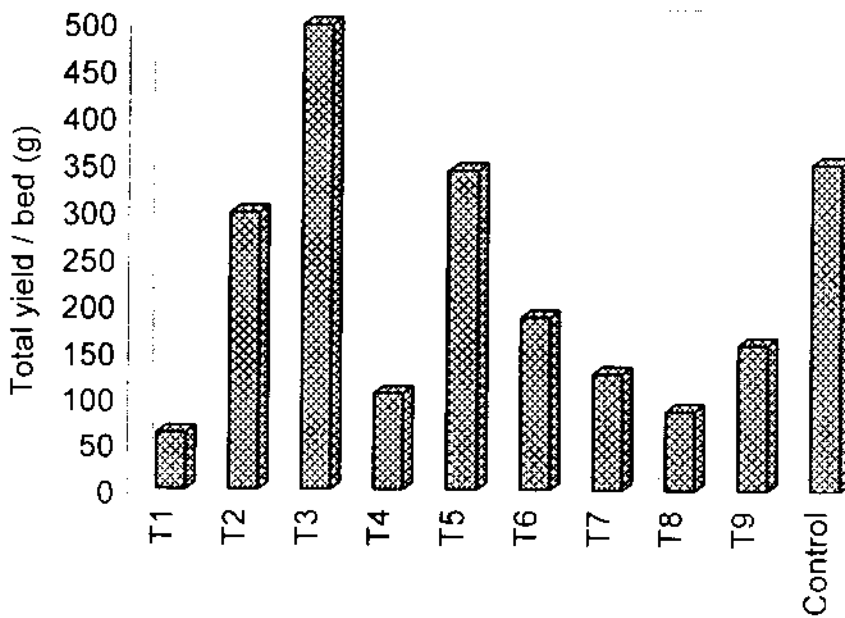


Fig. 11 Efficacy of non-retted coirpith on milky mushroom production

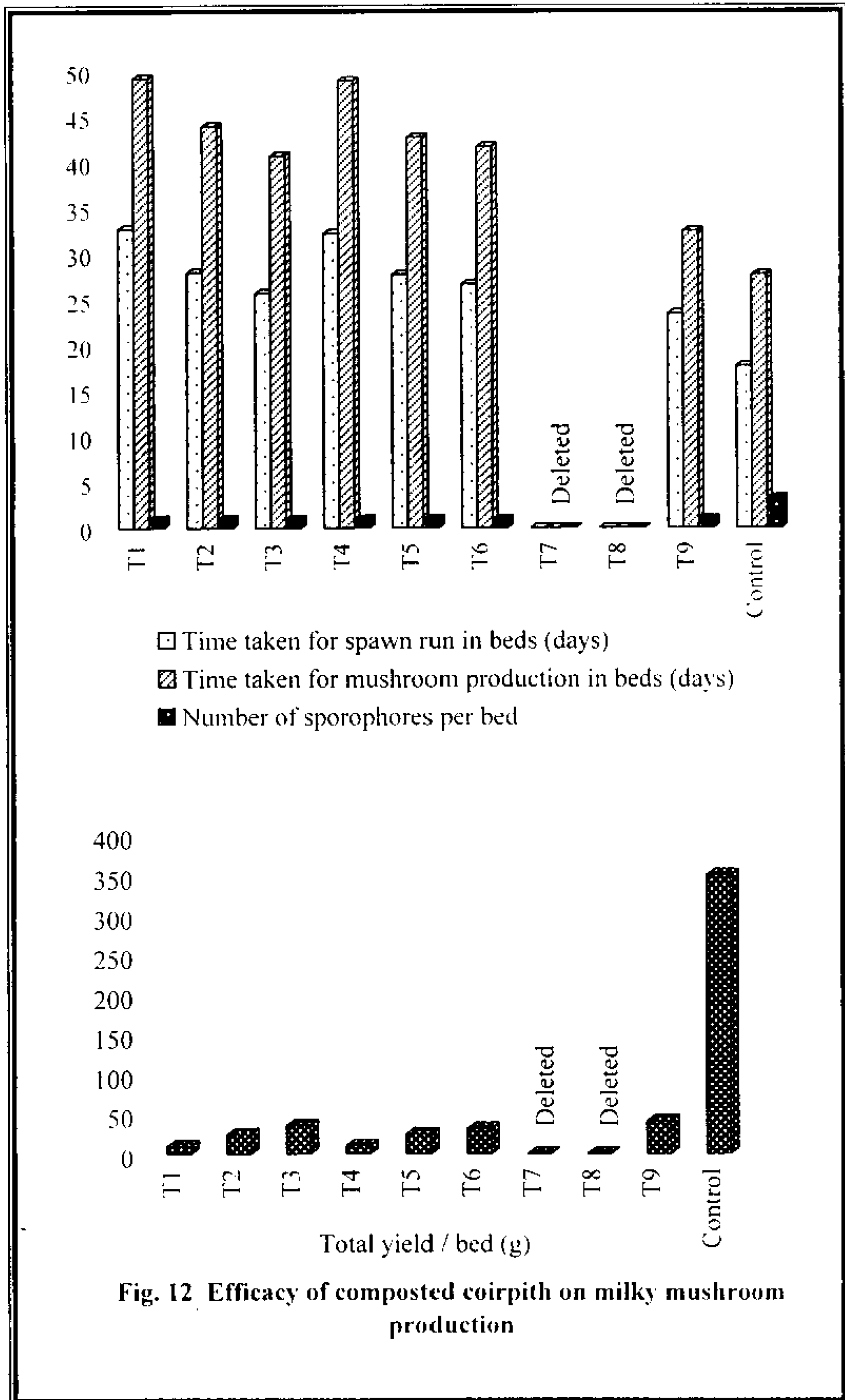


Fig. 12 Efficacy of composted coirpith on milky mushroom production

Table 14 Efficacy of composted coirpith on milky mushroom production

Treatments	Time taken for spawn run in beds (days)	Time taken for mushroom production in beds (days)	Number of sporophores per bed	Total yield / bed (g)
T_1	32.75	49.25	1	9.88
T_2	28.00	44.00	1	24.93
T_3	25.75	40.75	1	36.45
T_4	32.25	49.00	1	11.10
T_5	27.75	42.75	1	25.23
T_6	26.75	41.75	1	32.08
T_7	-	-	-	-
T_8	-	-	-	-
T_9	23.50	32.50	1	41.43
Control	17.75	27.75	3.31	350.25
SE	0.89	0.95	0.17	8.77
CD (0.05)	2.52	2.69	0.47	24.75

Table 15 Correlation between retted and non-retted coirpith on milky mushroom production

Particulars	RCP	NRCP	SE	CD
Time taken for spawn run in bed (days)	16.81	14.81	0.30	0.84
Time taken for mushroom production in beds (days)	29.89	32.28	0.32	0.90
Number of sporophores per bed	2.50	2.92	0.06	0.16
Total yield per bed (g)	163.06	206.67	2.92	8.25

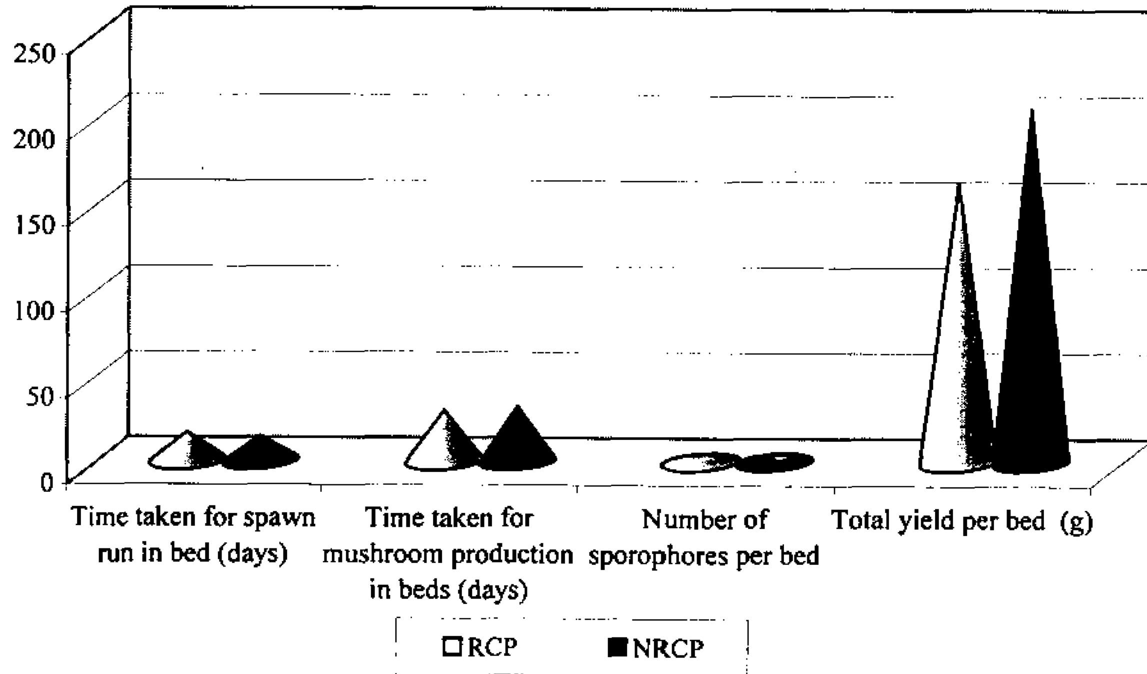


Fig. 13 Correlation between retted and non retted coirpith on milky mushroom production

other. This was followed by T_6 (CCP + SMS, 1 : 3), T_5 (CCP + SMS, 1 : 1) and T_2 (CCP + PS, 1 : 1) taking 26.75, 27.75 and 28 days respectively which were also on par with each other. T_1 (CCP + PS, 3 : 1) took the maximum time of 32.75 days for spawn run.

T_9 promoted faster mushroom production taking 32.5 days for the appearance of fruiting bodies, followed by T_6 and T_5 which were found to be on par with each other. The yield of milky mushroom obtained in composted coirpith ranged from 9.88 g (T_1) to 41.43 g (T_9) (Plate 18). None of the treatments had a biological efficiency of more than 10 per cent.

4.4.3.4 Comparative Efficacy of Retted and Non-retted Coirpith in Milky Mushroom Production

Milky mushroom preferred non-retted coirpith for its growth and production of sporophores than retted coirpith. This mushroom took two days more time for completely covering the bed and mushroom production in retted coirpith compared to non-retted coirpith. Similarly the yield obtained per bed in retted coirpith (163.06 g) was more than 20 per cent less compared to non-retted coirpith (206.67 g) (Table 15 and Fig.13).

DISCUSSION

5. DISCUSSION

Lignocellulosic substances are the most abundant naturally occurring organic polymers in the biosphere. This include different straws, sugarcane bagasse, cotton wastes, coirpith (coir wastes), cocoa pods, different oil cakes, sunflower straw, groundnut pod shell, tapioca starch waste, water hyacinth and certain other dry and wet land weeds, banana pseudostem and market wastes (Jandaik, 2002). All of these may constitute a perennial source of raw materials for mushroom production. Among this, the most common substrate utilized for mushroom production is paddy straw. But in Kerala paddy straw is a costly substrate, while coirpith is available in plenty.

One tonne of coirpith is produced from approximately 10,000 nuts (Nagarajan *et al.*, 1985). In India, 7.5 million tonnes of coirpith are produced annually (Kamaraj, 1994), out of which major contribution is from Kerala. At present coir industries are facing problems of disposal of this waste because of its low burning quality and resistance to microbial degradation. The low biodegradability is mainly due to the presence of soluble tannin related phenolic compounds (8-12 per cent) (Fan *et al.*, 1982), high C : N ratio and high lignin content (Kadalli and Nair, 2000). Because of these factors it is not being applied in the field as a manure in its raw form.

In India, mushrooms are mainly cultivated on wheat and paddy straw. These substrates have several other industrial and domestic uses such as cattle feed, raw material for paper making, for thatching mud houses, etc. Use of this costly substrate is the main reason for high cost of production of mushrooms in Kerala. The present study was therefore undertaken to screen the most efficient fungi for biodegradation of

coirpith and to find out the suitability of using coir pith as a substrate for oyster and milky mushroom production.

In the present study, the native microflora such as *Aspergillus niger*, *A. ochraceous*, *Rhizopus stolonifer* and *Trichoderma harzianum* were isolated from retted coirpith. These fungi were screened to find out their efficacy in biodegrading retted coirpith. Three types of mushroom were isolated from stumps of coconut and mango during a survey conducted in different parts of Thiruvananthapuram district of Kerala during South West monsoon period. In addition to these, *Pleurotus florida*, *Calocybe indica* and *Lentinus edodes* were also procured from Instructional Farm, College of Agriculture, Vellayani and All India Coordinated Mushroom Improvement Project, Tamil Nadu Agricultural University, Coimbatore and included in the study.

The carpophore of one of the collected mushrooms was sessile and bracket shaped, with deep orange-red colour tending to darken, pubescent which turn glabrous on maturity and with faint zonations. These descriptions were similar to that of *Pycnoporus sanguineus* (Fr.) Murrill and it was further confirmed by Peter Roberts, Mushroom Taxonomist, London. The carpophores of the second mushroom isolated were pure white, very hard textured, sessile, bracket-shaped, turning brown when touched, flesh cinnamon brown and felt like. Based on these characters it was identified as *Ganoderma applanatum* (Persoon) Patouillard. Similar findings reported by Findlay (1977) and Pacioni (1985) confirms the identity of this mushroom. The sporophore of the third mushroom isolated were medium to big sized, creamy white coloured with slight yellow in the centre, soft at immature stage and medium leathery at maturity. The characters of this mushroom was similar to *Pleurotus tuber-regium* (Fr.) Singer and it was further confirmed by Peter Roberts. *P. tuber-regium* is a new record of mushroom from India and is found to be a promising species for cultivation in Kerala with 46 per cent biological efficiency.

Organisms belonging to basidiomycotina like mushrooms, are capable of degrading coirpith by acting upon cellulose and lignin components. Out of the ten fungi used for the decomposition of retted coirpith, *T. harzianum* was the fastest in colonizing the substrate indicating its aggressive nature of growth. *G. applanatum* and *T. harzianum* were two fungi which were highly efficient in mineralising the organic carbon content of the coirpith. This is clear from the fact that these fungi could reduce the carbon content of the coirpith by 55.25 and 50.41 per cent over the control. Changes in the organic carbon content is an indirect estimate of organic matter content (OC x 1.724). Apart from reducing the carbon content of coirpith, *T. harzianum* also increased the nitrogen content of the coirpith to 1.66 per cent which is 441.50 per cent more than what is observed in undecomposed coirpith. High increase in the nitrogen content of the coirpith was also brought about by *L. edodes*. The decomposition of coirpith is effected by means of enzymes liberated by the organisms growing on it. Theradimani and Marimuthu (1992) observed a positive correlation between the enzyme produced by the fungi and rate of degradation of coirpith.

According to Ramamoorthy (1998) the cellulase production by *T. harzianum* is correlated with the biodegradability and a consequent reduction in C : N ratio, thus supporting the present observation. In the present study the lowest C : N ratio was recorded in coirpith degraded by *T. harzianum* (10.36 : 1) which confirmed the finding of Ramamoorthy *et al.* (1999). This finding is further supported by the work of Shirkot *et al.*, 2001). According to them *Trichoderma* sp. isolated from mushroom compost possessed good amount of cellulolytic activity and these produce fairly good amount of carboxy methyl cellulase, xylanase and beta glucosidase which have capacity to degrade plant polysaccharides to simple sugars.

In the present study *C. indica* was found to be the least efficient degrader of coirpith, when C : N ratio was used as a yardstick for assessing the efficacy of organism in degradation. This may be because of the poor enzyme production and activity by *C. indica* on the substrate (Ramamoorthy *et al.*, 1999). Coirpith, being a high lignocellulosic substance, inorder to be an efficient degrader, the fungus must produce sufficient amount of cellulase, laccase, polyphenol oxidase etc. to exploit the substrate completely and to convert it into a useful compost of low C:N ratio. Probably *C. indica* lacked such an efficient enzyme system.

The reduction of C : N ratio of retted coirpith may be due to microbial utilization of coirpith and by immobilization of nitrogen into the cells of the colonizers. While most of the carbon is released as carbon dioxide (Alexander, 1977) the nitrogen remained entrapped in the fungal cell and remained in the decomposed coirpith without loss. Decomposition by microbes also substantially reduced the volume of coirpith, which may result in an increased nutrient concentration in the final product (Reeja, 2002). Moreover, the nitrogen fixing ability of higher fungi may also contribute to increased nitrogen content of coirpith. The nitrogen fixing ability of *P. sajor-caju* and other higher fungi have been reported by Rangaswami *et al.* (1975) and Thayumanavan (1979 and 1980).

The decrease in cellulose content of the coirpith in the study, as a result of fungal decomposition ranged from 14.04 to 56.70 per cent. This is because these fungi preferred substrate rich in cellulose for their growth (Zadrazil, 1978; Rai and Saxena, 1991; Geetha and Sivaprakasam, 1998a). The organism which can produce cellulolytic and lignolytic enzymes were found to be efficient degraders of coirpith (Ramamoorthy *et al.*, 1999). Among the fungi tested, *T. harzianum*, *L. edodes* and *A. niger* reduced cellulose and lignin content of retted coirpith to the maximum extent. The lignin content of the coirpith as a result of fungal decomposition ranged

from 17.71 to 60.68 per cent over the control. A positive correlation was observed between the rate of lignin and cellulose degradation. All the fungi which were efficient degraders of lignin were also efficient cellulose degraders. This is clearly exhibited in the present investigation. *T. harzianum* was an efficient lignin and cellulose degrader while *P. sanguineus* was the most inefficient cellulose and lignin degrader. A similar result was reported by Saxena and Rai (1992). Lignin degradation by the fungus helps to increase the accessibility of native celluloses to cellulolytic enzyme and thereby increasing the rate of decomposition.

In Kerala, currently mushrooms are commercially cultivated using paddy straw as a substrate. Paddy straw being very costly, the farmers are finding it difficult to get better profits using this substrate. In the present investigation, attempts were made to use the most widely available organic matter in Kerala namely coirpith as a substrate for growing oyster and milky mushroom. Coirpith is available as retted and non-retted types. Mostly retted coirpith is available in Kerala, while non-retted types are popular in Tamil Nadu. In the present investigation efficiency of mushroom production on retted, non-retted and composted coirpith was studied. Among these three substrates tried, the composted coirpith did not support sporophore production of both *P. florida* and *C. indica*. Even in retted and non-retted coirpith, the production of sporophores was only 58.25 and 42.55 g for *P. florida* and 38.25 and 58.45 g for *C. indica* respectively. Relatively low yield in the coir pith could be due to the poor ability of fungus to degrade and utilize the hard lignocellulosic fibre. The structural features of coir pith limit the sites for enzymatic attack as the lignin surrounding the cellulose form a physical barrier and the cellulose present in the biomass possess a highly resistant crystalline structure. But improved yield has been reported in a mixture of substrates when compared to individual substrates (Burgarski *et. al.*, 1996). Thus, inorder to improve the sporophore producing ability of these substrates, they were

amended with paddy straw, spent mushroom substrate, neem cake, cellulose and starch in different proportions.

The spent mushroom substrate was the leftovers obtained after harvesting oyster mushroom on paddy straw. Before mixing it with coirpith it was sterilized using carbendazim (75 ppm) and formalin (500 ppm). Amending RCP with 50 per cent SMS was the best in realizing maximum number of sporophores and yield. This combination also produced sporophores within 16 days compared to 20.75 days in paddy straw. The suitability of SMS for mushroom production has been reported by Balakrishnan and Lulu Das (2001). The chemical analysis of SMS has clearly indicated that they contain easily hydrolysable nutrients in sufficient quantity (Thomas and Rajagopal, 2003). Apart from this it was found to be a ready source of enzyme such as cellulases and laccases. These enzymes apart from detoxifying the phenolic compounds present in coirpith but also helpful in releasing simple sugars which enhances the growth of mushrooms in the substrates. Thus in the study 1 : 1 combination of SMS and RCP accumulated all the advantages favourably and lead to the realization of maximum yield (627.59) and biological efficiency (125.50 per cent) over control (400 g and 80 per cent respectively). An increase in proportion of SMS in RCP from 25 to 50 per cent increased the yield. However, further increase of SMS to 75 per cent reduced the yield compared to 1 : 1 proportion but it was on par with the control where paddy straw was used as the substrate.

According to Zadrazil (1975) when *P. florida* was grown on paddy straw, 80 per cent of the nutrients was used by the fungus for its growth and only 20 per cent of the original substrate by weight remained as spent substrate. This spent substrate may contain nutrients in an available form as it had undergone partial decomposition during the mushroom production. When this SMS was incorporated in coirpith, the nutrients for the initial growth are present in an easily available form to the

mushrooms. For the remaining nutrients, the fungus had to depend upon the coirpith. The ability of the SMS to enhance the growth and production of mushroom is clear from the fact that 50 per cent SMS was better than 25 per cent. When the proportion of SMS was further increased there was no corresponding increase in yield, this may be due to the adverse effect of SMS such as increased bulk density and water holding capacity.

The neem cake (four per cent) amended beds produced fruit bodies much earlier than the other treatments. The suitability of neem cake supplementation on mushroom production was reported earlier (Hazarika, 1998; Srivastava and Singh, 1999). The present study with neem cake confirms the earlier findings. Comparatively high nitrogen content of the neem cake might be the reason for the positive effect of this amendment on mushroom production. Saroj Kumar and Singh (2002) reported that addition of nitrogenous organic substances to substrates increased yield of mushroom significantly.

When retted coirpith was supplemented with paddy straw, mushroom yield was increased with increase in the proportion of straw used from 25 to 75 per cent. The present study indicated the unsuitability of coir pith when used alone for mushroom production. However, mixing of coir pith with paddy straw enabled to obtain higher mushroom yield. Similar finding was observed by Thomas and Rajagopal (2003). The lignin rich plantation wastes formed a suitable substrate for oyster mushroom production as reported by Chandramohanan and Madhusudhanan (2002) with areca nut bunch waste, Kochu Babu and Nair (1991) with oil palm waste and Mathew *et. al.* (1991)with saw dust of rubber tree. Variation in mushroom yield in different combination of substrates could be attributed to the difference in the nutrient content.

The yield of *P. florida* in non-retted coirpith alone or when amended with different materials was less compared to similar treatment combinations

where RCP was used as the substrate. The phenolic compounds in retted coirpith is less compared to NRCP as most of the phenolic compounds are washed during the process of retting. In general, the production of mushroom in NRCP is less than the control either alone or when amended. Among the amendments, maximum yield was obtained in 1 : 1 proportion of NRCP and SMS. Amending NRCP with cellulose and starch supported least sporophore production. This may be due to the inability of the amendments to produce even small quantity of nitrogen. Nitrogen atleast in very small quantity is required for the faster production of sporophores (Hazarika, 1998; Srivastava and Singh, 1999).

When interaction between retted and non-retted coirpith was studied, it was found that RCP treatment combinations promoted oyster mushroom production as it boosted the yield parameters by minimizing the time for spawn run and mushroom production on bed and increased the number and yield of sporophores. This may be due to the fact that yield of sporophore was positively related to the cellulose content and cellulose-lignin ratio (Sivaprakasam and Kandaswamy, 1981). Also high cellulase enzyme activity of *P. florida* on the substrate which lead to higher yield of sporophores (Sivaprakasam, 1986; Geetha and Sivaprakasam, 1998b) and high water holding capacity of RCP (1109.28 per cent) compared to NRCP (886.66 per cent) reported by Reeja (2002).

When compared to RCP and NRCP, composted coir pith was very poor in supporting both oyster and milky mushroom production. This may be due to the presence of different antagonistic agents, which may suppress the growth of mushrooms. Often contaminants such as *Trichoderma* spp., *Coprinus comatus*, *Aspergillus niger* were observed dominant on all the beds. *Trichoderma* spp. have been reported to be the major fungal competitors which adversely affected the yield of edible fungi (Vijay and Sohi, 1987). The yield of mushrooms largely depended upon the nature of substrates. The reason for the poor growth and low

yield in composted coirpith could be related to the bulk density and water holding capacity of the substrates .In more and compact substrate aeration will be poor and proper gaseous exchange will not be possible. Similar observations were noticed by Krishnamoorthy and Muthusamy (1997) in the case of *C. indica*. Again, high nitrogen content of the substrate further pulled back the mushrooms in growing and establishing on the substrate. The work of Gogoi and Adhikary (2002) revealed that higher nitrogenous substrate such as tea leaves was less beneficial for mushroom growth also supports this observation.

The yield of sporophores of milky mushrooms was highest when the substrate used was paddy straw. Enriching coirpith with amendments such as paddy straw, SMS, cellulose, starch and neem cake did not increased the yield of mushroom in the case of retted coirpith. While in NRCP a higher yield than the control was recorded when paddy straw and coirpith were mixed in the ratio 3 : 1 and the yield was on par with the control when coirpith and SMS were mixed in the ratio 1 : 1. The reduced growth of milky mushroom in RCP may be due to its richness in lignin content (Owseph, 1999). Better growth of milky mushroom in NRCP may be due to its low water holding capacity (Reeja, 2002). Milky mushrooms require somewhat dry and sturdy substrate for better anchorage and growth. Milky mushrooms are in general low producers of enzymes and hence are poor degraders of complex organic matter.

The results of the study clearly indicate that it is possible to cultivate mushrooms in coirpith amended with SMS or paddy straw. Thereby, the cost of production of mushrooms can be brought down considerably by reducing the use of costly substrate namely paddy straw. For decomposition of coirpith *Pleurotus* spp. are being used. Instead the present investigation shows that *T. harzianum*. can be used as a better substitute.

SUMMARY

6. SUMMARY

This study was conducted to find out the most efficient fungi for degrading coirpith and to perfect methods for growing oyster and milky mushrooms on coirpith profitably.

Four different lignocellulolytic microflora *viz.*, *Aspergillus niger*, *A. ochraceous*, *Rhizopus stolonifer* and *Trichoderma harzianum* isolated from retted coirpith, three mushrooms *viz.*, *Pycnoporus sanguineus*, *Ganoderma applanatum* and *Pleurotus tuber-regium* collected during surveys in different parts of Thiruvananthapuram district, *P. florida* and *Calocybe indica* procured from Instructional Farm, Vellayani and *Lentinus edodes* from AICMIP, TNAU were used in the study. These fungi were brought into pure culture and screened to find out the most efficient fungi for degrading coirpith.

Among the fungi collected during the survey, *P. tuber-regium* is a new report of edible mushroom for India. This mushroom could be cultivated in paddy straw, produced sporophores within 18-20 days and gave a biological efficiency of 46 per cent.

In vitro studies revealed that *T. harzianum* was the fastest colonizer of retted coirpith followed by *G. applanatum* and *L. edodes.*.

T. harzianum reduced organic carbon content of retted coirpith from 34.49 to 17.09 per cent and C : N ratio from 112.21 : 1 to 10.36 : 1. *C. indica* was the least efficient as a degrader of coirpith.

The cellulose and lignin content reduced to their lowest values by *T. harzianum* followed by *L. edodes* and *A. niger*.

Four per cent neem cake amended beds even though promoted faster spawn run and mushroom production of *P. florida* in retted coirpith,

the maximum yield was obtained in a substrate containing 1 : 1 combination of retted coirpith and spent mushroom substrate.

A substrate containing non-retted coirpith and paddy straw in the 1 : 3 ratio promoted faster spawn run while faster rate of mushroom production and higher yield were noticed in beds containing a 1 : 1 ratio of non-retted coirpith and spent mushroom substrate and 1 : 3 ratio of non-retted coirpith and paddy straw.

Faster spawn run of milky mushroom in retted coirpith was noticed when it was supplemented with paddy straw (1 : 3 ratio) and spent mushroom substrate (1 : 1 and 1 : 3 ratio). The maximum yield of sporophore was realized on retted coirpith supplemented with paddy straw (1 : 3 ratio) and spent mushroom substrate (1 : 1 ratio). The yield obtained in retted coirpith substrate was significantly less than that of control where paddy straw was used as the substrate.

A combination of non-retted coirpith and spent mushroom substrate (1 : 1) promoted faster growth of mycelium of milky mushroom, while faster mushroom production and higher yield were seen in a substrate with a combination of non-retted coirpith and paddy straw (1 : 3) followed by non-retted coirpith and spent mushroom substrate (1 : 1 ratio).

Composted coirpith was found to be a poor substrate for oyster and milky mushroom production.

REFERENCES

7. REFERENCES

Abraham, S.P. 1991. Kashmir fungal flora – An overview. *Indian Mushrooms* (eds. Nair, M.C., Balakrishnan, S. and Gokulapalan, C.). Proceedings of National Symposium on Mushrooms, 1991. Kerala Agricultural University, Thrissur, pp. 12-24

*Alexander, M. 1977. Introduction to Soil Microbiology. Second edition. John Wiley and Sons Inc., London, p. 467

Anandh, K. and Prakasam, V. 2002. Studying the enzymology and substrate degradation pattern of wild edible mushroom accessions during growth and development. Third Indian Mushroom Conference, 6-7 March 2002. Tamil Nadu Agricultural University, Coimbatore. *Abstract* : 37

Anandh, K. and Prakasam, V. 2003. Some cultivable mushroom flora from Western Ghats. *Current Vistas in Mushroom Biology and Production* (eds. Upadhyay, R.C., Singh, S.K. and Rai, R.D.). Mushroom Society of India, National Research Centre for Mushroom, Solan, p. 289

*Argyropoulos, D.S. and Menachem, S.B. 1997. *Lignin. Adv. Biochem. Bioengg. Biotech.* 57 : 128-159

Arora, D.S. and Sandhu, D.K. 1985. Survey of some Indian soils for laccase producing fungi and their lignin degrading ability. Proceedings of the Indian Academy of Sciences. *Plant Sci.* 94 : 567-574

Bahram, H. 1989. Edible oyster mushroom on the straw. *Mushroom J.* 9 : 97-100

Bahukhandi, D.K. and Chamola, B.P. 1991. Effect of different doses of organic nutrients on yield of some species of Phoenix mushroom. *Indian Mushrooms* (eds. Nair, M.C., Balakrishnan, S. and Gokulapalan, C.). Proceedings of National Symposium on Mushrooms, 1991. Kerala Agricultural University, Thrissur, India, pp. 143-148

Balakrishnan, B. and Lulu Das. 2001. Low cost technique for cultivation of *Calocybe* – the milky white mushroom. *Kisan Wld.* 28 : 25

Banik, S., Bhttacharyya, S.K., Pandey, S.N. and Nandi, S.R. 1994. Residual slurry from biogas plant as a supplement for mushroom production. National Symposium on Mushroom, 8-10 April, 1994. Mushroom Society of India and National Centre for Mushroom Research and Training, Solan. *Abstract* : 69

Bano, Z. and Rajarathnam, S. 1982. Studies on the cultivation of *Pleurotus sajor-caju*. *Mushroom J.* 115 : 243-245

Bano, Z., Rajarathnam, S. and Natarajan, N. 1979. Some aspects on the cultivation of *Pleurouts flabellatus* in India. *Mushroom Sci.* 10 : 597-608

Baskaran, T.L., Sivaprakasam, K. and Kandaswamy, T.K. 1978. Compact bag method – a new method of increasing the yield of *Pleurotus sajor-caju*. *Indian J. Mush.* 4 (2) : 10-12

*Beg, S., Zafar, S.I. and Shah, F.H. 1986. Rice husk biodegradation by *P. ostreatus* to produce a ruminant feed. *Agric. Wastes* 17 (1) : 15-21

*Beguin, P. and Anbert, J.P. 1985. The biological degradation of cellulose. *Microbiol. Rev.* 13 : 25-28

*Berkeley, M.J. 1850. Decades of fungi XXV to XXX, Sikkim, Himalayan fungi collected by Dr. J.D. Hooker. *J. Bot. Kew Gard* 2 : 76-88

*Berkeley, M.J. 1852. Decades of fungi XXXVII, XXXVIII, Sikkim and Khassya fungi, collected by Dr. J.D. Hooker. *J. Bot. Kew-Gard.* 4 : 97-107

*Berkeley, M.J. 1854. Decades of fungi XLIX Indian fungi. *J. Bot. Kew. Gard.* 6 : 225-235

Bhatia, J.N. and Suhag, L.S. 1997. Studies on cultivation of *Pleurotus* sp. on different agricultural residues. Indian Mushroom Conference, 10-12 September, 1997. Mushroom Society of India and National Centre for Mushroom Research and Training, Solan. *Abstract* : 31

Bhavana, A.K. and Thomas, V.G. 2002. Yield pattern and nutrient composition of milky white mushroom (*Calocybe indica*) cultivated on fermented coirpith. Third Indian Mushroom Conference, 6-7 March 2002. Tamil Nadu Agricultural University, Coimbatore. *Abstract* : 87

Bhavani Devi. 1982. Studies on edible mushrooms of Kerala with special reference to paddy straw mushroom *Volvariella* spp. Ph.D. thesis, Kerala Agricultural University, Thrissur, p. 221

Bhowmic, B.B. and Debnath, C.R. 1985. Coir Fibre Part II. Potentiality of coir fibre products. *Indian Cocon. J.* 16 (3) : 7-10

Biddappa, C.C., Palaniswami, C., Upadhyay, A.K and Ramanujam, B. 1998. Comparison of techniques for organic manure production from plantation wastes. *J. Plant. Crops* 26 : 120-126

Bilgrami. K.S., Jamaluddin and Rizwi, M.A. 1979. Fungi of India – Part 1. Today and Tomorrows Printers and Publishers, New Delhi, India, p. 467

Blanchette, R.A. 1984. Screening wood decayed by white rot fungi for preferential lignin degradation. *Appl. Environ. Microbiol.* 48 : 647-653

Boominathan, K. and Reddy, C.A. 1992. Fungal degradation of lignin – Biotechnological applications. *Handbook of Applied Mycology, Vol. IV* (Eds. Arora, D.K., Elander, P.R. and Mukerji, K.G.) Marcel Dekka, Inc. New York, p. 763-822

Bose, S.R. 1920. Records of Agaricaceae from Bengal. *J. Asiatic Soc.* Bengal 16 : 347-354

Burgarski, D., Gvozdenovic, D., Takac, A., Jovicvic, D., Jevtic, S. and Lazic, B. 1996. *Acta Hort.* 462: 891-894

Butler, E.J. and Bisby, G.R. 1931. The fungi of India. *Imp. Counc. Agric. Res.*, India 1 : 237

*Cappaert, I., Vevdonck, O. and Boodt. 1976. Composting of agro residues. *Compost Sci.* 17 : 6-9

Chandramohanan, R. and Madhusudhanan, K. 2002. A partially underground permanent mushroom house for oyster mushroom (*Pleurotus* spp.) cultivation. Third Indian Mushroom Conference, 6-7 March 2002. Tamil Nadu Agricultural University, Coimbatore. *Abstract* : 78

Chandramohanan, R. and Moorthy, V.K. 1991. Utilization of Areca leaf as a substitute for cultivation of *Pleurotus sajor-caju*. *Indian Mushrooms*. (eds. Nair, M.C., Balakrishnan, S. and Gokulapalan, C.). Proceedings of National Symposium on Mushroom, 1991.Kerala Agricultural University, Thrissur, pp. 140-142

Chandrashekhar, B.S. and Veena Savalgi. 2003. Evaluation of sugarcane byproducts for the cultivation of oyster mushroom (*Pleurotus sajor-caju* (Fr.) Singer). *Current Vistas in Mushroom Biology and Production* (eds. Upadhyay, R.C., Singh, S.K. and Rai, R.D.). Mushroom Society of India, National Research Centre for Mushroom, Solan, p. 289

*Chang, S.T. and Miles, P.G. 1989. Edible mushrooms and their cultivation. CRC Press, Boca-Raton, p. 489

*Cooke, M.C. 1888. Exotic agarics. *Grev.* 16 : 105-106

Das, N., Mahapatra, S.C. and Chattopadhyay, R.N. 1997. Utilization of wild grasses as a potential substrate for Oyster mushroom cultivation in West Bengal. Indian Mushroom Conference. 10-12 September. 1997. Mushroom Society of India and National Centre for Mushroom Research and Training, Solan. *Abstract* : 37

Dessai, P.A.V., Eranna, N. and Shivappa Shetty, K. 1991. Pink *Pleurotus*- A new edible mushroom. *Indian Mushrooms* (eds. Nair, M.C., Balakrishnan, S. and Gokulapalan, C.). Proceedings of National Symposium on Mushrooms, 1991. Kerala Agricultural University, Thrissur, pp. 63-66

Doshi, A. and Sharma, S.S. 1995. Production technology of specialty mushrooms. *Advances in Horticulture Vol. 13 Mushroom* (eds. Chadha, K.L. and Sharma, S.R). Malhothra Publishing house, New Delhi p. 649

Doshi, A. and Sharma, S.S. 2001. Production of edible tubers and fruit bodies of *Pleurotus tuber-regium* (Fr.) Sing. on Rajasthan, India. *Mushroom Res.* 10:25-28

Doshi, A., Sidana, N. and Chakravarti, B.P. 1989. Cultivation of summer mushroom, *Calocybe indica* (P. & C.) in Rajasthan. *Mushroom Sci.* 12 : 395-400

Dubey, S.C. and Das, S.K. 1997. Effect of different substrates and application of organic nutrients and amendments on yield of *Pleurotus* sp. Indian Mushroom Conference, 10-12 September, 1997. Mushroom Society of India and National Centre for Mushroom Research and Training, Solan. *Abstract* : 28

*Falck, R. 1917. Uber die Waldkultur des Austernpilzes (*Agaricus ostreatus*) auf Laubholzstubben. *Zeitscchrift fur. Forst - und Jagdwesen* 49: 159-165

*Fan, L.S., Lel, Y.H. and Gharnhay, M.M. 1982. The nature of lignocellulosics and their pre-treatments for enzyme hydrolysis. *Adv. Biochem. Engg.* 23 : 157-187.

Findlay, W.P.K. 1977. *The observer's Book of Mushrooms Toadstools and other Common Fungi.* Frederick Warne Publishers Ltd., London, p. 186

*Fries, E.M. 1821. *Systema Mycologicum.* I Lundae Goyphiswaldae, p. 520

Geetha, D. 1994. Studies on oyster mushroom (*Pleurotus* spp.). Ph.D. thesis, Tamil Nadu Agricultural University, Madurai, p. 253

Geetha, D. and Sivaprakasam, 1997. Enzyme production and sporophore production potential of oyster mushroom (*Pleurotus* sp). Indian Mushroom Conference, 10-12 September, 1997. Mushroom Society of India and National Centre for Mushroom Research and Training, Solan. *Abstract* : 75

Geetha, D. and Sivaprakasam, K.1998a. Enzyme and sporophore production potential of Oyster Mushroom (*Pleurotus* spp.). *Mushroom Res.* 7 : 39-42

Geetha, D. and Sivaprakasam, K. 1998b. Degradative potential of Oyster Mushroom (*Pleurotus* spp.). *Mushroom Res.* 7 : 81-84

Geetha, D., Suharban, M. and Nair, H.K. 2002. *Effect of Different Casing Material on the Yield of Milky Mushroom.* Proceeding of XIV Science Congress, 29-31 January 2002 (ed. Das, M.R.). Kerala State Committee on Science Technology and Environment, Kochi, p. 719

Geiger, J.P., Rio, B., Nicole, M. and Nandris, D. 1986. Biodegradation of *Hevea brasiliensis* wood by *Rigidoporus lignosus* and *Phellinus noxius*. *European J. Forest Pathology* 16: 147-159

Ghosh, M. and Nandi, B. 1995. Dynamics of extracellular enzymes during lignocellulose degradation of water hyacinth by *Pleurotus* sp. *Mushroom Res.* 4 : 53-58

Gogoi, G. and Adhikary, R.K. 2002. Bio-efficacy of certain plant wastes for production of oyster mushroom. Third Indian Mushroom Conference, 6-7 March 2002. Tamil Nadu Agricultural University, Coimbatore. *Abstract* : 60

Gokulapalan, C., Geetha, D., Suharban, M. and Nair, M.C. 1989. Effect of chemically treated paddy straw on yield of oyster mushroom. *Indian J. Mush.* 15 (1&2) : 23

Gunasegaran, K. and Graham, M. 1987. Effect of organic additives on yield of the phoenix mushroom grown on cellulose waste. *Mushroom J. Tropics* 7 : 101-106

Hazarika, D.K. 1998. Effect of substrates and supplements on yield of oyster mushroom (*Pleurotus sajor-caju*). *Mushroom Res.* 7 (1) : 47-48

Hiroi, T. 1981. Enzymatic hydrolysis of woods VI. Susceptibility of rotted woods to cellulase from *T. viride*. *Mokuzai Gakkashi* 27 : 684-690

Isikhuemhen, O.S. and Okhuoya, J.A. 1998. Effect of substrate supplementation with NPK fertilizer on sporophore yield in *Pleurotus tuber-regium* (Fr.) Sing. *Mushroom Res.* 7 (1) : 35-38

Jackson, M.L. 1973. Soil Chemical Analysis. Prentice Hall of India (Pvt.) Ltd., New Delhi, p. 498

Jacob, P.J.S. and Abraham, T.K. 1998. Utilization of some common weeds for the cultivation of mushrooms. *Mushroom Res.* 7 (2) : 99-100

Jandaik, C.L. 1989. Response of *Pleurotus sajor-caju* to supplementation prior to and after pasteurization of straw. *Indian Phytopath.* 42 : 284-285

Jandaik, C.L. 2002. Recent advances in cultivation technology of Oyster Mushroom. Asian Congress of Mycology and Plant Pathology at 1-4 October, p.270

Jandaik, C.L. and Kapoor, J.N. 1975. *Pleurouts sajor-caju* (Fr.) Singer from India. *Indian J. Mush.* 1 : 1-2

Janshekar, H. and Fiechter, A. 1983. Lignin : Biosynthesis application and biodegradation. *Adv. Biochem. Eng. Biotechnol.* 27 : 119-178

Janshekar, H. and Fiechter, A. 1985. Cultivation of *Phanerochaete chrysosporium* and production of lignin peroxidases in submerged stirred tank reactors. *J. Biotechnol.* 8 : 97-112

Johnson, L.F. and Curl, E.A. 1972. Isolation of groups of microorganisms from soil. Methods for research in the ecology of soil borne plant pathogens. Burgess Publishing Co. pp., 1-33

Joseph, K., Kothandaraman, R. and Mathew, J. 1991. Comparative studies on cellulolytic enzyme production and yield of three species of oyster mushrooms. *Indian Mushrooms* (eds. Nair. M.C., Balakrishnan, S. and Gokulapalan, C.). Proceedings of National Symposium on Mushrooms, 1991. Kerala Agricultural University, Thrissur, pp. 187-189

Kadalli, G.G. and Nair, S. 2000. Manurial value and efficiency of coir dust based enriched super compost. *Indian Cocon. J.* 30 (7) : 49-50

Kadari, M. 1994. Effect of Additives on mycelial growth and fructification of *P. squrrosulus*. *Rewister-de-Biologia trop.* 42 : 1-2, 49-52

*Kalberer, P and Vogel, E. 1974. *Untersuchungen sur kultur von Pleurotus Gemusebau* 4 : 37-44

Kamaraj, C.M. 1994. Exportable coir products in Tamil Nadu. *The Cocon. Wealth* 1 : 6-8

Kattan, M.H., Helmy, Z.A., Mahmoud, B.H. and Kawi, K.A. 1991. Effect of additives on Oyster mushroom production. *Mush. J. Tropics* 11 : 67-74

Kaur, M.J. and Lakhanpal, T.N. 2001. Japanese mushroom cultivation technology on indigenous substrates in India. *Everyman's Sci.* 36 (1) : 10-15

*Kawase, K.J. 1962. Chemical components of wood decayed under natural conditions and their properties. *J. Fac. Agric. Hokkaido Univ.* 52: 186-245

*Kirk, T.K. and Farrell, R.L. 1987. Enzymatic combustion : the microbial degradation of lignin. *Ann. Rev. Microbiol.* 41 : 465-505

Kochu Babu, M. and Nair, K.R. 1991. Mushroom cultivation on oil palm factory wastes and performance of six species of *Pleurotus* (oyster mushroom). *Indian Mushrooms.* Proceedings of National Symposium on Mushroom, Thiruvananthapuram, pp. 84-86

Kothandaraman, R., Kochuthresiamma Joseph, Mathew, J. and Jayarathnam, K. 1991. Preliminary investigations into the feasibilities of growing oyster mushroom (*Pleurotus florida*) on rubber wood saw dust. *Indian Mushrooms* (eds. Nair, M.C., Balakrishnan, S. and Gokulapalan, C.). Proceedings of National Symposium on Mushrooms, 1991. Kerala Agricultural University, Thrissur, pp. 99-101

Krishnamoorthy, A.S. 2003. Commercial prospects of milky mushroom (*Calocybe indica*) in the tropical plains of India. *Current Vistas in Mushroom Biology and Production* (eds. Upadhyay, R.C., Singh, S.K. and Rai, R.D.). Mushroom Society of India, NRCM, Solan, p. 289

Krishnamoorthy, A.S. and Muthusamy, M. 1997. Yield performance of *Calocybe indica* (P. & C.) on different substrates. *Mushroom Res.* 6 (1) : 29-32

Krishnamoorthy, A.S., Marimuthu, T. and Muthusamy, T. 2002a. Casing soil requirements for the cultivation of *Calocybe indica*. Third Indian Mushroom Conference, 6-7 March 2002. Tamil Nadu Agricultural University, Coimbatore. *Abstract* : 107

Krishnamoorthy, A.S., Marimuthu, J. and Muthusamy, M. 2002b. Enzyme related biodegradative potential of *Calocybe indica*. Third Indian Mushroom Conference, 6-7 March 2002. Tamil Nadu Agricultural University, Coimbatore. *Abstract* : 36

Krishnamoorthy, A.S., Muthusamy, M., Marimuthu, T. and Narasimhan, V. 1997 Evaluation of different natural and synthetic growth media for culturing *Calocybe indica*. Indian Mushroom Conference, 10-12 September, 1997. Mushroom Society of India and National Centre for Mushroom Research and Training, Solan. *Abstract* : 65

Krishnamoorthy, A.S. and Nakkeeran, S. 2002. Biodiversity and strain variation in *Calocybe indica*. Third Indian Mushroom Conference, 6-7 March 2002. Tamil Nadu Agricultural University, Coimbatore. *Abstract* : 16

Kumar, M., Kumar, A., Kumar, H. and Singh, V.K. 1997. Effect of different substrates and supplementation on the yield of *Pleurotus sajor-caju* and *P. ostreatus*. Indian Mushroom Conference, 10-12 September, 1997. Mushroom Society of India and National Centre for Mushroom Research and Training, Solan. *Abstract* : 26

Kumuthakalavalli, R. and Daniel, T. 1991. Effect of germinated pulses on sporophore yield of oyster mushroom (*Pleurotus sajor-caju*). *Indian Mushrooms*. (eds. Nair, M.C., Balakrishnan, S. and Gokulapalan, C.). Proceedings of National Symposium on Mushrooms, 1991. Kerala Agricultural University, Thrissur, pp. 115-116

*Kurtzman, R.H. Jr. 1979. Metabolism and culture of *Pleurotus*, the oyster mushroom. *Taiwan Mushrooms* 3 : 1-13

Kushwaha, K.P.S., Banu, C., Bhatt, N., Sharma, G., Bhatt, P. and Bajaj, A. 2002. Fleshy fungi from Uttaranchal : Collection and cultivation. Third Indian Mushroom Conference. 6-7 March 2002. Tamil Nadu Agricultural University, Coimbatore. *Abstract* : 11

*Leal-Lara, H., Ramirez-Canilo, R., Eger-Hummill, G. and Padilla-Hustado, F. 1989. Production of *Pleurotus ostreatus* strains for selective degradation of lignin. International Symposium on microbial ecology, 16-18 February, 1989, Japan. *Abstract* : 5

Madan, M. and Bisaria, R. 1983. Biochemical changes associated with bioconversion of plant residues into food through *Pleurotus sajor-caju*. *Biotech. Lett.* 5 : 601-604

Mane, A.G., Bhedkar, V.D., Deshmukh, V.R. and Mohite, P.U. 1997. Effect of supplementation of vermicompost on spawn run, yield, biological efficiency and protein conversion efficiency of *Pleurotus sajor-caju* grown on selected substrates. Indian Mushroom Conference, 10-12 September, 1997. Mushroom Society of India and National Centre for Mushroom Research and Training, Solan. *Abstract* : 29

Manjula, B. 1983. *A Revised List of the Agaricoid and Boletoid Basidiomycetes from India and Nepal.* Proceedings of the Indian Academy of Sciences, Madras, pp. 81-125

Marimuthu, T., Krishnamoorthy, A.S. and Jeyarajan, R. 1991. Glimpses of mushroom Research in TNAU. Tamil Nadu Agricultural University, Coimbatore, p. 63

Masroor, M.K. and Bihari Lal. 2002. Effect of change of micro-organisms in compost due to weed supplements on mushroom yield. Third Indian Mushroom Conference, 6-7 March 2002. Tamil Nadu Agricultural University, Coimbatore. *Abstract* : 62-63

Mathai, G. and Suharban, M. 1994. Growth response of *Pleurotus sajor-caju* (Fr.) Singer in different substrates used for spawn and sporophore production. National Symposium on Mushroom, 8-10 April, 1994. Mushroom Society of India and National Centre for Mushroom Research and Training, Solan. *Abstract* : 64

Mathew, J., Kothandaraman, R. and Joseph, K. 1991. Cultivation of oyster mushroom on rubber processing factory waste – A possible solid waste utilization method. *Indian Mushrooms* (eds. Nair, M.C., Balakrishnan, S. and Gokulapalan, C.). Proceedings of National Symposium on Mushrooms, 1991. Kerala Agricultural University, Thrissur, pp. 97-99

Moorthy, V.K. 1981. Microbial and chemical studies on the cultivation of *Pleurotus sajor-caju.* (Fr.) singer (Oyster mushrooms). M.Sc (Ag.) thesis, University of Agricultural Sciences, Bangalore, p. 128

Nagarajan, R., Manickam, T.S. and Kothandaraman, G.V. 1985. Manurial value of coir pith. *Madras agric. J.* 72 (9) : 532-533

Nagarajan, R., Ramasamy, K., Savithri, P. and Manickam, T.S. 1990. Coir waste in crop production. Agricultural college and Research Institute Madurai and Central Coir Research Institute, Coir Board Kalavoor, p.39

Nallathambi, P. and Marimuthu, T. 1993. *Pleurotus platypus* : a potent oyster mushroom for organic recycling of agricultural wastes. *Mushroom Res.* 2 : 75-78

Natarajan, K. 1978. South Indian Agaricales IV. *Kavaka* 6 : 65-70

Okhuoya, J.A., Isikhuemehn, O.S., Ogboe, E. and Osemwegie, O.O. 1998. Effect of mulching on sporophore yield of *Pleurotus tuber-regium* (Fr.) Sing. *Mushroom Res.* 7 (2) : 89-92

*Oki, T., Senba, Y. and Ishikawa, H. 1982. The biodegradation of soft wood lignin and guaiacylglycerol-beta-guaiacyl ether by extra-cellular enzyme in shiitake *Lentinus* (Lentinula) *edodes* (Birk.) Sing. *J. Japan Wood Res. Soc.* 28 : 800-805

Owseph, A. 1999. Enzyme production and composting potential of oyster mushroom (*Pleurotus* spp.). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, p. 67

Owseph, A., Geetha, D. and Suharban, M. 2001. Lignocellulose degradation by oyster mushroom. *Mushroom Res.* 10 (1) : 37-40

Owseph, A., Geetha, D. and Suharban, M. 2003. Enzyme production and yield characteristics of four species of *Pleurotus* on paddy straw, non-retted and retted coirpith. *Current Vistas in Mushroom Biology and Production* (eds. Upadhyay, R.C., Singh, S.K. and Rai, R.D.). Mushroom Society of India, National Research Centre for Mushroom, Solan, p. 289

Pacioni, G. 1985. *Encyclopedia of Mushrooms and Toadstools*. Macdonald and Co., London, p. 511

Pandey, M. and Tewari, R.P. 1991. Evaluation of tea and coffee wastes for *Pleurotus florida* cultivation. *Indian Mushrooms*. (eds. Nair, M.C., Balakrishnan, S. and Gokulapalan, C.). Proceedings of National Symposium on Mushrooms, 1991. Kerala Agricultural University, Thrissur, pp. 88-90

Patra, A.K., Pani, B.K. and Panda, S.N. 1997. Effect of leaf and oil cake amendments on paddy straw on the sporophore yield of *Pleurotus sajor-caju* (Fr.) Singer. Indian Mushroom Conference, 10-12 September, 1997. Mushroom Society of India and National Centre for Mushroom Research and Training, Solan. *Abstract* : 30

*Pegler, D.N. 1976. Agric flora of Sri Lanka. *Kew Bull. Additional series* 12 : 43-45

*Poincelot, R.P. 1974. A scientific examination of the principles and practice of composting. *Compost Sci.* 15 (3) : 24-31

*Pometto, A.L. and Crawford, D.L. 1986. Effects of pH on lignin and cellulose degradation by *Streptomyces viridosporus Appl. Environ. Microbiol.* 52 : 246-250

Prasad, G. and Govindarajan, K. 2001. Determination of lignin. Laboratory manual on bioconversion of cellulose wastes (eds. Doraisamy, P., Marimuthu, P. and Govindarajan, K.). Tamil Nadu Agricultural University, Coimbatore, pp. 7-8

Purkayastha, R.P. and Chandra, A. 1974. Studies on *Calocybe India*, an edible mushroom of India. Proceedings of Sixth European Mycological Congress 8-12 January, 1974. Avignon, France. *Abstract* : 74

Purkayastha, R.P. and Chandra, A. 1985. Manual of Indian Edible Mushrooms. Today and Tomorrow's Printers and Publishers, New Delhi, p. 267

Purkayastha, R.P., Mondal, T. and Jana, K.K. 1981. An improved method of cultivation of *Calocybe indica* an edible white mushroom. *Indian J. Mushrooms* 7 : 3-10

Purkayastha, R.P. and Nayak, D. 1979. A new method of cultivation of *Calocybe indica* – an edible summer mushroom. *The Taiwan Mushrooms* 3 : 14-18

Purkayastha, R.P. and Nayak, D. 1981. Development of cultivation method and analysis of protein of a promising edible mushroom *Calocybe indica*. P & C. *Mushroom Sci.* 9 : 697-713

Rai, R.D. and Saxena, S. 1991. Influence of forms and levels of cellulose on production of cellulases by *Pleurotus sajor-caju* (Fr.) Singer. *Indian Mushrooms* (eds. Nair, M.C., Balakrishnan, S. and Gokulapalan, C). Proceedings of National Symposium on Mushrooms 1991. Kerala Agricultural University, Thrissur, pp. 202-206

Rajarathnam, S., Wadkhede, D.B. and Patwardhan, M.V. 1979. Some biochemical and chemical changes in straw constituents during growth of *Pleurotus flabellatus* (Berk & Br.) *European J. Appl. Microbiol. Biotechnol.* 8 : 125- 134

Rajarathnam, S., Wankhede, D.B. and Bano, Z. 1987. Degradation of rice straw by *Pleurotus flabellatus*. *J. Chem. Technol. Biotech.* 37 : 203-214

Ramamoorthy, V. 1998. Composting coirpith using lignocellulolytic fungi for the management of root rot of black gram. M.Sc. thesis, Tamil Nadu Agricultural University, Coimbatore, p. 175

Ramamoorthy, V., Meena, B., Muthusamy, M., Seetharaman, K. and Alice, D. 1999. Composting coirpith using lignocellulolytic fungi for the management of root rot of black gram. *Mushroom Res.* 8 (2) : 13-18

Ramamoorthy, V., Muthusamy, M., Meena, B., Sethuraman, K. and Alice, D. 2000. Biodegradation of coirpith using native microflora. *J. Plantn. Crops* 28 (3) : 218-221

Rangaswami, G., Kandaswamy, T.K. and Ramasamy, K. 1975. *Pleurotus sjaor-caju* (Fr.) Singer; a protein rich nitrogen fixing mushroom fungus. *Curr. Sci.* 40 : 403-404

Rao, S.C. 1991. Evaluation of substrates and supplements for the oyster mushroom (*Pleurotus florida*) cultivation in the state of Goa. *Indian Mushrooms*. (eds. Nair, M.C., Balakrishnan, S. and Gokulapalan, C.). Proceedings of National Symposium on Mushrooms, 1991. Kerala Agricultural University, Thrissur, pp. 93-96

Rawal, R.P., Singh, R.D. and Khander, R.R. 1991. Effect of different crop industrial residues on mushroom production. Advances in Mushroom Sciences. *Abstract* : 37

Reddy, M.V.S. 1985. Enzymology of lignin degradation in coir dust by *Pleurotus sajor-caju*. M.Sc. (Ag.) thesis, Tamil Nadu Agricultural University, Coimbatore, p. 110

Reeja, R.S. 2002. Comparative efficiency of lignocellulolytic fungi for bioconversion of coirpith. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, p. 96

Reeja, R.S., Lulu Das and Balakrishnan, B. 2002. A native strain of *Pleurotus eous* – an efficient decomposer of coirpith. Souvenir cum Abstracts III. Indian Mushroom Conference from March 6-7, p. 54

Sarbhoy, A.K., Agarwal, D.K. and Varshney, J.L. 1986. Fungi of India 1977-1981. Associated Publishing Company, New Delhi, India, p. 274

Saroj Kumar and Singh, S.P. 2002. Influence of supplements on the yield of paddy straw mushroom *Volveriella diplasia* (Berk. & Br.) Singer. *Mushroom Res.* 11 (1) : 35-38

Savithri, P. and Khan, H.H. 1994. Characteristics of coconut coirpith and its utilization in agriculture. *J. Plantn. Crops.* 22 : 1-18

Saxena, S. and Rai, R.D. 1992. Effect of nitrogen on production of extra cellular degradative enzymes by *Pleurotus sajor-caju.* (Fr.) Singer on wheat straw. *Mushroom Res.* 1 : 45-48

Seth, P.K. 1976. Supplementation of organic substrates to synthetic composts for increased mushroom yields. *Indian J. Mush.* 2 (1) : 36-39

Sharma, S.S., Doshi, A., Trivedi, A. and Kothari, K.L. 1994. Factors affecting sporophore production of *Calocybe indica* (P. & C.). National Symposium on Mushrooms 8-10 April, National Centre for Research on Mushroom and Training, Solan. *Abstract* : 73

Sharma, V.P. and Jandaik, G.L. 1991. Yield response of *Pleurotus ostreatus* and *P. florida* to spent straw and spent compost. *Indian Mushrooms.* (eds. Nair, M.C., Balakrishnan, S. and Gokulapalan, C.). Proceedings of National Symposium on Mushrooms, 1991. Kerala Agricultural University, Thrissur, pp. 124-127

Sharma, S.G., Jyoti and Singh, V.K. 1999. Biological efficiency and cellulase activities of early and later fruiting *Pleurotus* spp. *Mushroom Res.* 8 : 23-26

Shirkot, C.K., Jarial, R.S. and Poonam Shirkol. 2001. Isolation of cellulolytic micro organisms from mushroom compost. *Indian J. Mush.* 19 (1&2) : 33-38

*Sinden, J.W. 1932. Mushroom spawn and methods of making the same. *U.S. Patent* 1 : 869-917

*Sinden, J.W. 1934. Mushroom spawn and methods of making the same. *U.S. Patent* 2 : 844-861

*Singer, R. 1986. The Agaricales in modern Taxonomy, 4th edition. *J. Cramer Wein Keim.* p. 821

Singh, M.P. 2002. Yield performance of *Pleurotus florida* on various sizes of cereal reside. Third Indian Mushroom Conference, 6-7 March 2002. Tamil Nadu Agricultural University, Coimbatore. *Abstract* : 61

Singh, R.P. and Dwivedi, R.R. 1991. Standardisation of substrates for the production of *Pleurotus sjaor-caju*. *Indian Mushrooms*. (eds. Nair, M.C., Balakrishnan, S. and Gokulapalan, C.). Proceedings of National Symposium on Mushrooms, 1991. Kerala Agricultural University, Thrissur, pp. 102-103

Singh, R.P. and Singh, A. 1991. Studies on the utilization of soybean straw for production of *Pleurotus sjaor-caju* (Fr.) Sing. *Indian Mushrooms*. (eds. Nair, M.C., Balakrishnan, S. and Gokulapalan, C.). Proceedings of National Symposium on Mushrooms, 1991. Kerala Agricultural University, Thrissur, pp. 90-93

Sivaprakasam, K. 1986. Constituents of substrates in relation to sporophore yield of *Pleurotus sajor-caju*. *Madras agric. J.* 73 : 601-605

Sivaprakasam, K. and Kandaswamy, T.K. 1981. Influence of the growth of *Pleurotus sajor-caju* (Fr.) Singer on cellulose content of the substrates. *Madras agric. J.* 68 (9) : 628-630

Sivaprakasam, K., Rajan, F.S. and Jeyarajan, R. 1986. *Pleurotus citrinopileatus* – a new Indian edible mushroom. *Curr. Sci.* 55 : 1203

Srivastava, D.K. and Singh, S.P. 1999. Effects of different supplements in paddy straw on yield of *P. citrinopileatus* (Fr.) Singer. *Mushroom Res.* 8 (2) : 47-48

Suharban, M. 1987. Monographic studies on edible species of *Pleurotus* and standardisation of the techniques for large scale cultivation. Ph.D. thesis, Kerala Agricultural University, Thrissur, p. 195

Suharban, M., Geetha, D. and Nair, M.C. 1997. Degradation of coirpith by Oyster mushroom (*Pleurotus* spp.) and its effect on yield of bhindi. *Mushroom Res.* 6 (2) : 101-102

Suharban, M., Geetha, D., Nair, H.K. and Babu Mathew. 1997. Comparative suitability of different varieties of banana pseudostem for production of *Pleurotus sajor-caju*. *Mushroom Res.* 7 (2) : 101

Suharban, M. and Nair, M.C. 1991. Cultivation of oyster mushroom on wooden logs of common trees. *Indian Mushrooms*. (eds. Nair, M.C., Balakrishnan. S. and Gokulapalan, C.). Proceedings of National Symposium on Mushrooms, 1991. Kerala Agricultural University, Thrissur, pp. 114-115

Suharban, M., Nair, M.C. and Gokulapalan, C. 2002. *Pleurotus* species from Kerala. Third Indian Mushroom Conference, 6-7 March 2002. Tamil Nadu Agricultural University, Coimbatore. *Abstract* : 17

Sutherland, J.B. 1984. Biodegradation of lignocelluloses from cotton-gin trash by *Pycnoporus cinnabarinus*. *Mycolgia* 76 : 369-372

Tewari, R.P. and Pandey, M. 1994. Studies on cultivation of white button mushrooms (*Agaricus bisporus*). National Symposium on Mushrooms 8-10 April. Mushroom Society of India and National Centre for Mushroom Research and Training, Solan. *Abstract* : 45

Tewari, R.P. and Pandey, M. 2002. Sizeable income generating venture. The Hindu Survey of Indian Agriculture, pp. 165-167

Thakur, M.P., Godara, D.R., Shukla, C.S. and Sharma, R.L. 2002. Recent advances in the production technology of paddy straw mushroom. Third Indian Mushroom Conference, 6-7 March 2002. Tamil Nadu Agricultural University, Coimbatore. *Abstract* : 81

Thayumanavan, B. 1979. Biochemical changes brought about by the growth of *Pleurotus sajor-caju* (Fr.) Singer on some natural substances. *Farm Sci.* 6 : 33-36

Thayumanavan, B. 1980. Nitrogen fixation by the fungus *Pleurotus sajor-caju* (Fr.) Singer. *Indian J. Biochem. Biophys.* 17 : 75-77

Thayumanavan, B. 1982. Extracellular cellulase and laccase enzymes from *Pleurotus sajor-caju* (Fr.) Singer. *Madras agric. J.* 69 : 132-134

Theradimani, M. and Marimuthu, T. 1991. *Pleurotus platypus*, an efficient decomposer of coconut coirpith. *Indian Mushrooms*. (eds. Nair, M.C., Balakrishnan, S. and Gokulapalan, C.). Proceedings of National Symposium on Mushrooms, 1991. Kerala Agricultural University, Thrissur, pp. 198-201

Theradimani, M. and Marimuthu, T. 1992. Utilization of *Pleurotus* spp. for decomposing coconut coir pith. *Mushroom Res.* 1 (1) : 49-51

Thomas, G.V., Prabhu, S.R., Reeny, M.Z. and Bopaiah, B.M. 1997. Evaluation of lignocellulosic biomass from coconut palm as substrate for cultivation of *Pleruotus sajor-caju* (Fr.) Singer. Indian Mushroom Conference, 10-12 September, 1997. Mushroom Society of India and National Centre for Mushroom Research and Training, Solan. *Abstract* : 28

Thomas, G.V. and Rajagopal, V. 2003. Oyster mushroom cultivation for recycling of ligno-cellulosic biomass from coconut palm. *Current Vistas in Mushroom Biology and Production* (eds. Upadhyay, R.C., Singh, S.K. and Rai, R.D.). Mushroom Society of India, NRCM, Solan, p. 289

Trivedi, A., Sharma, S.S. and Doshi, a. 1991. Cultivation of *Calocybe indica* under semi- arid conditions. *Indian Mushrooms* (eds. Nair, M.C., Balakrishnan, S. and Gokulapalan, C.). Proceedings of National Symposium on Mushrooms, 1991. Kerala Agricultural University, Thrissur, India, pp. 160 – 168

Ulmer, D.C., Leisola, M.S.A. and Fiechter, A. 1984. Possible induction of the ligninolytic system of *Phanerochaete chrysosporium*. *J. Biotechnology* 1 : 13-24

Upadhyay, R.C. and Verma, R.N. 2000. Non-conventional substrates for growing oyster mushrooms. *Mushroom Res.* 9 (1) : 35-38

Updegraff, D.M. 1969. Utilization of cellulose from waste paper by *Myrothecium verrucariae*. *Analy. Biochem.* 32 : 420-424

Vadivel, M., Ambikapathy, V. and Paneerselvam, A. 2002. Cultivation and biochemical analysis of *Pleurotus sajor-caju*. Third Indian Mushroom Conference, 6-7 March 2002. Tamil Nadu Agricultural University, Coimbatore, *Abstract* : 32

172187

Veenasavalgi, Vijayakumarsavalgi and Kulkarni, J.H. 1994. Bioconversion of agrowastes by *P. florida* with special reference to legume weed *Cassia hirsuta*. National Symposium on Mushrooms, 8-10 April, 1994. Mushroom Society of India and National Centre for Mushroom Research and Training, Solan. *Abstract* : 63

Vijay, B. and Sohi, H.S. 1987. Cultivation of oyster mushroom (*Pleurotus sajor-caju* (Fr.) Singer) on chemically sterilized wheat straw. *Mushroom J. Tropics* 7 : 67-75

Vijayakumari, J., Murthy, N.K. and Usha, K. 1991. Biodegradation of coirpith. National Symposium on Mushrooms, 22-24 January, 1991. Kerala Agricultural University, Thrissur. *Abstract* : 66

Wood, D.A., Matcham, S.E. and Fermor, T.R. 1988. Treatment of lignocellulosics with White Rot Fungi. Elsevier Applied Science, London, p. 445

*Zadrazil, F. 1974. The ecology and industrial production of *Pleurotus ostreatus*, *P. florida*, *P. cornucopiae* and *P. erynyii*. *Mushroom Sci.* 9 : 621-652

*Zadrazil, F. 1975. *Die Zersetzeng des stroh- Zellulose- lignin- Komplexes mit P. florida and dessn. Netzung. Z. Pflanzener nahs. Bodenkd.* 138 : 263-278

*Zadrazil, F. 1978. Cultivation of *Pleurotus*. The Biology and cultivation of Edible Mushrooms (Eds. Chang, S.T. and Hayes, W.A). Academic Press, New York, pp. 521-557

* Original not seen

APPENDICES

APPENDIX – I

Key to the identification of mushrooms

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 used for the study

1. Potato Dextrose Agar

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

2. Martin's Rose Bengal Agar

Dextrose	-	10 g
Peptone	-	5 g
Potassium dihydrongen phosphate	-	1 g
Magnesium sulphate	-	0.5 g
Rose Bengal	-	33 mg
Streptomycin solution (1 per cent)	-	3 ml
Distilled water	-	1000 ml
pH	-	7.0

APPENDIX – III

Glossary

Annulus	–	Ring – like structure on stipe, derived from partial veil
Carpophore	–	The fruit-body or conspicuous and familiar part of the higher fungi, which bears the reproductive structures (asci and basidia)
Decurrent	–	Gills that descend stipe to some degree
Gills	–	Radially arranged plate – like structures on the under sides of the caps of the gilled fungi, which bears the basidia
Glabrous	–	Without any hair or other ornamentation
Imbricate	–	Overlapping, like roof tiles
Infundibuliform	–	Funnel shaped
Pore	–	The orifice of the tubes that form the hymenium of the Boletaceae and the Polyporaceae
Pubescent	–	Covered with very fine, thin, soft hairs
Rugose	–	Wrinkled, rough
Sessile	–	Without a stipe
Stipe	–	The part of the carpophore that supports a cap or the hymenium in general; the stem or stalk of the fungus
Suberose	–	Corky, rubbery
Tube	–	Tubular structures lined by the basidia in the boletes and polypores
Veil	–	The tissue covering the immature carpophore of certain fungi; on expansion of the fungus this veil breaks and leaves patches or remnants of the cap or a sac – like cup or remnants about the base of the stipe
Volva	–	The remains of the universal veil which stay at the base of the skin of certain fungi

UTILIZATION OF FUNGI FOR COMPOSTING AND MUSHROOM PRODUCTION ON COIRPITH

SHERIN. A. SALAM

Abstract of the
thesis submitted in partial fulfilment of the requirement
for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2003

Department of Plant Pathology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM 695522

ABSTRACT

The present study entitled "Utilization of fungi for composting and mushroom production on coirpith" was conducted during 2001 to 2003 at College of Agriculture, Vellayani, Thiruvananthapuram district with the objective to isolate most efficient fungi for degradation of coirpith and standardization of technology for mushroom production on coirpith.

Four lignocellulolytic fungi *viz.*, *Aspergillus niger, A. ochraceous, Trichoderma harzianum* and *Rhizopus stolonifer* were isolated from retted coirpith. Three mushrooms *viz.*, *Pycnoporus sanguineus, Ganoderma applanatum* and *Pleurotus tuber-regium* collected during surveys conducted in different parts of Thiruvananthapuram district, and *Lentinus edodes, Pleurotus florida* and *Calocybe indica* were procured from TNAU and College of Agriculture, Vellayani.

P. tuber-regium, a mushroom collected during the study is a new report from India. This edible mushroom has a biological efficiency of 46 per cent and is ideally suited for cultivation in Kerala.

T. harzianum was the fastest colonizer on retted coirpith followed by *G. applanatum* and *L. edodes*. The maximum reduction of organic carbon, C:N ratio, cellulose and lignin and the maximum increase of nitrogen content in retted coirpith was recorded when it was degraded by *T. harzianum*.

Among the different substrates used for mushroom production, the maximum yield of *P. florida* was realized in a substrate containing 1:1 combination of retted coirpith and spent mushroom substrate while the maximum yield of *C. indica* was observed in a substrate containing 1:3 combination of non-retted coirpith and paddy straw.

Composted coirpith was found to be unsuitable as a substrate for large scale cultivation of oyster and milky mushrooms.