"STUDIES ON GROWTH, YIELD AND BIOCHEMICAL COMPOSITION OF *Lentinula edodes* (SHIITAKE MUSHROOM) UNDER CHHATTISGARH CONDITION "

M.Sc. (Ag) Thesis

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By

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CERTIFICATE- I

This is to certify that the thesis entitled **"STUDIES ON GROWTH, YIELD AND BIOCHEMICAL COMPOSITION OF *LENTINULA EDODES* (SHIITAKE MUSHROOM) UNDER CHHATTISGARH CONDITION"** submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** of the Indira Gandhi Agricultural University, Raipur (C.G.) is a record of the bonafide research work carried out by **Mr. UGRESH KUMAR DEWANGAN** under my guidance and supervision. The subject of the thesis has been approved by Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma (certificate awarded etc.) or has been published/published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

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CERTIFICATE – II

This is to certify that the thesis entitled **"STUDIES ON GROWTH, YIELD AND BIOCHEMICAL COMPOSITION OF *LENTINULA EDODES* (SHIITAKE MUSHROOM) UNDER CHHATTISGARH CONDITION"** submitted by **Mr. UGRESH KUMAR DEWANGAN** to the Indira Gandhi Agricultural University, Raipur (CG) in Partial fulfilment of the requirements for the Degree of **M.Sc. (Ag)** in the **Department of Plant Pathology** has been approved by the Student's Advisory Committee after an oral examination in collaboration with the external examiner.

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LIST OF ABBREVIATION

ABBREVIATION	DISCRIPTIONS
@	at the rate
%	Per cent
PS	Paddy straw
D+F	Dextrose + Fructose
NS	Non significant
S	Significant
RB	Rice bran
SD	Sawdust
DAI	Days after inoculation
OS	Oak wood sawdust
WS	Wheat straw
CC	Corn cob
Wt.	Weight
C.G.	Chhattisgarh
PDA	Potato dextrose agar
CD	Critical difference
BE	Biological efficiency
WB	Wheat bran
SEm	Standard error of means
g	Gram
Mg	Milli gram

CHAPTER -I

INTRODUCTION

Mushroom science is the discipline, which is concerned with the principles and practices of mushroom cultivation. It is essential to establish the facts upon which principles can be derived for future developments of the discipline. Consistent production of successful mushroom crops is based upon scientific knowledge and practical experience (Chang and Miles, 1989). Mushrooms are large reproductive structures of edible fungi, which belongs to the division *Ascomycotina* or *Basidiomycotina*. The vegetative part of the mushroom consists of thread like, thin, long mycelia which under suitable conditions form fruit bodies or basidiocarp. Mushrooms have been in existence for millions of years and were known to us even before the origin of man (Kohlii, 1990).

There are at least 12,000 species of fungi that can be considered as mushroom with at least 2000 species showing various degrees of edibility (Chang, 1999a). Furthermore, over 200 species of mushroom have been collected from the wild and utilized for various traditional medical purposes mostly in the far East. About 35 mushroom species have been cultivated commercially and of these about 20 are cultivated in an industrial scale. The majority of these

cultivated species are both edible and possess medicinal properties. However, two of the major medicinal mushrooms, *viz*. *Ganoderma lucidum* and *Trametes spp*. (*Coriolus*) are distinctly inedible. Overall, the world production of cultivated edible and/or medicinal mushroom was recorded as 4909.3 x 10^3 tons in 1994, 6158.4 x 10^3 tons in 1997 with an estimated value of 14 billion US dollars (Chang, 1999b).

Lentinula edodes (Berkeley) Pegler = *Lentinus eodoes* (Berkeley) Singer is best known by its Japanese name (Shiitake), Chinese name (*Xiangu*) and French name (*Lentin*). In China, it was originated and called as *Xiangu*, *Xiangu* means mushroom with great aroma (*Xian* means aroma, *gu* means mushrooms). Two highly prized forms of *Xiangu* are dongu, the winter Shiitake (dong means winter) and huagu, the flower Shiitake (hau means flower). Both form thick meaty mushroom caps. Huagu, the most sought-after Shiitake and the most expensive, is a form of dongu, with flower like cracking pattern on the upper surface of the mushroom cap (Wu, 2000).

Among the cultivated mushroom, *Lentinula edodes* (Berk.) Sing., known as Shiitake is the second most important edible mushroom in the world whose share varied from 14.09% to 25.4% in 1997. In 1980, Japan was the only country growing Shiitake in bulk and accounted for about 84% of the total Shiitake production in the

world. But, in 1999, China started producing Shiitake mushroom and within no time, it becomes the largest producer, exporter and consumer of Shiitake in the world. In 1997, the production of Shiitake in China was 13.30 lakh tones, which was increased to 16.00 lakh tones in the year 2000. It is the most popular fungus cultivated in Japan, China and other East Asian countries.

For a long period of time, this mushroom has been valued for its unique taste, flavors and as a medicinal tonic. The mushroom was used not only as a food but was taken as a remedy for respiratory diseases, poor blood circulation, liver trouble, exhaustion, weakness, premature ageing and to boost chi or life energy. *Lentinula edodes* is capable to expresse the modern terminology of generating stamina, curing colds and may play a role in the cure and prevention of heart diseases, cancer and AIDS.

Cultivation of Shiitake on natural logs begins in China almost a thousand years ago. Wu, Sang Kwuang in Zhejian figured out how to enhance fruiting in Shiitake that grew wild in nature (Chang and Miles, 1989). Traditionally, Shiitake is produced on logs with relatively low yields varying with climatic events and the whole cycle is very long (3 to 5 years). In the last decades, indoor Shiitake production was introduced in USA and in Europe substituting wood

logs by synthetic media in which sawdust is the basic ingredient for substrate formulation (Pryzbylo Wicz and Donoghue 1998). Very strict controlled conditions of cultivation are required for both mycelial propagation and fruiting and therefore very costly investment are required.

Today, Shiitake cultivation, using synthetic log is widely practiced, not only in South East Asia but also in North America, Europe, Australia and New Zealand. In India, some sporadic efforts on cultivation of Shiitake have been made by NRCM, Solan and AICRP, Udaipur. But, no systematic work has been carried out to understand the Shiitake mushroom at length. Hence, some efforts in understanding its growth, yield and biochemical composition were directed to derive some comprehensive information, which may help in standardization of production technology of Shiitake mushroom under Chhattisgarh condition. The present study was undertaken with the following objectives:

1. To study the vegetative growth and biomass production of *Lentinula edodes* on different media:

(a) Effect of different solid media on growth and colony characters.

(b) Effect of individual broth media on biomass production.

(c) Effect of mixed broth media composition on biomass production.

2. To study the antimicrobial activity of culture filtrate of *Lentinula edodes* against competitor molds.
3. Evaluation of different spawning and fruiting substrates on growth, development and yield of *Lentinula edodes*:
   (a) Evaluation of different spawning substrates on spawn development and yield of *Lentinula edodes*.
   (b) Evaluation of different fruiting substrates on growth and yield of *Lentinula edodes.*
4. To see the changes in biochemical composition of substrates with the activity of *Lentinula edodes* at different intervals.
5. To study the biochemical constituents of mycelium and basidiocarp of *Lentinula edodes*.

CHAPTER-II

REVIEW OF LITERATURE

This chapter deals with review of research work done by earlier workers on production technology and other parameters related to the mycelial growth, yield, nutritive value and anti-microbial activities of *Lentinula edodes* (Berk.) Pegler. In India, work on cultivation of *Lentinula edodes* is very meagre. The literature pertaining to proposed studies and other mushroom was thoroughly reviewed, wherever required.

2.1 Studying the vegetative growth and biomass production of *Lentinula edodes* in different media.

2.1.1 Effect of different solid media on vegetative growth.

Bilay *et al.* (2000) used four commercially available media (malt extract agar, wart agar, yeast malt extract agar and experimental agar medium) for the mycelial growth of edible and medicinal mushrooms. They found potato dextrose agar medium to be most suitable for the growth of edible fungi. According to Kapoor *et al.* (1997) malt extract agar and potato dextrose agar media exhibited good growth of *P. fossulatus.* Garibova *et al.* (1999) characterized some morphological and cultural peculiarities of *Lentinula edodes* isolates such as the differences in growth on various media.

Ziombra *et al.* (1991) grew the fungal species on four different media *viz*., Hansen, wheat, reproductive or rye grass. They found more intensive

growth of *P. ostreatus* mycelium but the growth was least intensive in *Agaricus bisporus.* The reproductive medium was best for *Pleurotus ostreatus* and *Lentinula edodes* strains.

2.1.2 Effect of different individual broth media on biomass production.

Pacumbaba and Pacumbaba (1999) demonstrated YVMBS (yeast extract, V-8 vegetable juice, multigrain oat meal, brown sugar), YVMS (Yeast extract, V-8 vegetable juice, sucrose) and MVBS (multigrain oatmeal, V-8 vegetable juice, brown sugar) to be excellent broth for growing Shiitake mushrooms. Similarly, Aleksandrova *et al.* (1998) obtained high yield of submerged mycelium of *Lentinula edodes* (up to 32g/litre of medium) in a wheat grain substrate followed by oak wood sawdust substrate medium. Maziero *et al*. (1995) screened *Lentinula edodes* (strain CCBO72) for biomass production and found better biomass yields of 8.0-15.0 g DW/litre.

Tan and Moore (1992) compared biomass production of *Lentinula edodes* strain in between stationary and shake flask cultures. They showed strain Le-465 to produce higher yields from shaken culture than from stationary cultures while, no differences were recorded in Le-11 and Le-103.

2.1.3 Effect of different mixed broth on the vegetative growth.

Kaur and Lakhanpal (1995) found glucose as the best carbon source for mycelial growth of *Lentinula edodes* followed by fructose and sucrose. Similarly, Sakamoto *et al.* (1978) found good biomass of mycelium (*Lentinula edodes*) on a medium containing glucose as the carbon source (10g/l). The maximum dry weight of mycelium (*Lentinula edodes*) on a medium containing starch, glucose and corn steep liquor was 25g/litre at 14DAI.

Song *et al*. (1987) showed glucose and ammonium chloride as the suitable carbon and nitrogen source for optimal mycelial growth at pH 4.3-4.8. Similarly, khan *et al*. (1995) found starch as the best source of carbon while, urea and asparagines as the best source of nitrogen at pH 5. According to Miyauchi *et al.* (1998), *Lentinula edodes* and *Grifola frondosa* grew well at the initial pH of 4.0 and 5.0 but, it grew poorly at pH higher than 6.0

2.2 Antimicrobial activity of *Lentinula edodes*

Pacumbaba and Pacumbaba (1999) showed that the mycelial leachate of Shiitake mushroom (*Lentinula edodes*) inhibiled the growth of *P. syringae pv. glycinea, P. siringae pv. tabaci, X. campestris pv. campestris, Erwinia amylovora, Ralstonia solanacearum, Curtobacterium flaccumfaciens pv. flaccumfaciens, Bacillus cereus, Escherichia coli,*

Listeria monocytogenes, Salmonella typhimurium and Staphylococcus aureus, due to presence of antibiotic.

Dried extracts of *Lentinula edodes* from chloroform, ethyl acetate and water exhibited good activity against S*treptococcus spp., Actinomyces spp., Lactobacillus spp., Prevotella spp. and Porphyromonas spp. while, Enterococcus spp., Staphylococcus spp., Escherichia spp., Bacillus spp. and Candida spp.* were relatively resistant to these extract (Hirasaw *et al.* 1999). Aqueous extracts of sawdust and rice bran media on which *Lentinula edodes* or *Flammulina velutipes* had grown strongly, inhibited the TMV infection (Takagi and Shimada, 1977 and Takagi and Sugimura, 1977).

2.3 Effect of different spawning and fruiting substrates on growth, development and yield of *Lentinula edodes.*

2.3.1 Effect of different spawning substrates on vegetative growth.

Philippoussis *et al*. (2003) cultivated two selected *Lentinula edodes* strains (S 4080 and SIEFO231) on Oakwood sawdust (OS), wheat straw (WS) and corn cobs (C.C.) in order to examine the influence of these residues on mycelial growth and basidiome production.They found fastest colonization in OS and WS media. Yang *et al.* (2003) used different sawdust (*Larix leptolepis, Pinus densiflora* and *Pinus koraiensis*) added with carbon nutrition's (sucrose, xylose, glucose, paper pellet) nitrogen nutrients (potassium nitrate, ammonium chloride, asparagines, glutamic acid) and

vegetable oil (rice bran oil). They found highest mycelial growth on sawdust medium.

Rathod *et al.* (2002) reported bajra and wheat grains as better substrate for commercial spawn production of *Pleurotus florida*. Sharma (2003) used five different grains (Jowar, Kutki, Kodo, Maize and Wheat) for spawn preparation of *P. djamor* and reported early spawn development on kutki grains, however, wheat grain based spawn produced more yield of *P. djamor*. Similarly, Prasuna (2002) studied different grain substrate for spawn production of *Calocybe indica* and reported early spawn development on wheat grains followed by jowar grains.

Salmones *et al.* (1999) evaluated the mycelial growth of two strain of *Leutinula edodes* on barley straw, corn cobs, corn stover, rice bran, pine apple crown bracts, coffee husks, sugarcane bagasse and sugarcane leaves. They found best mycelial growth in sugarcane bagasse, pineapple crown bracts, coffee husks and sugarcane leaves. According to Palomo *et al.* (1998) most suitable substrate for production of fruiting bodies was *Eucalyptus spp.* sawdust alone or in combination with rice dust (90% sawdust: 10% rice dust) or C*edrelinga catenaeformis* sawdust alone.

2.3.2 Effect of different fruiting substrate on the growth and yield.

Pire *et al*. (2001) assayed 8 types of wood *viz*., Coihae *(Nothofagus dombeyi),* Lenga *(N. Pumilio),* Nire *(N. antaretica),* Roble pellin *(N.*

obliqua), Eucalyptus *(Eucalyptus camaldulensis),* Pine *(Pinus elliottii),* Parana pine *(Araucaria angustifolia)* and Willow *(Salix babylonica)* with two strain of Shiitake (BAFC-1318 and BAFC-2250). They obtained fruit bodies from most wood, with the exception of Parana pine and Pine. The strain, BAFC-2250 had higher biological efficiencies in Roble pellin (60.4%), Lenga (52.3%) and Eucalyptus (26.5%). Leskobiro (1991) reported best substrates for mycelial growth and vigour in the descending order, walnut wood shavings, beech wood shavings and ground maize cobs. Chopped wheat straw alone was unsuitable but it could be used if mixed with other materials.

Shieh *et al*. (1991) tested different sawdust mixture of C*unninghamia lanceolata* and C*astanopsis hystrix* and found 100% C*unninghamia* to be unsuitable but mixture of C*unninghamia* sawdust and C*astanopsis* sawdust in 1:1 ratio increased the yield of *Lentinula edodes.* Similarly, Kim *et al*. (1987) obtained best yields on mixed sawdust media with oak sawdust (50% V/V) and poplar sawdust (50% V/V), acron powder (5% V/V) and rice bran (10% V/V).

2.4 Change in biochemical constituents of substrates with the activity of *Lentinula edodes.*

According to Santra and Nandi (1980) L*entinus praerigidus, Trametas cingulata* and *Polyporus orcularius* progressively removed all the major wood components. Lignin was generally removed preferentially to cellulose and hemicellulose and glucan faster than hemicellulose.

Kaviyarasan and Natarajan (1997) estimated the activities of various extra cellular enzymes like laccase, phenoloxidase, cellulase amylase and pectinase at five different stages of the growth and fruiting on straw and in the submerged culture condition on *Pleurotus cornucopiae var. citrinopileatus.* They found that the phenoloxidase and laccase activity was more during vegetative mycelial growth but, declined sharply at the onset of fruiting, while cellulase and amylase activities were more during fruiting and declined as soon as the harvesting was over. The pectinase activity was more during the mycelial growth and declined sharply at the onset of fruiting like that of phenoloxidase. Similarly, Sharma *et al.* (1999) estimated the percent loss in lignin, cellulose and hemicellulose content of paddy straw inoculated with *Pleurotus spp.* They showed 17.60% loss in lignin, 13.89%, loss in cellulose and 32.70% loss in hemicellulose after 12 days of inoculation.

Enoki *et al*. (1988) tested 8 of 12 white rot fungi, which degraded the lignin model dimers, a pure cellulose substrate, and wood block of

C*ryptomeria japonica* and *Fagus crenata. Poria sabacida,* a white rot fungus, failed to degrade filtar paper, but degraded the three dimers and removed about 70% of the lignin, only 1.8% of the cellulose and 24.8% of the total weight loss of *Fagas crenata*, whereas 8 of 12 brown rot fungi failed to degrade the dimers or the pure cellulose, but caused substantial weight losses of wood and degraded the cellulose component as well as the lignin component in the wood.

Dill and Kraepelin (1986) analyzed that South Chilean "Palo podrido" (rotten wood in forest trees used as food and forage by the south American Indians) results from a white rot fungus causes highly selective and extensive delignification of 14 samples, 11 had extremely low klason lignin values, ranging from 6.1 to 0.4% (dry wt.). They suggested that the extremely low nitrogen content (0.037 to 0.073% dry wt.) of the investigated wood species was the primary cause for the extensive delignification as well as the concomitant suppression of cellulose break down. Geigar *et al*. (1986) used two white rot fungi agents' *viz*., *Rigidoporus lignosus and Phellinus noxious*, which degraded the lignin fraction of rubber tree wood *in vitro*. Lignin assays showed the tendency of *R. lignosus* to degrade the lignin fraction preferentially, while *P. noxious* preferentially degraded the polysaccharide fraction.

Henningsson *et al. (*1972) reported that in decay experiment, the white rot fungus rapidly decomposed Birch wood and in the initial stages lignin was chiefly attacked e.g. with loss of 15% of the wood, 50% of the lignin

2.5 Biochemical composition of *Lentinula edodes* basidiocarp and mycelium.

2.5.1 Moisture

Moisture content did not have any nutritional significance but, it considerably influenced the nutritional values of any food item, which have high content of moisture i.e. fruits and vegetables. Moisture content of basidiocarp varied depending upon the environmental conditions, stage of growth, time of harvesting and post harvest storage. Cui *et al.* (2004) reported 17.66% moisture (dry wt. basis) in the fruit body of *Lentinula edodes*. Upadhyaya and Rai (1999) reported 89.80% moisture in the basidiocarp of *L. squarrosulus.*

2.5.2 Ash

Ash content of *Lentinula edodes* also varied considerably. According to Cui *et.al.* (2004) ash content on fruit body of *Lentinula edodes* was 4.6% on dry weight basis. Ash content of 7.74% (Dry wt. basis) on fruit body of *Lentinula squarrosulus* was reported by Upadhyay and Rai (1999).

2.5.3 Protein

Mushrooms are considered as good source of quality protein. In general, mushrooms contain 17.35% protein (dry wt. basis). Cui *et al.* (2004) recorded 17.66% crude protein content in fruit body of

Lentinula edodes whereas submerged mycelia contained 23-24% protein (Osadchaya and Lopatento, 2002). Upadhyay and Rai (1999) recorded 24.03% protein in fruit body of *Lentinula squarrosulus*. Longvah and Deosthale (1998) found considerably low amount of protein, 23% on dry weight basis.

2.5.4 Fat

In general crude fat includes lipid compounds, free fatty acids, mono, di and triglycerides, sterols, esters and phospholipids. According to Chang and Quimo (1982) all edible mushrooms contained low amount of fat. In cultivated mushrooms, fat content varied from 2-6% on dry weight basis. Cui *et al*. (2004) recorded 2.03 % of fat in *Lentinula edodes* fruit body. Upadhyay and Rai (1999) found 5.98% fat in *Lentinula squarrosulus* fruit body. Fat content of 3-4% in fruit body of *Lentinula edodes* was reported by Feofilova *et al*. (1998) whereas, Longvah and Deosthale (1998) found considerably low amount of fat (2% on dry wt. basis).

Osadcnaya and Lopatenoto (2002) recorded 8-9% fat content on submerged mycelia of *Lentinula edodes* whereas, Feofilova *et.al*. (1998) found high amount of fat (20%) on submerged mycelium as against 3-4% on fruiting bodies of *Lentinula edodes.*

2.4.5 Carbohydrate

Crisan and Sands (1978) recorded 3.28% (on fresh wt. basis) carbohydrate in various species of mushroom. In general, carbohydrate consisted of large range of compounds i.e. pentose, hexose, disaccharides, amino-sugars, sugar alcohols and sugar acids. Cui *et al* (2004) recorded about 31.66% of carbohydrate on fruiting body of *Lentinula edodes*, while Upadhayay and Rai (1999) recorded

considerably high amount of carbohydrate on fruiting body of *Lentinula squarrosulus.*

CHAPTER – III

MATERIALS AND METHODS

3.1 Experimental site

All the research experiments were conducted in the Mushroom Research Laboratory, Department of Plant Pathology, Indira Gandhi Agricultural University, Raipur (C.G.). The work on nutritional and biochemical constituents were carried out in the Department of Medicinal and Aromatic Plants and Department of Forestry, I.G.A.U., Raipur.

3.2 Cleaning, washing and sterilization

During the present investigation, glasswares *viz.* petridishes, conical flasks made of BOROSIL and ASGI were used. The glassware prior to use were properly washed and cleaned with detergent powder followed by rinsing with tap water. All the glasswares were first air dried and then sterilized at 180^0C for 2 hrs in hot air oven. The other instruments *viz*., blade, scalpel, scissors, inoculation needle, cork borer were sterilized by dipping them in 95% alcohol followed by heating over the flame.

3.3 Source of materials

The pure cultures of two strains of *Lentinula edodes* were procured from Edible Fungi Research Institute, Shanghai (China) and National Research Center for Mushroom, Chambaghat, Solan (H.P.). These pure cultures were subsequently transferred on PDA slants and maintained at

25-26^{O}C in an incubator during the entire course of investigation. The other materials i.e. paddy straw, cotton waste, wheat bran, rice bran, paddy grain, maize grain, polypropylene bags, non absorbent cotton, empty glucose bottles and other related chemicals were procured from the Department of Plant Pathology, College of Agriculture, Raipur and sawdust of different woods were obtained from local sawmill of Raipur city.

3.4 Preparation of media

The following media were used during the study period and their constituents are as follows:

3.4.1 Solid agar media

(I) Potato dextrose agar

Potato (peeled and sliced)	200g
Dextrose	20 g
Agar agar	20 g
Distilled water	1000 ml

(II) Malt extract agar

Malt extract	200g
Dextrose	20 g
Agar agar	20 g
Distilled water	1000 ml

(III)	Wheat extract agar	
	Wheat extract	200g
	Dextrose	20 g
	Agar agar	20 g
	Distilled water	1000 ml
(IV)	Saw dust extract	
	Saw dust extract	200g
	Dextrose	20 g
	Agar agar	20 g
	Distilled water	1000 ml
(V)	Paddy straw extract	
	Paddy straw extract	200g
	Dextrose	20 g
	Agar agar	20 g
	Distilled water	1000 ml
(VI)	Czapek (Dox agar) medium	
	Sodium nitrate	2g
	Potassium hydrogen phosphate	1g
	Magnessium sulphate	0.5 g
	Potassium chloride	0.5 g
	Ferrous sulphate	0.01 g
	Sucrose	30 g
	Agar agar	20 g
	Distilled water	1000 ml

(VII) Wheat + paddy straw extract

Wheat + paddy straw (1:1)	200g
Dextrose	20 g
Agar agar	20 g
Distilled water	1000 ml

(VIII) Corn meal extract medium

Maize	200g
Dextrose	20 g
Agar agar	20 g
Distilled water	1000 ml

3.4.2 Liquid broth media

(I) Potato dextrose broth

Potato (peeled and sliced)	200g
Dextrose	20 g
Distilled water	1000 ml

(II) Wheat extract broth

Wheat	200g
Dextrose	20 g
Distilled water	1000 ml

(III) Malt extract broth

Malt extract	200g
Dextrose	20 g
Distilled water	1000 ml

(IV) Maize extract broth

Maize extract	200g
Dextrose	20 g
Distilled water	1000 ml

Prepared media were sterilized in an autoclave at 15 lbs/inch^2 for 20 minutes.

3.5 Statistical analysis

All the experimental data were statistically analyzed using completely randomized design. The critical differences (CD) were calculated at five percent probability level.

3.6 General methods

3.6.1 Culturing of mycelium

Potato dextrose broth was taken in 250 ml of conical flask. It was sterilized and then inoculated using 5mm diameter disc of pure culture of *Lentinula edodes* mycelium. Precaution was taken to place the bit in the center of the flask. The inoculated flasks were incubated at $25^{O}C \pm 1^{0}C$ till the mycelium covered the entire flask. The mycelial mat was filtered by ordinary filter paper and weighed for fresh weight of mycelium. It was packed in a polyethylene (100 gauge) bag and kept in refrigerator for further study.

3.6.2 Multiplication

Two strains of *Lentinula edodes* were multiplied during the course of study. The entire work of multiplication was carried out in an inoculation chamber, which was sterilized with UV tube or alcohol prior to use. The cultures were transferred in to sterilized poured petridishes with the help of

inoculation needle. The petridishes were then incubated at 25-26^{O}C in an incubator for further investigation.

3.6.3 Preparation of mother spawn

Mother spawn was prepared by transferring pure culture of *Lentinula edodes* on wheat grains and sawdust. In case of wheat grains, clean, healthy and bold sized wheat grains were taken for preparation of mother spawn. These were washed and soaked in water over night and then grains were boiled for 10-15 minutes till they become soft and the seed coat remained intact. These grains were air dried by spreading them on the floor. There after, the grains were mixed with 2 percent calcium carbonate and 2 percent gypsum on wet weight basis.

In case of sawdust, clean, uniform particle size (0.85 mm) sawdust was taken for preparation of mother spawn. It was wetted for 24 hrs. and then mixed with wheat or rice bran (20 percent), sucrose and calcium carbonate (1 percent).

Mixed grains/sawdust was then filled up to half capacity in 250 ml conical flask and plugged with non absorbent cotton and sterilized in an autoclave at 15 lbs/inch^{2} for 30 minutes. After cooling, the flasks were inoculated with small bit of pure culture and incubated for 30 days at 24 $\pm$ 2^{O}C. The spawn prepared in this way was termed as mother spawn and used throughout the period of study.

3.6.4 Mushroom cultivation

Various hardwood sawdust *viz., Shorea robusta (Sal), Tectona grandis (Teak) and Pterocarpus marsupium* (Biza) was used as substrate for cultivation of *Lentinula edodes*. The sawdust was first moistened in plain water for over night, to maintain 60% moisture. It was then supplemented with nitrogen rich additives such as rice bran/wheat bran and then compacted into special autoclavable polypropylene bags of various dimensions (Stamets, 2000). The bags were autocalved to ensure complete internal sterility at 121^0C for 2hrs. and allowed to cool. They were aseptically inoculated with the fresh sawdust/wheat grain, spawn @ 2% on wet weight basis of substrate under the laminar flow. The spawned bags were transferred to incubation room for spawn run, where $21\text{-}27^0$C temperature, 80-90% relative humidity, proper ventilation, >10000 ppm CO_2 and darkness were maintained. Spawn run comprised of five stages of vegetative growth *viz.*, mycelial growth stage, mycelial coat formation stage, bump formation stage, browning stage, and bark formation stage. After bark formation stage, the bags were shifted to growing house with $16\text{-}21^0$C temperature, 80-90% relative humidity and 4-7hrs/day ventilation. For induction of primordial, shock treatment was given at 12^0C for 2-4 hrs. and then after 5-8 days of incubation, primordia developed into young mushroom button. Matured sporophores were picked up when the

mushroom cap is only partly extended (60-70%). Picking was done by slight pulling and twisting of the sporophore.

3.6.5 Weighting of the sporophore

The single pan electronic balance "ATCO" make with a sensitivity of 0.01 g was used for weighing of freshly harvested sporophores.

3.6.6 Yield of the mushroom

Yield was calculated taking fresh weight of two or three flushes, from a unit area of bag.

3.6.7 Biological efficiency

The yield was expressed in terms of biological efficiency (B.E.) and calculated using following formula (Chang *et al*. 1981)

$$\text{Biological efficiency (BE\%)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

3.7 Specific methods

3.7.1 Studying the vegetative growth of *Lentinula edodes*

3.7.1.1 Effect of different media on diametric growth

Eight different media *viz*. potato dextrose agar, wheat extract agar, paddy straw extract agar, malt extract agar, saw dust extract agar, Czapek's dox agar, wheat + paddy straw extract agar and corn meal agar were studied to find out the suitable medium for obtaining higher diametric growth of *Lentinula edodes*. Twenty ml of each medium was poured in sterilized

petridishes and a little quantity of streptomycin sulphate was added to avoid bacterial contamination. The plates were inoculated with 7 mm disc of actively growing mycelium of *Lentinula edodes*. Thereafter, plates were incubated at $25^0C \pm 1^0C$ and the observations on colony diameter and characters were recorded when the growth of *Lentinula edodes* completely covered the plates in any of the treatments. Each treatment had four replication.

3.7.1.2 Effect of different spawning substrate

Seven substrates *viz*., wheat grain, sorghum grain, paddy grain, sawdust, paddy straw + saw dust (20% WB), sawdust + wheat straw + paddy straw (20% WB) and sawdust + cotton waste (20% WB) were used for spawn development. The grains and other substrates were processed in view of their nature and rest of the standard procedure was followed for preparation of spawn. Three replications were maintained. Each bottle contained 125g of grains and these were sterilized. After cooling, these were inoculated with 7mm disc of pure culture of *Lentinula edodes* and incubated at $25^OC \pm 1^0C$ in dark place. The observations on percent colonization of substrate were recorded when the individual substrates were completely covered by the mycelial growth in any one of the treatments. The observations were also recorded on number of days taken for spawn run, bump formation, yield and biological efficiency.

3.7.2 Studying the biomass production of *Lentinula edodes*

3.7.2.1 Effect of different broth medium on biomass

Five individual broth media *viz*., potato dextrose broth, wheat extract broth, malt extract broth, maize extract broth and rice extract broth were studied to find out the suitable medium for obtaining higher biomass. In a 250 ml flask, 100 ml of above medium was taken and sterilized in an autoclave. The media were then inoculated and incubated for 35 days. Thereafter, the broth was filtered and mycelial mat was collected on Whatman filter paper No.1 and fresh weight was weighted on "ATCO" make electronic top pan balance. The mycelial mat was dried in an oven at $60^{O}C$ till the constant weight was obtained and again the dry weight of mycelial mat was recorded.

3.7.2.2 Effect of mixed broth media composition on biomass

Ten different broth media as detailed below were used to obtain higher biomass of *Lentinula edodes*

T_1	Dextrose	20g	T_2	Dextrose	25g
	Ammonium sulphate	3.0 g		Ammonium sulphate	3.0 g
	K_2HPO_4	5.0 g		K_2HPO_4	5.0 g
	Vita "B" complex	100 mg		Vita "B" complex	100 mg
	pH	5		pH	5
	Distilled water	1000ml		Distilled water	1000ml

T_3	Dextrose	30g	T_4	Dextrose+ fructose	(15+15g)
	Ammonium sulphate	3.0 g		Peptone	3 g
	K_2HPO_4	5.0 g		K_2HPO_4	5 g
	Vita “B” complex	100 mg		Vita “B” complex	100 mg
	pH	5		pH	6
	Distilled water	1000ml		Distilled water	1000ml
T_5	Dextrose+ fructose	(15+15g)	T_6	Dextrose+ fructose	(15+15g)
	Peptone	3.0 g		Peptone	3.0 g
	K_2HPO_4	5.0 g		K_2HPO_4	5.0 g
	Vita “B” complex	100 mg		Vita “B” complex	100 mg
	pH	4		pH	5.5
	Distilled water	1000ml		Distilled water	1000ml
T_7	Fructose	20g	T_8	Fructose	25g
	Peptone	3.0 g		Peptone	3 g
	K_2HPO_4	5.0 g		K_2HPO_4	5 g
	Vita “B” complex	100 mg		Vita “B” complex	100 mg
	pH	5.5		pH	5.5
	Distilled water	1000ml		Distilled water	1000ml
T_9	Fructose	30g	T_{10}	Potato dextrose broth	
	Peptone	3 g		Potato (Peeled)	200g
	K_2HPO_4	5 g		Dextrose	20g
	Vita “B” complex	100 mg		pH	7.0
	pH	5.5		Distilled water	1000ml
	Distilled water	1000ml			

In a 150 ml flask, 75 ml of above medium was taken and sterilized in an autoclave. The media were then inoculated and incubated for 45 days. Thereafter, the broth was filtered and mycelial mat was collected on Whatman filter paper No.1 and fresh weight and dry weight was weighed on “ATCO” make electronic top pan balance.

3.7.3 Changes in biochemical constituents of fruiting substrates with the activity of *Lentinula edodes*

From ten fruiting substrates, samples were derived at three stages *viz*. initial stage, mycelial stage (55DAI) and popcorn stage (after 80 DAI). The samples were then processed for degradation of cellulose, phenol and lignin component. Each treatment had three replications.

Determination of cellulose

(I) Materials

1. Acetic/nitric reagent: mixed 150 ml of 80% acetic acid and 15 ml of concentrated nitric acid.
2. Anthrone reagent: Dissolved 200 mg anthrone in 100 ml concentrated sulphuric acid, prepared fresh and chill for 2 hrs. before use.

(II) Procedure

1. Added 3 ml acetic/nitric reagent to a known amount (0.5 g) of the sample in a test tube and mixed in a vortex.
2. Placed the tube in a water bath at 100^0C for 30 min.
3. Cooled and then centrifuged the contents for 15-20 min.
4. Discarded the supernatant.
5. Washed the residue with distilled water and again centrifuged it and discard the supernatant from tube.

6. Added 10 ml of 67% sulphuric acid and allowed it to stand for 1 hr.
7. Diluted 1 ml of the above solution of 100 ml of distilled water.
8. To 1 ml of this diluted solution, added 10 ml of anthrone reagent and mixed well.
9. Heated the tubes in a boiling water bath for 10 min.
10. Cooled and measured the colour at 630 nm.
11. Set a blank with anthrone reagent and distilled water

(III) Calculation

Cellulose content (mg/g)

$$= 344.83 \times OD \times \frac{100}{1} \times \frac{10}{1} \times \frac{1}{0.5} \times \frac{100}{1000} \times \frac{1}{1000}$$

Determination of phenol

(I) Materials

1. 0.2 M Borate buffer (pH 7.6).
2. 80% Alcoholic borate buffer.
3. Saturated Na_2CO_3.
4. Folin - ciocalteau reagent

(II) Procedure

1. Weighed exactly 0.5g of the sample and grind it with a pestle and mortar in 7 ml of 80% alcoholic borate buffer.

2. Centrifuged the homogenate at 10000 rpm for 20 min. and saved the supernatant.

3. Pipetted out different aliquots to make up the volume 3 ml in to test tube as follows:

Sample extract	100 µl
Distilled water	800 µl
Folin - cioculteau regent	100 µl
Saturated Na_2CO_3	2000 µl

4. Mix thoroughly, placed the tubes in boiling water for exactly one min, cooled and recorded the observation at 660 nm against a blank.

5. Set a blank with distilled water + folin cioculteau reagent + saturated Na_2CO_3

(III) Calculation

Phenol content (mg/g)

$$= 363.63 \times 2 \times \frac{7000}{100} \times \frac{10}{0.5} \times \frac{1}{1000} \times OD$$

Estimation of Lignin

For estimating lignin content, the collected sawdust samples (0.5 g in weight) were digested in hot concentrated sulphuric acid and the insoluble residue obtained by filtration were oven dried and weighted (Effland, 1977).

3.7.4 Estimation of biochemical constituents of mycelium and basidiocarp of *Lentinula edodes*

Mycelium obtained from potato dextrose broth medium and sporophores from wheat grains and sorghum grains were subjected to chemical analysis for various constituents as under:

Estimation of moisture

Procedure

1. Weighed the sample accurately and placed in an oven at $60^{O}C$ for 24 hrs.
2. Weighed the oven-dried sample and again dried in an oven in same way, till the constant weight was obtained. The percentage of moisture was estimated by subtracting the final dried weight from the initial weight of sample.

$$\text{Moisture (\%)} = \frac{\text{Wt. loss}}{\text{Wt. of sample}} \times 100$$

Estimation of Ash

The crude ash of the test sample was analyzed as per the method described by Bano *et al*. (1981). Test sample 1-gram powder was charred in silica dish then transferred in a muffle furnance for 5 hrs. at 600 ^{0}C till complete ashing. The final weight of ash was recorded using following formula:

$$\text{Ash content (\%)} = \frac{\text{Wt. of crucible with ash - Wt. of empty crucible}}{\text{Wt. of crucible with sample - Wt. of empty crucible}} \times 100$$

Estimation of fat

Fat was estimated as per the method given by Sadasivam and Manikam (1992) using "Soxhlet apparatus". The fat content was calculated using following formula:

Fat content = Wt. of flask with fat – Wt. of empty flask.

Estimation of total protein content

Nitrogen content was determined by micro-kjeldhal method according to AOAC (1980) procedure using Gerhardt Digestion and Distillation (Model VAP-30), West Germany. The estimated nitrogen was converted into protein by multiplying protein factor 6.25 (N x 6.25). In each case, defatted sample was taken for estimation.

Determination of carbohydrate

(I) Apparatus

1. Micro pipette
2. Water bath
3. Spectrophotometer
4. Test tube

(II) Reagents

1. 2.5 N HCL
2. Anthrone (2 mg/ml)

(III) Procedure

1. 0.1 g extracted oven dried sample was taken in to 10 ml test tube and added 5 ml 2.5 N HCL.

2. Kept in water bath for 3 hrs.
3. After 3 hrs, cooled and neutralized with Na_2CO_3 till the fan not stop.
4. Make up the volume to 100 ml by adding distilled water.
5. Pipetted out 10 ml of above solution in test tube and centrifuged it for 10-15 minutes at 4000 rpm. Also made set a blank with distilled water.
6. Pipetted out 0.1 ml extract from test tube in another 10 ml test tube and added 1.9 ml distilled water to make 2 ml volumes.
7. In 2 ml volume, added 4 ml anthrone reagent and kept it on water bath for 8 min. at $100^{O}C$ temperature.
8. Cooled and observed reading at 630 nm on spectro-photometer.

Calculation

Carbohydrate content (g/100g)

$$= 131.06 \times OD \times \frac{100}{0.1} \times \frac{1}{0.1} \times \frac{1}{1000} \times \frac{100}{1000}$$

3.7.5 Studying the antimicrobial activity of *Lentinula edodes* culture filtrate

The effect of *Lentinula edodes* culture filtrates was studied against different contaminants. *Lentinula edodes* culture filtrate was prepared on potato dextrose broth in 250 ml flask. These flask were inoculated by 5mm

disc of *Lentinula edodes* culture, and then incubated at 25 ± 1^0C for 45 days. Afterward, the mycelium was filter out in sterilized condition and remaining filtrates was used as an anti microbial substance. Four contaminants *viz., Trichoderma viride, Sarocladium oryzae*, C*oprinus spp*. and S*clerotium rolfsii* were tested. A disc of 5 mm diameter was cut from pure culture of contaminants and inoculated in culture filtrate of *Lentinula edodes.* These contaminants inoculated flask were incubated at 25 ± 1^0C and observed the growth of contaminants on broth (% of flask covered by the mycelium) extract at different time intervals. Three replications were maintained.

3.7.6 To evaluate the effect of different fruiting substrates on the growth and yield of Shiitake mushroom

The sawdust from different woods and composition of different treatments were as follows:

T_1	Sawdust (Biza)	50%	T_2	Sawdust (Teak)	50%
	Rice bran	23%		Rice bran	23%
	Sugar	1.5%		Sugar	1.5%
	Gasso	2%		Gasso	2%
	$CaCO_3$	0.5%		$CaCO_3$	0.5%
T_3	Sawdust (Sal)	50%	T_4	Sawdust (Mix) (1:1:1) Biza: Teak: Sal	50%
	Rice bran	23%		Rice bran	23%
	Sugar	1.5%		Sugar	1.5%
	Gasso	2%		Gasso	2%
	$CaCO_3$	0.5%		$CaCO_3$	0.5%

T_5	Sawdust (mix) (1:1:1)	50%	T_6	Sawdust (mix) (1:1:1)	50%
	Rice bran	15%		Rice bran	20%
	Sugar	1.5%		Sugar	1.5%
	Gasso	2%		Gasso	2%
	$CaCO_3$	0.5%		$CaCO_3$	0.5%
T_7	Sawdust (Teak)	50%	T_8	Sawdust (Teak)	50%
	Rice bran	15%		Rice bran	20%
	Sugar	1.5%		Sugar	1.5%
	Gasso	2%		Gasso	2%
	$CaCO_3$	0.5%		$CaCO_3$	0.5%
T_9	Sawdust (mix of all)	50%	T_{10}	Sawdust+ Paddy straw (1:1)	50%
	Rice bran	23%		Rice bran	20%
	Sugar	1.5%		Sugar	1.5%
	Gasso	2%		Gasso	2%
	$CaCO_3$	0.5%		$CaCO_3$	0.5%

Mixed the ingredient of each treatments and made up the volume 9 Kg (wet basis). Thereafter, the mixed substrates were filled in polyethylene bags (1.5 Kg/bag) and plugged the mouth of bag with cotton via plastic ring. These bags were sterilized in an autoclave at 20 lbs/inch^2 for 2 hrs. After cooling, the bags were spawned @ 2% on wet weight basis of substrate aseptically and were shifted to growing room where the prevailing temperature of 21-27^{0}C, relative humidity of 95-100%, proper ventilation and darkness were maintained. Six replications for each treatment were maintained and

the observations on number of days taken for mycelial growth and popcorn stage were recorded.

CHAPTER –IV

RESULTS AND DISCUSSION

4.1 Effect of different media on vegetative growth and biomass production.

4.1.1 Effect of different solid media on vegetative growth

Effect of different solid media on growth and colony characters of two strain of *Lentinula edodes* was studied under *in vitro* conditions and the results are presented in Table –1.

There was significant difference in radial growth of *Lentinula edodes* on different media evaluated. The radial growth of *Lentinula edodes* in American strain was significantly higher (87.50 mm) in potato dextrose agar medium followed by corn meal agar medium, (86.75), malt extract agar, (81.75 mm) and wheat extract agar, (76.25 mm). However, it was least (14.00 mm) in Czapeks dox agar medium. The mycelial growth on potato dextrose agar was white, fluffy with concentric rings and regular margin and other media showed varying pattern from white to dull white, with rings and without rings, very thick, scattered, with regular to irregular margin. In case of Chinese strain of *Lentinula edodes*, significantly higher radial growth (83.25 mm) was recorded in malt extract agar. The radial growth in potato dextrose agar medium, (76.75 mm) and corn meal agar, (74.75mm) was statistically at par among themselves. It was significantly less (22.75 mm) in wheat + Paddy straw extract agar medium. The mycelial growth on malt

extract agar was very thick, white, strandy with irregular margin. However, the growth on wheat + paddy straw extract agar medium was dull white, thin, flat, raised in periphery with irregular margin. In rest of the medium, the growth pattern varied from white to dull white, flat to fluffy, thick to thin with irregular margins.

In the present study, potato dextrose agar, malt extract agar and corn meal agar media were found to be excellent in promoting higher mycelial growth in both the strains of *Lentinula edodes*. However, Czapeks dox agar, wheat + paddy straw extract agar and paddy straw extract agar did not perform well. The reproductive medium as suitable for culturing of *Pleurotus ostreatus* and *Lentinula edodes* strain was also reported by Ziombra *et al.*(1991). Potato dextrose agar as suitable medium for the growth of edible and medicinal mushroom was reported by Bilay (2000).

4.1.2 Effect of individual broth media on biomass production

Effect of liquid media on biomass production of *Lentinula edodes* strains was studied and the data is presented in Table -2.

Fresh mycelial weight and dry mycelial weight of *Lentinula edodes* differed significantly on different liquid culture media used. Both the strains under study exhibited different trends in fresh and dry mycelial weight with regards to different culture media studied. Maximum fresh mycelial weight in American strain (13.12g) was recorded on wheat extract broth. The fresh biomass in malt extract broth (8.63), maize extract broth (7.72g) and potato

dextrose broth was though less than wheat extract but was statistically at par among themselves. Minimum fresh mycelial weight (5.42g) was recorded in rice extract broth. Similarly, maximum dry mycelial weight (0.53g) was recorded in wheat extract broth, while it was minimum (0.12g) in rice extract broth. The average biomass in American strain of *Lentinula edodes* was significantly higher (6.82g) in wheat extract broth with least biomass (2.773g) in rice extract broth.

On the other hand, Chinese strain of *Lentinula edodes* showed maximum fresh mycelial weight (14.72g) in potato dextrose broth, while, it was minimum (2.44g) in malt extract broth. Maximum dry mycelial weight (0.93g) was obtained in potato dextrose broth while, it was lowest (0.24 g) in malt extract broth. The average biomass was also maximum (7.83g) in potato dextrose but, it was minimum (1.34g) in malt extract broth. From the results, it appears that both the strains of *Lentinula edodes* differed in the requirement of nutrients as a result of which they showed different trends in biomass production. The culture media suitable for American strain was found to be unsuitable for Chinese strain of *Lentinula edodes*. Aleksandrova *et al*. (1998) reported higher yield of submerged mycelium of *Lentinula edodes* (up to 32 g/liter of medium) in wheat grain extract confirming the present results. Similarly, potato dextrose agar as a suitable medium for the growth of edible and medicinal mushroom was also reported by Bilay (2000).

4.1.3 Effect of mixed broth media composition on biomass production

Biomass production of *Lentinula edodes* as influenced by media composition is presented in Table - 3.

American strain and Chinese strain of *Lentinula edodes* showed different trends in fresh mycelial weight and dry mycelial weight with respect to different media composition studied. American strain of *Lentinula edodes* showed maximum fresh mycelial weight (4.75g) in Dextrose (D) +Fructose (F) at pH 4.0 followed by D+F at pH 5.5 (4.29g). However, fresh mycelial weight was minimum (0.54 g) in dextrose @ 25 g^{-1} . Similarly, dry mycelial weight was significantly higher in D+F at pH 4.0 (0.295g), followed by D+F at pH 5.5 (0.279 g). Minimum dry mycelial weight was recorded in dextrose @ 25 g^{-1} (0.083g). The average biomass was also higher in D+F at pH 4.0 (2.52g) and D+F at pH 5.5 (0.083g) but, it was lower in dextrose @ 25g^{-1}.

On the other hand, Chinese strain showed maximum fresh (0.090g) and dry mycelial weight (0.083g) in potato dextrose broth. The minimum fresh and dry mycelial weight, 0.019g and 0.016g respectively was recorded in dextrose @ 20g^{-1}. The average biomass of *Lentinula edodes* in both the strain showed same trend as noticed in fresh and dry biomass. Miyauchi *et al*. (1998) reported *Lentinula edodes* and *G. frondosa* to grow well at the initial pH of 4.0 and 5.0 but very poor growth was recorded at pH higher than 6.0. Kaur and Lakhanpal (1995) reported that the media containing

glucose was best followed by fructose and sucrose. Potato dextrose agar as a suitable medium for the growth of edible and medicinal mushroom was also reported by Bilay (2000).

4.2 Anti-microbial activity of *Lentinula edodes*

Vegetative growth of different contaminants on the mycelial extract of *Lentinula edodes was* studied and the results obtained are presented in Table - 4.

Mycelial growth of different contaminants on mycelial extract of American strain of *Lentinula edodes* after 20 and 30 days of inoculation did not show the significant difference, but significant differences in mycelial growth of contaminants were observed after 10 days of inoculation. Minimum mycelial growth (1.0%) was observed in S*clerotium rolfsii* followed by *Sarocladium oryzae* (1.0%) wheareas, it was maximum (3.6%) in *Trichoderma viride.* No growth was observed in C*oprinus spp*. indicating high sensitivity to the mycelial extract of *Lentinula edodes*. Contaminants in mycelial extract of Chinese strain of *Lentinula edodes* showed significant difference after 10,20 and 30 days of inoculation. After 10 days of inoculation, least mycelial growth (5.0%) was observed in *Sarocladium oryzae* followed by *Trichoderma viride* (11.60%), while it was maximum (60.00%) in *Sclerotium rolfsii.* But, no growth was observed in *Coprinus spp*. The mycelial growth of *Sclerotium rolfsii* completely covered (100%) the mycelial extract of *Lentinula edodes* after 20 and 30 days of inoculation

indicating resistance to extract of *Lentinula edodes*. The mycelial growth of *Trichoderma viride* was 71.66 per cent at 20 and 30 DAI and least mycelial growth (20%) was observed in *Sarocladium oryzae* at 20 and 30 DAI, but no growth was observed in *Coprinus spp*. From the results presented, it appears that American strain of *Lentinula edodes* was more effective than Chinese strain in inhibiting the mycelial growth of most of the contaminants. Both the strain of *Lentinula edodes* were highly effective against *Coprinus spp.* which is a major mold fungus being encountered during mushroom cultivation. *Sarocladium oryzae* also showed inhibitory effect, but the growth of *Sclerotium rolfsii* was very little influenced due to the mycelial extract of *Lentinula edodes.* Pacumbaba and Pacumbaba (1999) reported that the mycelial leachate of *Lentinula edodes* inhibited the growth of *Pseudomonas syringae Pv glycinea*, *Bacillus cereus* and *Escherichia coli* due to the presence of antibiotic. Dried extract of *Lentinula edodes* exhibited good activity against *Actinomyces, Prevotella spp*. and *Porphyromonas spp*. while *Bacillus spp., Staphylococcus spp*. and *Candida spp*. were relatively resistant to these extract.

4.3. Effect of different spawning and fruiting substrate on growth, development and yield of *Lentinula edodes*

4.3.1 Effect of different spawning substrate on mycelial development.

Effect of spawning substrates on percent mycelial colonization of American and Chinese strains of *Lentinula edodes* was studied at different intervals and the results obtained are depicted in Table -5

The percent colonization in both the strains of *Lentinula edodes* differed considerably with respect to different spawning substrates used. It was noticed that the spawning substrates suitable for the mycelial development of American strain of *Lentinula edodes* were unsuitable for Chinese strain of *Lentinula edodes*. The mycelial colonization in American strain of *Lentinula edodes* at 20 days after inoculation was significantly higher (95%) in sawdust + paddy straw substrate followed by wheat grains (88.33%), sorghum grains (86.66%) and saw dust + wheat straw + paddy straw substrate (85.00%), whereas, the percent colonization was significantly less (54.73%) on saw dust + cotton waste. The percent mycelial development was further enhanced and reached to 100% on sawdust + paddy straw at 25 days after inoculation. At 30 days after inoculation, the complete mycelial colonization (100%) in American strain of *Lentinula edodes* was recorded on wheat grains and saw dust + paddy straw substrates. The percent colonization in American strain of *Lentinula edodes* was though less but satisfactory in other substrates *viz*. sorghum grains (98.33%), sawdust + wheat straw + paddy straw (98.33%), saw dust alone (96.67%) and saw dust + cotton waste (96.66%). No colonization in American strain of *Lentinula edodes* was recorded on paddy grains.

The rate of growth/day in American strain of *Lentinula edodes* was considerably higher (3.33%) on wheat grains and saw dust + paddy straw (3.33%) followed by sorghum grains (3.28%) and saw dust + wheat straw +

paddy straw (3.28%). The rate of growth/day was considerably less (3.22%) on sawdust alone and saw dust + cotton waste (3.22%). However, no growth was recorded on paddy grains.

The mycelial colonization in Chinese strain of *Lentinula edodes* at 20 days after inoculation was significantly higher (83%) on wheat grains followed by sorghum grains (66.60%) whereas, the percent colonization was significantly less (15%) on saw dust + cotton waste. No colonization of *Lentinula edodes* was recorded on paddy grains and sawdust + Wheat straw + Paddy straw substrates. The percent mycelial development was further enhanced and reached to 91.66% on wheat grains and sorghum grains at 25 days after inoculation. At 30 days after inoculation, the complete mycelial colonization (100%) in Chinese strain of *Lentinula edodes* was recorded on wheat grains and sorghum grains whereas, the percent colonization was significantly less (33.33%) on saw dust + cotton waste.

The rate of growth/day in Chinese strain of *Lentinula edodes* was considerably higher (3.33%) on wheat grains and sorghum grains. However, it was considerably less (1.11%) on sawdust + cotton waste and sawdust alone (1.94%). No growth was recorded on paddy grains and saw dust + wheat straw + paddy straw substrate. From the results, it can be said that both the strain of *Lentinula edodes* differed considerably with respect to different substrates used. Saw dust + wheat straw+ paddy straw supporting good colonization of American strain of *Lentinula edodes* did not at all

support the Chinese strain of *Lentinula edodes*, but wheat and sorghum grains were found to be quite suitable substrate for the growth of both the strain of *Lentinula edodes*. Wheat grains and sorghum grains as suitable spawning substrate for *Pleurotus* spp. have also been reported by several workers (Rotainh and Swargiary (1994), Mathew *et al*. (1996), Rathod *et al*. (2002), Khatri and Agrawal (2002). Philippoussis *et al*. (2003) found fastest mycelial colonization of *Lentinula edodes* strain (S 4080 and SIEF 0231) on oak wood sawdust and wheat straw media. Yang *et al*. (2003) also found highest mycelial growth on sawdust medium.

4.3.2 Effect of different spawning substrate on spawn development and yield.

Effect of spawning substrates on spawn development, bump formation and yield of Chinese strain of *Lentinula edodes* was studied and the results are presented in Table -6.

The period required for spawn run and bump formation in Chinese strain of *Lentinula edodes* was significantly influenced with different kinds of spawning substrates used. Wheat grains and sorghum grains took significantly less time (25.00 days) for spawn run followed by Saw dust + Paddy straw (33.33 days) substrate whereas, other substrates required significantly more time (46.66 to 62.33 days) for spawn run. Sorghum and wheat grains could also take less time (65.66 days) for bump formation while, other substrates did not support bump formation. The biological efficiency was significantly higher on wheat grains (12.53%) followed by

sorghum grains (10.13%) whereas, other substrates did not produce the basidiomata.

Wheat and sorghum grains were found to be most suitable substrates for spawn development of *Lentinula edodes* as it took significantly less time. However, spawn development was delayed in sawdust + paddy straw and sawdust alone. Rathod *et al*. (2002) reported bajra and wheat grain as better substrate for commercial spawn production of *Pleurotus florida*. Early spawn development of *Calocybe indica* on wheat grains followed by jowar grain was also reported by Prasuna (2002). Sharma (2003) found that wheat grain based spawn produce more yield of *Pleurotus djamor*.

4.3.3 Effect of different fruiting substrate on the growth and yield of *Lentinula edodes*

Mycelial colonization and spawn run as influenced by different fruiting substrates was studied and the results are presented in Table - 7.

Ten different types of sawdust-based substrate were used to know the mycelial colonization. There was significant difference in percent colonization (recorded at 80 DAI) and the period required for spawn run. Maximum mycelial colonization (94.16%) was obtained in substrate with Sal sawdust + RB (22%), followed by 81.66 per cent in Teak sawdust + RB (20%) and 78.33 per cent in Biza sawdust + RB (22%). The mycelial colonization was minimum (41.66%) in substrate with Teak sawdust + RB (22%). The period required for spawn run (mycelial stage) and pop corn stage was minimum, 70 and 75 days, respectively in sawdust of Sal + RB

(22%) whereas, it was significantly delayed in rest of the substrate (95.33 to 134.33 days). Thus, it can be said that sawdust of Sal + RB (22%) was the best fruiting substrate as it allowed maximum mycelial colonization and minimum time for spawn run and pop corn stage. Pire et *al*. (2001) reported higher biological efficiencies of *Lentinula edodes* strain, BAFC-2250 in roble pellin (60.4%), lenga (52.3%) and eucalyptus (26.5%). Leskobiro (1991) reported best substrates for mycelium growth and vigour in the descending order, walnut wood Shavings, beech wood Shavings and ground maize cobs. Shieh *et al*. (1991) found that mixture of *Cunninghamia* saw dust and Castanopsis sawdust in 1:1 ratio increased the yield of *Lentinula edodes*.

4.4 Change in biochemical constituents of substrates with the activity of *Lentinula edodes* (Chinese strain)

Degradation of biochemical constituents of different fruiting substrate at varying growth stage of *Lentinula edodes* was studied and the results are depicted in Table -8.

Initial content of lignin, cellulose and phenol in different fruiting substrates differed significantly. The initial lignin content was significantly higher (63.68 mg/g) in sawdust of Biza wood + RB (22%) while, it was significantly less 52.09mg in Mixed wood +22% RB followed by Mixed wood + 15% RB (52.22mg/g) and Teak wood + 15% RB (52.4 mg/g). The lignin content was found to be degraded rapidly in active mycelial stage and popcorn stage in majority of the fruiting substrate. The degradation of lignin

in active mycelial stage was less but the rate of degradation increased rapidly when the fungus entered in the popcorn stage. In case of Teak wood sawdust +22 % RB and Teak wood sawdust + 15% RB, the rate of lignin degradation was more in active mycelial stage but it was less during popcorn stage.

The initial content of cellulose was significantly higher on Sal wood sawdust + 22% RB (19.31 mg/g) while, it was significantly lower (5.44 mg/g) on all Mixed wood sawdust + 15% RB. The rate of degradation of cellulose was considerably more than lignin and phenol content. The percent reduction in cellulose content varied from 59.97-74.41 percent in active mycelial stage to 90.77 to 92.12 percent in popcorn stage in Sal wood sawdust + 22% RB, Mixed wood sawdust + 22% RB and Sawdust + Paddy straw + 20% RB. It indicates that *Lentinula edodes* mainly utilizes cellulose as an important source of food. In other fruiting substrates too, the percent reduction in cellulose was quite high.

Initial phenol content was significantly higher in Sal wood sawdust + 22%RB (62.59 mg/g) whereas, it was significantly lower (26.19 mg/g) on Sawdust + Paddy straw + 20% RB. The phenol content showed marked variation in different fruiting substrates. In five substrates, there was continuous increse in phenol content both at active mycelial stage and popcorn stage. In first two substrates, there was increse in percent phenol content at active mycelial stage but, it declined at popcorn stage. In Teak

wood sawdust + 15% RB and Teak wood sawdust + 20% RB, there was continuous decrease in percent phenol content at both active mycelial stage and popcorn stage. However, all mixed wood sawdust + 15% RB showed percent decrease in phenol content at active mycelial stage but, it was increased at popcorn stage. In 50% of the fruiting substrates, percent increse in phenol content both at active mycelial stage and popcorn stage may be associated with dark browning of the substrate. Browning was more intense at popcorn stage, which might be due to more content of phenol.

Thus, change in different biochemical constituents and the speed at which they changed is an indication of good growth and development of fungus in the substrate. Similar changes in lignin, cellulose and phenol content of paddy straw due to the activity of *Pleurotus cornucopiae* var. *citrinopileatus* at growth and fruiting stage were reported by Kaviyarasan and Natarajan (1997) and Sharma *et al*. (1999) in case of *P.djamor* and *P.ostreatus*.

4.5 Nutritional composition of *Lentinula edodes* basidiocarp and mycelium.

The basidiocarp and mycelium of Chinese strain and mycelium alone of American strain of *Lentinula edodes* were analyzed for nutritional constituents and the results obtained are presented in Table – 9.

It was found that the basidiocarp and mycelium of Chinese strain and mycelium of American strain of *Lentinula edodes* did not show the significant differences in fat, carbohydrate and ash content. However, the

content of fat varied from 4.27 to 2.76 percent, carbohydrate varied from 25.03 to 22.86 percent and ash varied from 4.23 to 3.94 percent. Protein and moisture content in Chinese as well as American strain were found to differ significantly. The protein content was significantly higher 32.81 percent in basidiocarp of Chinese strain of *Lentinula edodes* followed by mycelium of Chinese strain (20.65%). The protein content was significantly less (17.01%) in mycelium of American strain of *Lentinula edodes*. Similarly, moisture content was significantly higher (13.14%) in mycelium of Chinese strain, while it was significantly lower (7.78%) on mycelium of American strain of *Lentinula edodes*. Cui *et al*. (2004) reported moisture content of 17.66 per cent (on dry weight basis) and crude protein content of 17.66 percent on fruit body of *Lentinula edodes,* which are slightly different from the present study. Similarly, Osadchya *et al*. (2002) and Upadhayay and Rai (1999) reported 23.24% protein in submerged mycelia of *Lentinula edodes* and *Lentinula squarrosulus,* which was higher than present investigation.

CHAPTER – V

SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

The present investigation was undertaken to study the vegetative growth and biomass production of *Lentinula edodes* on different media; to study the anti-microbial activity of culture filtrates of *Lentinula edodes* against competitor mould; to evaluate different spawning and fruiting substrates on growth, development and yield of *Lentinula edodes*; to see the changes in biochemical composition of substrates with the activity of *Lentinula edodes* at different intervals and to study the biochemical constituents of mycelium and basidiocarp of *Lentinula edodes*.

The radial growth of *Lentinula edodes* in American strain was significantly higher (87.50 mm) in potato dextrose agar medium but, it was least (14.00 mm) in Czapeks dox agar medium. In Chinese strain of *Lentinula edodes*, significantly higher radial growth (83.25 mm) was recorded in malt extract agar and it was significantly less (22.75 mm) in wheat straw + paddy straw extract agar medium.

On different culture media, American strain of *Lentinula edodes* showed maximum fresh (13.12g) and dry mycelial biomass (0.53g) in wheat extract broth and minimum fresh (5.42g) and dry biomass (0.12g) in rice extract broth. Similarly, the average biomass also followed the same

trend. On the other hand, Chinese strain of *Lentinula edodes* showed maximum fresh (14.27g) and dry mycelial biomass (0.93g) in PDA and minimum fresh (2.44 g) and dry biomass (0.24g) in malt extract broth. Average biomass also followed the same pattern.

On different media composition, American strain of *Lentinula edodes* showed maximum fresh (4.75g), dry (0.295g) and average biomass (2.52g) in D+F at pH 4.0 and D+F at pH 5.5 (4.25, 0.279 and 2.28g) and minimum biomass in dextrose @ 25 g^{-1} (0.54, 0.083 and0.31g). But, Chinese strain showed maximum fresh (0.090g) , dry (0.83g) and average biomass (0.087g) in pototo dextrose broth and the minimum fresh (0.019g), dry (0.016 g) and average biomass (0.018g) in Dextrose @ 20g^{-1}.

Mycelial growth of different contaminants on mycelial extract of American strain of *Lentinula edodes* shows significant differences after 10 days of inoculation, but there was no difference at 20 and 30 days of inoculation. After 10 days of inoculation minimum mycelial growth (1.0%) was observed in *Sclerotium rolfsii* followed by *Sarocladium oryzae* (1.0%) Whereas, it was maximum (3.6%) in *Trichoderma viride*. No growth in *Coprinus spp*. was observed. The growth of contaminants in mycelial extract of Chinese strain of *Lentinula edodes* showed significant difference after 10, 20 and 30 days of inoculation. After 10 days of inoculation, least mycelial growth (5.0%) was observed in *Sarocladium oryzae* followed by

Trichoderma viride (11.60%), while it was maximum (60.00%) in *Sclerotium rolfsii* and no growth was obsvered in case of *Coprinus spp*. The mycelial growth of *Sclerotium rolfsii* completely covered (100%) the mycelial extract of *Lentinula edodes* after 20 and 30 days of inoculation. The mycelial growth of *Trichoderma viride* was 71.66 per cent at 20 and 30 DAI and least mycelial growth (20%) was observed in *Sarocladium oryzae* at 20 and 30 DAI but no growth was observed in *Coprinus spp*. indicating sensitivity of extract to *Coprinus spp., Sarocladium oryzae* and resistant to *Sclerotium rolfsii* and *Trichoderma viride.*

On spawning substrates, mycelial colonization of American strain of *Lentinula edodes* at 20 days after inoculation was higher (95%) in sawdust + paddy straw, wheat grains (88.33%), Sorghum grains (86.66%) and sawdust+ wheat straw + paddy straw (85.00%) and was least (54.73%) on saw dust + cotton waste. Complete mycelial colonization (100%) was recorded on sawdust + paddy straw substrate at 25 DAI and 30 DAI on wheat grains. No mycelial colonization was recorded on paddy grains. The rate of growth per day in American strain of *Lentinula edodes* was higher (3.33%) on wheat grains, sawdust + paddy straw, sorghum grains (3.28%) and sawdust + wheat straw + paddy straw (3.28%). On the other hand, mycelial colonization in Chinese strain of *Lentinula edodes* at 20 days after inoculation was higher (83%) in wheat grains and sorghum grains (66.60%)

whereas, less colonization (15%) was noticed on sawdust + cotton waste. After 25 days of inoculation, the percent mycelial colonization was 91.66% on wheat and sorghum grains. At 30 DAI, the complete mycelial colonization (100%) was noticed on wheat grains and sorghum grains, whereas it was less (33.33%) on sawdust + cotton waste. No mycelial colonization was recorded on paddy grains and sawdust + wheat straw + paddy straw. The rate of growth/day was higher (3.33%) on wheat grains and sorghum grains.

Different spawning substrates were found to significantly influence the period of spawn run, bump formation and fresh yields in Chinese strain of *Lentinula edodes*. Wheat grains and sorghum grains took less time (25 day) for spawn run and bump formation (65.66 days), but other substrate did not support bump formation. Biological efficiency was significantly higher in wheat grains (12.53%) followed by sorghum grains (10.13%) whereas, other substrate did not produce the basidiomata.

On different sawdust fruiting substrates, the maximum percent mycelial colonization (94.16%) at 80 DAI was obtained in sawdust of Sal + RB (22%) and the minimum (41.66%) in sawdust of Teak + RB (22%). In sawdust of Sal + RB (22%) took less time for spawn run and popcorn stage (75 days) while, other substrate took more time for spawn run and popcorn stage which varied from 95.33 to 134.33 days.

The Chinese strain of *Lentinula edodes* fruitbody and mycelium and American strain of *Lentinula edodes* mycelium did not show significant difference in fat, carbohydrate and ash content but, higher protein content (32.81%) was found in sporophore of Chinese strain of *Lentinula edodes* followed by mycelia of Chinese strain (20.65%) and less (17.01%) on American strain mycelia. Higher moisture content (13.14%) was found on mycelia of Chinese strain followed by fruit body of Chinese strain (10.29%) and least (7.78%) on mycelium of American strain of *Lentinula edodes.*

Conclusions:

The present investigation entitled "Studies on growth, yield and biochemical composition of *Lentinula edodes* (Shiitake Mushroom) under Chhattisgarh condition" was undertaken in the Mushroom Research Laboratory of Department of Plant Pathology, Department of Medicinal and Aromatic Plants and Department of Forestry, College of Agriculture, IGKV, Raipur (C.G.). The results of the study summarized are concluded below:

1. The radial growth in American strain and Chinese strain of *Lentinula edodes* was higher in potato dextrose agar and malt extract agar medium respectively.
2. Higher biomass of American strain of *Lentinula edodes* was recorded on wheat extract broth while, Chinese strain of *Lentinula edodes* produced more biomass on potato dextrose broth.

3. Media with dextrose + fructose at pH 4.0 and pH 5.5 were found to be superior in obtaining higher biomass of American strain of *Lentinula edodes*, while potato dextrose broth of pH 7.0 was superior in obtaining higher biomass of Chinese strain of *Lentinula edodes*.
4. Mycelial extract of American strain of *Lentinula edodes* completely inhibited the growth of *Coprinus spp.* and partially suppressed the growth of *Sclerotium rolfsii* and *Sarocladium oryzae*, while mycelial extract of Chinese strain of *Lentinula edodes* could only suppress the growth of *Coprinus spp*.
5. The complete mycelial colonization (100%) and higher rate of growth/day in both the strain of *Lentinula edodes* was recorded on wheat grains, sawdust+ paddy straw and sorghum grains substrates at 30 DAI.
6. Wheat grains and sorghum grains took less time for spawn run (25 days) bump formation (64.33 to 65.66 days) and gave higher yield (10.13 to 12.53% BE) as compared to other spawning substrates.
7. Higher mycelial colonization (94.16%) was noticed on sawdust of Sal wood + RB (22%) at 80 DAI. Minimum time (70 days) for spawn run and popcorn stage (75 days) was required in sawdust of Sal + RB (22%) as compared to other substrates.
8. The Chinese strain of *Lentinula edodes* was found to be superior than American strain of *Lentinula edodes* with respect to protein, fat, carbohydrate and ash content.

Suggestions for future work

1. The work on cultural conditions for increasing higher biomass of *Lentinula edodes* should be studied.
2. Environmental condition of Chhattisgarh inducing fruiting needs to be thoroughly studied.
3. Locally available substrates and their fermentation should be exhaustively studied for vegetative growth and fruiting of *Lentinula edodes.*
4. Supplementation of substrates for production of *Lentinula edodes* should be studied.
5. Strain of *Lentinula edodes* suitable for higher temperature, condition needs to be evaluates under Chhattisgarh condition.

"STUDIES ON GROWTH, YIELD AND BIOCHEMICAL COMPOSITION OF *Lentinula edodes* (SHIITAKE MUSHROOM) UNDER CHHATTISGARH CONDITION "

BY

UGRESH KUMAR DEWANGAN

ABSTRACT

Cultivation of Shiitake mushroom was attempted in Chhattisgarh under AICRP on mushroom, with least success. Hence, an efforts were further made to systematically understand its growth, yield and changes in biochemical constituents of the substrate during cultivation and biochemical constituents percent in the basidiocarp and mycelium of *Lentinula edodes* strain to derive comprehensive information which may help in standardization of production technology of Shiitake mushroom under Chhattisgarh condition. The diametric growth of American strain and Chinese strain of *Lentinula edodes* was higher in potato dextrose agar (87.50mm) and malt extract agar medium (83.25mm), respectively. On the other hand, potato dextrose broth produced higher biomass among different liquid media studied. Mixed liquid media with dextrose + fructose at pH 4.0 and pH 5.5 was found to be superior in obtaining higher biomass (4.75g) and (4.29g), respectively, of American strain of *Lentinula edodes* as compare to other mixed broth media. However, potato dextrose broth at neutral pH was found to be best for obtaining higher biomass in Chinese strain of *Lentinula edodes.* Mycelial extract of American strain of *Lentinula edodes* was found to completely inhibit the growth of *Coprinus spp*., partially suppressed the growth of *Sclerotium rolfsii* and *Sarocladium oryzae* but, mycelial extract of Chinese strain of *Lentinula edodes* could only suppress the growth of *Coprinus spp*. The complete mycelial colonization (100%) and higher rate of growth/day in both the strain of *Lentinula edodes* was recorded on wheat grains, sawdust + paddy straw and sorghum grains substrate at 30DAI. Wheat grains and sorghum grains took less time for spawn run, bump formation and gave higher yield as compared to other spawning substrates. On different fruiting wood substrates, higher mycelial colonization (94.16%) as well as minimum time (70 days) for spawn run and popcorn stage (75 days) was noticed on sawdust of Sal wood + RB (22%). Nutritionally, the Chinese strain of *Lentinula edodes* was found to be superior than American strain of *Lentinula edodes* with respect of protein, fat, carbohydrates and ash content.

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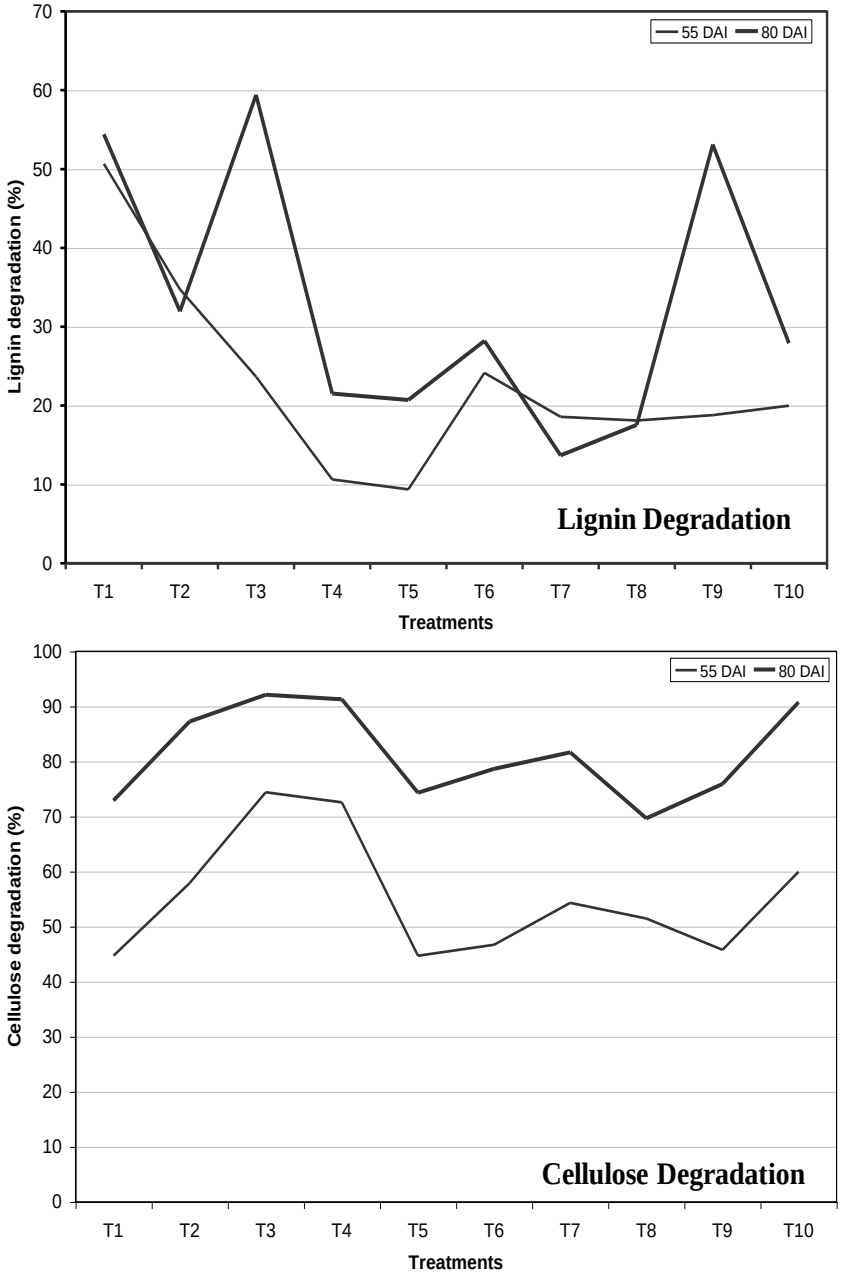
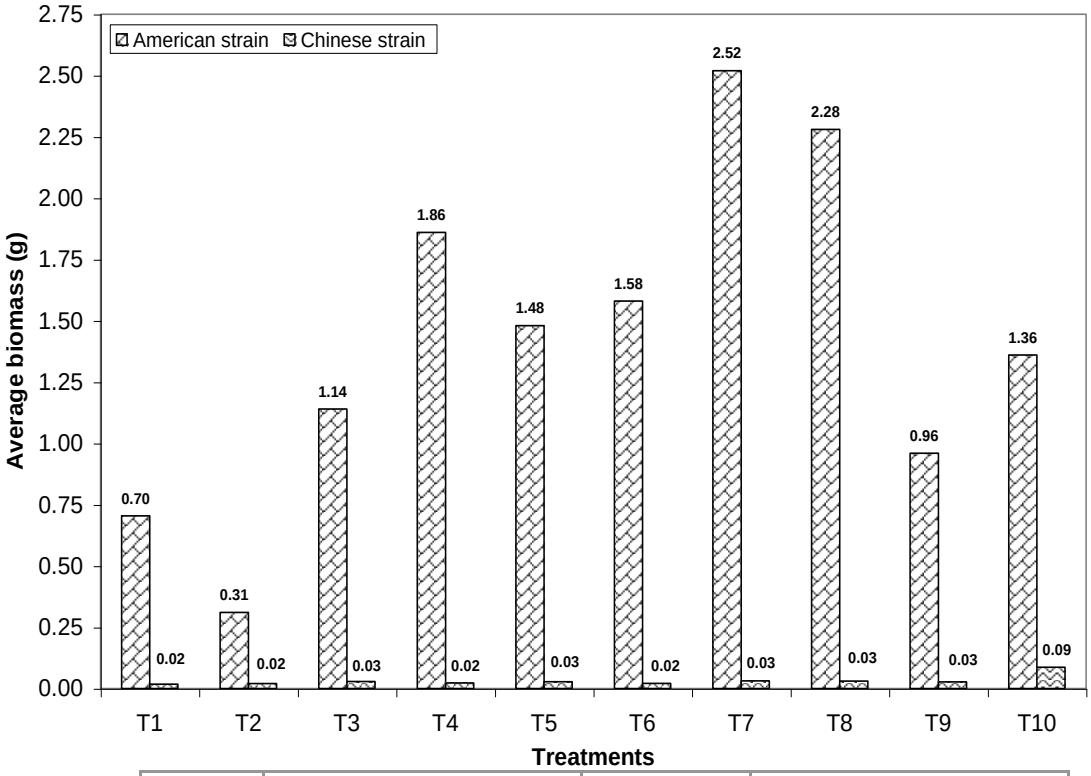


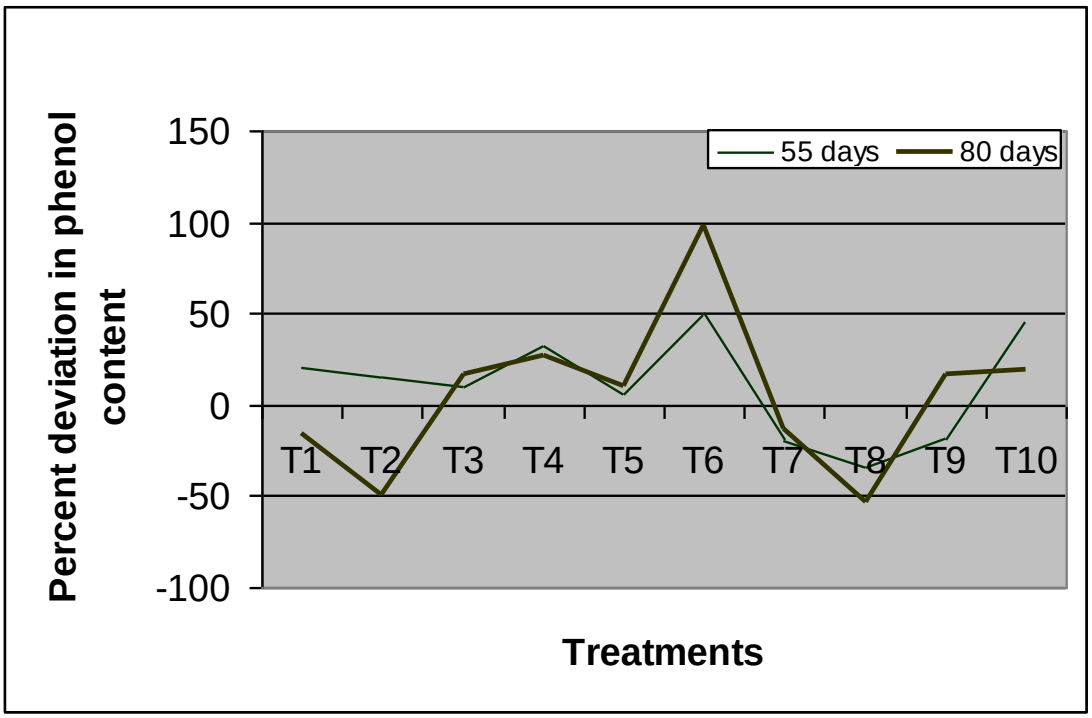
T1	Biza (S.D.)+R.B.(22%)	T6	Mix(S.D.)+R.B.(20)
T2	Teak(S.D.)+R.B.(22%)	T7	Teak(S.D.)+R.B.(15%)
T3	Sal(S.D.)+R.B.(22%)	T8	Teak(S.D.)+R.B.(20%)
T4	Mix(S.D.)+R.B.(22)	T9	Mix of all (S.D.)+ R.B.(15%)
T5	Mix(S.D.)+R.B.(15%)	T10	S.D.+P.S.+R.B.(20%)

Fig. 3: Percent degradation of lignin and cellulose content of sawdust substrate due to the activity of *Lentinula edodes*



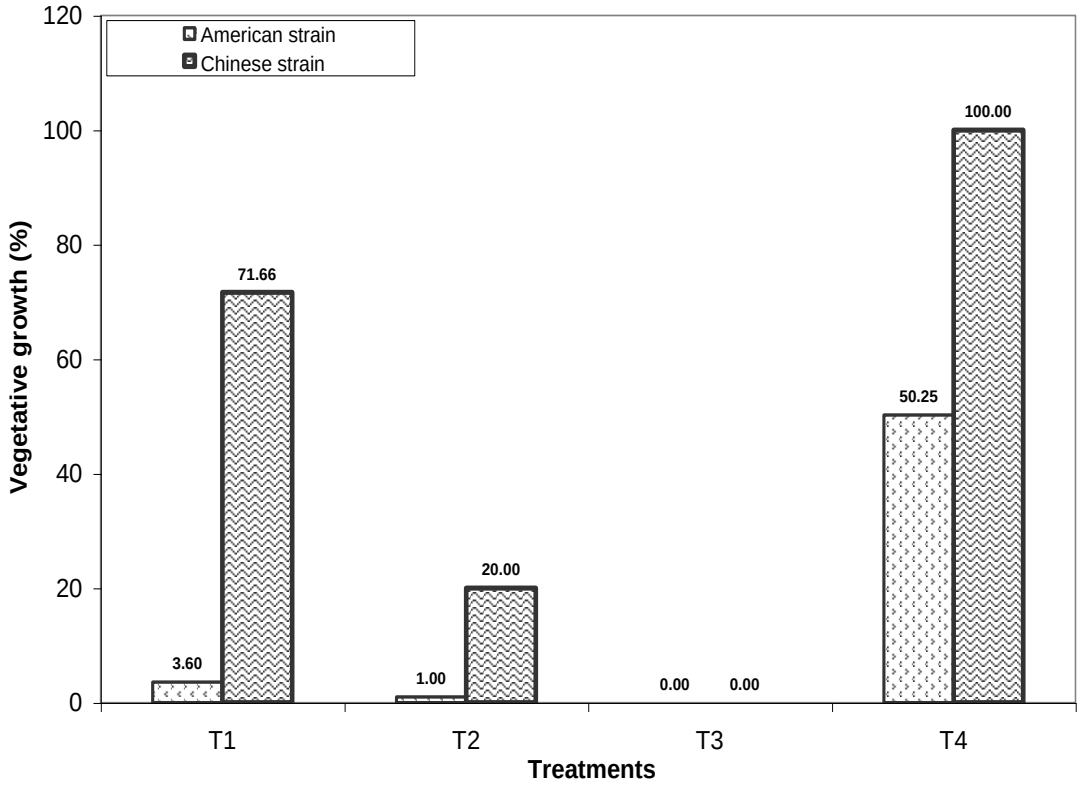
T1	Dextrose-20g-l	T6	Fructose-30g-l
T2	Dextrose-25g-l	T7	D+F-at pH 4.0
T3	Dextrose-30g-l	T8	D+F-at pH 5.5
T4	Fructose-20g-l	T9	D+F-at pH 6.0
T5	Fructose-25g-l	T10	Potato dextrose broth (Control)

Fig.1: Effect of mixed media composition on average biomass production of *Lentinula edodes*



T1	Biza (S.D.)+R.B.(22%)	T6	Mix(S.D.)+R.B.(20)
T2	Teak(S.D.)+R.B.(22%)	T7	Teak(S.D.)+R.B.(15%)
T3	Sal(S.D.)+R.B.(22%)	T8	Teak(S.D.)+R.B.(20%)
T4	Mix(S.D.)+R.B.(22)	T9	Mix of all (S.D.)+ R.B.(15%)
T5	Mix(S.D.)+R.B.(15%)	T10	S.D.+P.S.+R.B.(20%)

Fig. 4: Percent change in phenol content in sawdust substrate due to the activity of *Lentinula edodes*



T1	*Trichoderma viride*
T2	*Sarocladium oryzae*
T3	*Coprinus spp.*
T4	*Sclerotium rolfsii*

Fig. 2: Vegetative growth of different contaminants in the mycelial extract of *Lentinula edodes*

Plate 3A: Spawn development of American strain of *Lentinula edodes* in different spawning substrates.

Plate 3B: Spawn development of Chinese strain of *Lentinula edodes* in different spawning substrates.

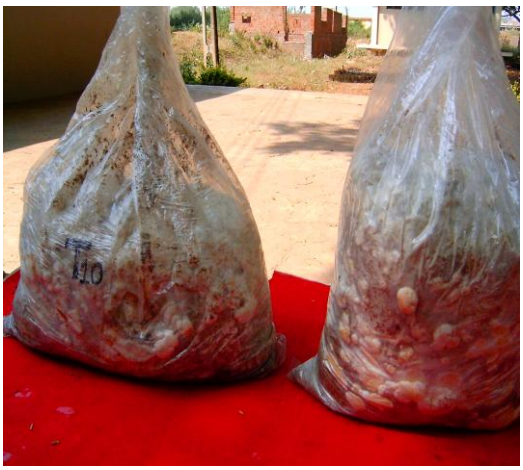
Plate 1A: Effect of individual broth media on biomass production of American strain of *Lentinula edodes.*

Plate 1B: Effect of individual broth media on biomass production of Chinese strain of *Lentinula edodes.*

Plate 4A: Primordial stage of Chinese strain of *Lentinula edodes* on wheat and jowar grains.

Plate 4B: Basidiocarp of Chinese Strain of *Lentinula edodes* on wheat grains and jowar grains.

Plate 5A: Mycelial growth of Chinese strain of *Lentinula edodes* on different fruiting substrates.



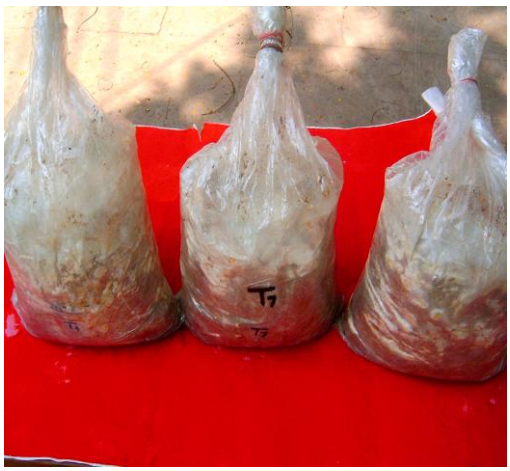


Plate 5B: Popcorn stage of Chinese strain of *Lentinula edodes* on different fruiting substrates.

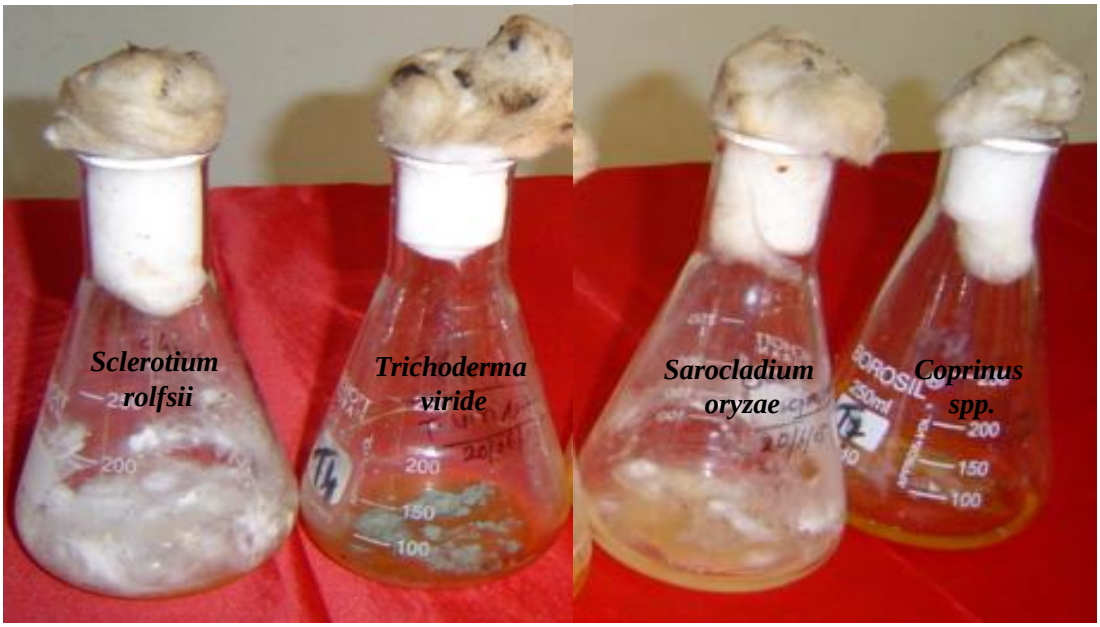


Plate 2A: Vegetative growth of different contaminants on mycelial extracts of American strain of *Lentinula edodes*.

Plate 2B: Vegetative growth of different contaminants on mycelial extracts of Chinese strain of *Lentinula edodes*.