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Advances in Applied Science Research, 2012, 3 (4):1938-1949

ISSN: 0976-8610
CODEN (USA): AASRFC

Cultivation and study of growth of oyster mushroom on different agricultural waste substrate and its nutrient analysis

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ABSTRACT

The cultivation of edible fungi is a controlled bioconservation of agro industrial lingo-cellulosic waste & residues. Mushroom cultivation fits in very well with sustainable farming & has several advantages. It uses agricultural waste products. A high production per surface area can be obtained, after picking the spent substrate is still a good soil conditioner. The Mushrooms are good cash crop. The development of Oyster mushroom (Grey and pink) production methodologies on agricultural waste like Paddy straw and wheat straw gives very high yield as well as the nutritional contain like carbohydrate, protein, ash, calcium, magnesium, crude fibers and lipid were checked

Keywords Oyster, Agricultural waste, nutrition, medicinal values

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INTRODUCTION

Mushroom with their great variety of species, constitute a cost-effective means of both supplementing the nutrition to human kinds. 4-5 species of mushroom are of industrial significance throughout the world (Chang and Miles, 1991). In India, only 3 species, namely, *Agaricus bisporus*, *Pleurotus sajorcaju* and *Volveriell* are preferred for commercial cultivation. Of the three cultivated species, the white button mushrooms have the highest consumer preference and account for about 90 per cent of total mushroom production. The Oyster mushroom grows during winter months only therefore, it needs proper preservation techniques to promote their consumption among common people and excess mushroom is processed into food products acceptable to consumers. Mushrooms contain 90 per cent moisture. Oyster mushroom (*Pleurotus* sp.) belonging to Class Basidiomycetes and Family Agaricaceae is popularly known as 'Dhingri' in India and grows naturally in the temperate and tropical forests on dead and decaying wooden logs or sometimes on dying trunks of deciduous or coniferous woods. It may also grow on decaying organic matter. Produces protein rich food Oyster mushrooms are the third largest cultivated mushroom. The economic importance of the mushroom lies primarily in its use as food for human consumption. It is rich in Vitamin C and B complex and the protein content varies between 1.6 to 2.5 percent & mineral salts required for the human body. The niacin content is about ten times higher than any other vegetables.

The folic acid present in oyster mushrooms helps to cure anemia. It is suitable for people with hyper-tension, obesity and diabetes due to its low sodium: potassium ratio, starch, fat and calorific value. Alkaline ash and high fiber content makes them suitable for consumption for those having hyperacidity and constipation & cholesterol inhibitors mushrooms. Mushrooms are rare vegan sources of vitamin D and conjugated linoleic acid. Mushrooms have antioxidant property due to presence of compounds like Ergothioneine. Oyster mushroom can grow at moderate temperature ranging from 20 to 30^0 C and humidity 55-70% for a period of 6 to 8 months in a year. It can also be cultivated in summer months by providing the extra humidity required for its growth in hilly areas above 900m.

(m.s.l.), the best growing season is during March/April to September/October and in the lower regions from September/October to March/April. In this study we are going to observe growth of same species on different raw material, Nutritional qualities of same species on different raw material and physico-chemical characteristics of oyster mushroom.

Classification of Oyster Mushroom-
Scientific classification-
Kingdom – Fungi
Phylum – Basidiomycota
Class - Agaricomycetes
Order - Agaricales
Family - Pleurotaceae
Genus – *Pleurotus*
Species - *ostreatus*

MATERIALS AND METHODS

Materials-
1. Spawn (Source:- Agriculture College, Pune)
2 Paddy Straw, Wheat Straw as raw materials.
3 Water bath
4 Hot Air Oven
5 Plastic Bags

Chemicals-
1. 1N NaOH
2 Chloroform : Methanol(2:1)

Methods-
OYSTER MUSHROOM CULTIVATION:
- Substrate preparation

Oyster mushroom was grown on various substrates viz., paddy straw, Wheat straw, vegetable plant residues etc. Since paddy straw is easily available and cheap, it is widely used. Paddy straw used was fresh and well dried.

- Soaking-

Wheat and paddy straw were chopped into 3-5 cm pieces and soaked in fresh water for 8-16 hours. Excess water from straw was drained off by spreading it on filter paper.
- Heat Treatment-

Heat treatment of substrate results in minimizing contamination problem and gives higher and almost constant yields. It can be done e by pasteurization.

- Pasteurization-

Water was boiled in a wide mouth container such as tub or drum. The wet substrate was filled in gunny bags. The filled bag was dipped in hot water of 80-85C for about 10-15 minutes. To avoid floating, it was pressed with some heavy material or with the help of a wooden piece. After pasteurization, excess hot water was drained off from container so that it can be reused for other sets & hot water temperature was maintained at 80-85C for all sets to achieve pasteurization.

- Spawning-

When the pasteurized substrate had cooled down to room temperature, it was ready for filling and spawning. At this stage, substrate moisture content was about 70%. Polythene bags (35 x 50 cm, 150 gauges) or polypropylene bags (35 x 50 cm, 80 gauges) were used for its cultivation. One 500 ml bottle spawn (200-250 g) can be used for 10-12 kg wet straw (3 bags). Spawning can be done in layer spawning or through spawning.

In case of layer spawning, substrate was filled in bag, pressed to a depth of 8-10 cm and broadcasted with a handful of spawn above it. Similarly, 2nd and 3rd layers of substrate were put and simultaneously after spawning, the bags

were closed. After spawning, pasteurized straw was mixed with 2% spawn and filled in bags. After that it was gently pressed, and the bags were sealed for spawn running (development).Spawned bags were stacked on racks in neat and clean place, in closed position. Temperature at 25-35 C and humidity at 70-85% was maintained by spraying water twice a day on walls and floor. It took 15- 20days when bags were fully covered with white and pink mycelium respectively.

- Cropping and harvest-

After 20-22 days, when bags were fully impregnated with white mycelium, they were transferred into cropping room and the polythene covers were removed. The open blocks were kept on racks about 20 cm apart with gap of 50-60 cm between two shelves. Mushrooms were grown in a temperature range of 20-33 C. Relative humidity was maintained by spraying water twice a day on the walls and floor of the room. A light spray of water was given on blocks as soon as the small pin heads appeared. Once pinheads were 2-3 cm big a little heavier watering was done on blocks and watering of blocks was stopped to allow them to grow. Mushrooms were plucked before they shed spores to maintain quality. After 1st flush of harvest, 0.5 to 1 cm outer layer of the block was scrapped. This helped to initiate 2nd flush which appeared after 10 days.

After harvesting they were packed in perforated (5-6 small holes) polythene bags to keep them fresh. It loses freshness after about 6 hours, which can be enhanced by keeping them in refrigerator. Oyster mushroom can be shed dried for 2 days and dried product marketed in polythene bags. Dried mushrooms were soaked in water for 10 minutes before use.

After the growth of *oyster* mushrooms following biochemical test were carried out –

- Determination of Moisture-

The moisture content is determined by measuring the of a material before & after the water removed by evaporation Moisture content was determined by following –

%Moisture =initial –dried/initial *100

Here M initial & M dried are the mass of sample before & after drying respectively To obtain an accurate measurement of the moisture content of material evaporation method necessary to remove all water molecules.

- Determination of total protein-

Protein content was measured by using Biuret method (Burtis and Ashwood, 2006).

- Determination of total lipid-

Total lipid was determined by slight modified method of Folch et al. (1957). Five gram of each sample was suspended in 50ml of chloroform: methanol (2:1) mixture then mixed thoroughly and let stand for 3 days. The solution was filtrated and centrifuged at 1000g by a centrifuge machine. The upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by heating. The remaining was the crude lipid.

- Determination of total ash-

One gram of the sample was weighed accurately into a crucible. The crucible was placed and heated first over a low flame till all the material was completely charred, followed by heating in an oven for about 6 hours at 6000C. It was then cooled and weighed.

Then total ash was calculated as following equation (Raghuramalu et al., 2003):

Ash content (g/100g) = weight of ash *100/ weight of sample

- Determination of crude fiber-

Crude fiber determined using Soxhelt extraction method.

- Determination of total carbohydrate-

The content of the available carbohydrate was determined by the following equation (Raghuramalu et al., 2003):

Carbohydrate (g/100g sample) = [100 – (Moisture + Fat + Protein + Ash + Crude Fiber)]

- Determine Metabolizable energy-

The content of availed or carbohydrates can supply energy.

ME (Kcal/100g) = [(3.5*CP)+(8.5*CF)+(8.5*NFE)]

Where
ME = metabolic energy CP =crude protein
CF = crude fate (lipid) NFE = % nitrogen free extract(carbohydrate)

- Determination of calcium and magnesium content**:**

1)Estimation of total hardness(Ca & Mg) :
- Take 25 ml of sample in a conical flask & add 1ml of buffer solution.
- Add a pinch of Erichrome black T indicator in it, The solution turns red
- Titrate the whole content against 0.01M EDTA solution.
- Take 3 readings; the end point is wine red to blue.
- Note the mean burette reading, and calculate the total hardness using following formula –

Total Hardness (mg/ml) = MBR*M*1000/Volume of sample (ml)

2)Estimation of Conc. of Calcium :
- Take 25ml of sample in a conical flask and add 2ml of 1N NaOH to it.
- Add a pinch of Muroxide indicator to it which turns solution into pink.
- Titrate the whole content against 0.01 M EDTA solution.
- Take 3 readings; the end point is pink solution turns to purple.
- Note the mean burette reading, and calculate amount of Calcium in the sample using following formula

Conc. of Calcium (mg/ml) = MBR*M*1000/Volume of sample

Conc. of Magnesium (mg/ml) = Conc. of total hardness - Conc. of Calcium

I. GREY MUSHROOM-

a) Substrate: Wheat Straw


DAY 1: Spawning & Bagging


DAY 12: Initial budding


DAY 13: Initial fruiting



DAY 16: Initial growth **DAY 17 : Grown stage** **DAY 18: Full grown grey Mushroom**

b) Substrate: Paddy Straw



DAY 13: Budding **DAY 15: Initial fruiting** **DAY 17: Initial growth**


DAY 18: Full grown grey Mushrooms **DAY 20: Visible gills of mushroom**

II. PINK MUSHROOM-

a) Substrate: Wheat Straw



DAY 12: Initial Budding **DAY 14: Initial Fruiting** **DAY 15: Fruiting**



DAY 17: Initial Growth **DAY 18: Grown mushrooms** **DAY 20: Full grown flower Shaped mushroom**

b) Substrate: Paddy Straw



DAY 17: Budding **DAY 18: Initial Fruiting** **DAY 19: Fruiting**


DAY 20: Full grown mushrooms **DAY 21: Visible gills seen in mushroom**

- **Determination of protein content -**

Test Tube No	Vol. of BSA (ml)	Vol. of Saline (ml)	Vol. of $CuSO_4$ (ml)	Stand For 15Min. At R.T.	Vol. of FC reagent (ml)	Stand For 30 Min. At R.T.	Conc. Of BSA (µg)	O.D at 660 nm (gray wheat straw)	O.D at 660 nm (gray paddy straw)	O.D at 660 nm (pink wheat straw)	O.D at 660 nm (pink paddy straw)
Blank	0.0	1.0	5		0.5		00	0.0	0.0	0.0	0.0
1	0.2	0.8	5		0.5		20	0.20	0.20	0.20	0.20
2	0.4	0.6	5		0.5		40	0.33	0.33	0.33	0.33
3	0.6	0.4	5		0.5		60	0.47	0.47	0.47	0.47
4	0.8	0.2	5		0.5		80	0.60	0.60	0.60	0.60
5	1.0	0.0	5		0.5		100	0.74	0.74	0.74	0.74
6	0.5 (Sample)	0.5	5		0.5		-	0.81	0.13	0.50	0.17

- **Determination of Sugar Content-**

Test Tube No.	Std. glucose (ml)	Disstelled Water	Phenol	Conc.H_2SO_4 (ml)	Stand For 30 Min. At R.T	Conc. Of Glucose	O.D at 660 nm (gray wheat straw)	O.D at 660 nm (gray paddy straw)	O.D at 660 nm (pink wheat straw)	O.D at 660 nm (pink paddy straw)
Blank	0.0	1	1	5		00	0.0	0.0	0.0	0.0
1	0.2	0.8	1	5		20	1.06	1.06	1.06	1.06
2	0.4	0.6	1	5		40	1.19	1.19	1.19	1.19
3	0.6	0.4	1	5		60	1.42	1.42	1.42	1.42
4	0.8	0.2	1	5		80	1.50	1.50	1.50	1.50
5	1.0	0.0	1	5		100	1.63	1.63	1.63	1.63
6	0.5 (Sample)	0.5	1	5		-	0.18	1.52	1.30	0.99

- **Determination of Moisture Content-**

Mushroom	W_1 (Wt. of mushroom before drying)	W_2 (Wt. of mushroom after drying)	Moisture Content $(W_1 - W_2)/W_1 * 100$
Gray Wheat Straw	6	0.75	0.875 = 87.5%
Gray Paddy Straw	20	4.5	15.5 = 77.5%
Pink Wheat Straw	8.660	0.690	0.92 = 92%
Pink Paddy Straw	18.00	2.5	0.8611 = 86.11%

Determination of Crude Fiber -

Mushroom	W_1= Initial Wt. of beaker (in gm)	W_2 = Final Wt. of beaker (in gm)	Crude Fiber $W_2 - W_1$ (in gm)
Gray Wheat Straw	111.0	111.81	0.81
Gray Paddy Straw	100.750	101.560	0.81
Pink Wheat Straw	102.0	103.260	1.260
Pink Paddy Straw	98.950	99.040	0.09

- **Determination of Lipid Content-**

Mushroom	W_1= Initial Wt. of beaker (in gm)	W_2 = Final Wt. of beaker (in gm)	Lipid content $W_2 - W_1$ (in gm)
Gray Wheat Straw	96.870	97.580	0.71
Gray Paddy Straw	141.20	142.47	1.27
Pink Wheat Straw	150.260	151.260	1
Pink Paddy Straw	157.250	157.620	0.37

Determination of Ash Content-

Mushroom	W1= Initial Wt. (in gm)	W2 = Wt. of Ash (in gm)	Ash content W2 / W1*100 (in gm)
Gray Wheat Straw	5	4.090	81.8
Gray Paddy Straw	10	5.730	57.3
Pink Wheat Straw	10	0.740	7.4
Pink Paddy Straw	10	5.73	57.3

RESULTS AND DISCUSSION

1. GREY MUSHROOM

A) Substrate- Wheat Straw

- **Determination of Calcium and Magnesium Content-**

Part I-Estimation of Total Hardness

Readings	Burette Readings			MBR
	I	II	III	
Final	9	9	9	9
Initial	0	0	0	
Difference	9	9	9	

Calculations:
Total Hardness (mg/l)
= MBR*M*1000/ Vol. of sample (ml)
= 9*0.01*1000/25 = 3.6 mg/l

Part II- Estimation of Conc. of Calcium

Reading	Burette Reading			MBR
	I	II	III	
Final	1	1	1	1
Initial	0	0	0	
Difference	1	1	1	

Calculations:
Conc. of Calcium (mg/l)
= M.B.R *M* 1000/Vol. of sample (ml)
=1*0.01*1000/25 = 0.4 mg/ml

Conc. of Magnesium (mg/l) = Conc. of total hardness- Conc. of Calcium
= 3.6 - 0.4 = 3.2 mg/ml

- **Determination of metabolise energy-**

ME (Kcal/100g) = [(3.5*C.P) + (8.5*CF) + (8.5*NFE)]
= [(3.5*10.3) + (8.5*0.71) + (8.5*1.6)] = 55.685

B) Substrate - Paddy Straw

- **Determination of Calcium and Magnesium Content-**

Part I- Estimation of Total Hardness

Reading No.	Burette Reading			MBR
	I	II	III	
Final	11.8	11.5	11.5	11.5
Initial	0	0	0	
Difference	11.8	11.5	11.5	

Calculations:
Total Hardness (mg/l)
= MBR*M*1000/ Vol. of sample (ml)
= 11.5*0.01*1000/25
= 4.6 mg/l

Part II- Estimation of Conc. of Calcium

Reading	Burette Reading			MBR
	I	II	III	
Final	3	3	3	3
Initial	0	0	0	
Difference	3	3	3	

Calculations:
Conc. of Calcium (mg/l)
= M.B.R *M* 1000/Vol. of sample (ml)
=3*0.01*1000/25
= 1.2 mg/ml
Conc. of Magnesium (mg/l) = Conc. of total hardness- Conc. of Calcium
= 4.6 - 1.2 = 3.4 mg/ml

- **Determination of metabolise energy** -

ME (Kcal/100g) = [(3.5*C.P) + (8.5*CF) + (8.5*NFE)]
= [(3.5*1.6) + (8.5*1.27) + (8.5*13.2)] = 128.595

2. PINK MUSHROOM

A) Substrate – Wheat Straw

- **Determination of Calcium and Magnesium Content-**

Part I-Estimation of Total Hardness

Reading No.	Burette reading			MBR
	I	II	III	
Final	8.5	8.5	8	8.33
Initial	0	0	0	
Difference	8.5	8.5	8	

Calculations:
Total Hardness (mg/l)
= MBR*M*1000/ Vol. of sample (ml)
= 8.33*0.01*1000/25
= 3.32 mg/l

Part II- Estimation of Conc. of Calcium

Reading	Burette Reading			MBR
	I	II	III	
Final	3	2.7	3	2.8
Initial	0	0	0	
Difference	3	2.7	3	

Calculations:
Conc. of Calcium (mg/l)
= M.B.R *M* 1000/Vol. of sample (ml)
= 2.8*0.01*1000/25
= 1.12 mg/ml
Conc. of Magnesium (mg/l) = Conc. of total hardness- Conc. Of Calcium
= 3.32 - 1.12 = 2.2 mg/ml

- **Determination of metabolise energy-**

ME (Kcal/100g) = [(3.5*C.P) + (8.5*CF) + (8.5*NFE)]
= [(3.5*2.1) + (8.5*0.37) + (8.5*8.4)] = 81.895

B) Substrate – Paddy Straw

- **Determination of Calcium and Magnesium Content-**

Part I-Estimation of Total Hardness

Reading	Burette reading			MBR
	I	II	III	
Final	10	10	10	10
Initial	0	0	0	
Difference	10	10	10	

Calculations:
Total Hardness (mg/l)
= MBR*M*1000/ Vol. of sample (ml)
= 10*0.01*1000/25
= 4 mg/ml

Part II- Estimation of Conc. Of Calcium

Reading	Burette Reading			MBR
	I	II	III	
Final	1	1	1	1
Initial	0	0	0	
Difference	1	1	1	

Calculations:
Conc. of Calcium (mg/l)
= M.B.R *M* 1000/Vol. of sample (ml)
=1*0.01*1000/25
=0.4 mg/ml
Conc. of Magnesium (mg/l) = Conc. of total hardness- Conc. of Calcium
= 4 - 0.4 = 3.6 mg/ml

- **Determination of metabolise energy-**

ME (Kcal/100g) = [(3.5*C.P) + (8.5*CF) + (8.5*NFE)]
= [(3.5*2.1) + (8.5*0.37) + (8.5*8.4)]
= 81.895

DISCUSSION AND INTERPRETATION

1)Cultivation growth -
After the cultivation the growth was observed varyingly in both raw materials in 15-21 days. The comparative nutritional composition of mushrooms cultivated in different substrates is different. These figures show that all parameters (protein, lipid, carbohydrate, fiber and ash) have some extents of difference within mushrooms cultivated in different substrates.

2) Protein content -
From the observation table the Protein content of mushroom in paddy was significantly higher than in wheat straw. Amount of protein in wood scrap from the graph is 21mg/ml. & protein contain for wheat straw from the graph is 15 mg/ml.

3) Lipid content-
Lipid content of mushrooms was higher in wheat straw than paddy straw. The amount of lipid in grey mushroom in wheat straw was 0.710g that in paddy was1.27g & the amount of lipid in pink mushroom in wheat straw is 2g and that in paddy is0.37g.

4) Crude fiber contain-
The Crude fiber contents of oyster mushroom in grey mushroom in wheat straw was 0.81g that in paddy was0.81g. The pink mushroom in wheat straw contains 1.260g that in paddy straw was 0.09g

5) Ash content-
Total ash content in grey mushroom on wheat straw was 81.8(g/100) that in paddy is 57.3 (g/100) for pink mushrooms on wheat straw 7.4 (g/100g) and that in paddy 47.3(g/100g).

6) Moisture content-
The moisture contents of grey mushroom on wheat straw was 87.5% that on paddy straw was77.5%. For pink mushrooms on wheat straw was 92% and in paddy straw was 86.11%.

7) Total carbohydrate contain-
Total carbohydrate content of mushroom in wood scrap was (68.6g/100gm) & wheat straw was (66.7g/100gm).

8) Total metabolizable energy -
Using these data the highest metabolizable energy was found in wheat straw was (300 kcal/100g).The protein and lipid content of oyster mushroom on two different raw materials is near about similar to the finding of Sohi (1998) & Alam et al.(2007) but carbohydrate , fiber & Ash content are different. The metabolizable energy was also different.

Many factors may be involved in the difference of nutritional composition of mushrooms cultivated in different substrates. In present study suggest that oyster mushroom different from each other in nutritional composition although they are of same genus, however each species grow different raw material with same condition but give high protein contain & low Ash content. Hence fruiting bodies of oyster mushrooms can be taking regularly as a protein supplement or as an alternative protein supplement in their diet. The low lipid & high fiber content of the oyster mushroom make it health beneficial food items especially against heart disease and diabetes.

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