Evaluation of yield and nutritional status of oyster mushroom [*Pleurotus florida* (mont.) Singer] grown on different substrates

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Abstract

Background: This study was conducted to examine the effect of different substrates and to evaluate the yield and nutritional composition of mushroom obtained from each. **Methods**: The gathered substrates, sugarcane bagasse, lemongrass, vetiver roots, rubber sawdust, and neem cake were used for this. Statistics were applied to daily data on the weight of mushroom production on various substrates. **Findings:** Rubber sawdust from the three-month project work produced the highest yield, followed by lemongrass, sugarcane bagasse, and vetiver roots. Neem cake produces no yield, and no mycelial growth is seen in this. On nutrient analysis of mushrooms harvested from lemongrass and rubber sawdust, there were no much differences noticed. The availability of substrates at affordable prices in the specific location where oyster mushrooms are produced determines their characteristics. **Novelty and application** can create nutrient-dense mushrooms from various substrates and aid in their environmentally friendly disposal. Here used substrates which are available at free of cost or at cheap rate can be recommended for low-cost mushroom production for common man. The fact that diverse substrate wastes are recycled into vermicompost after mushroom harvesting has become interesting.

1. Introduction

A mushroom, or toad stool, is the fleshy, spore - bearing fruiting body of a fungus, typically produced above ground on soil or on its food source. Mushrooms are fleshy fungi which constitute a major group of lower plant kingdom. A mushroom is a general term applied to the fruiting bodies of the fleshy fungi and as such belongs to different groups of fungi. The majority of these mushrooms (fleshy fungi) belong to Hymenomycetes of Basidiomycotina, characterized by the presence of spore - bearing layer known as Hymenium. Mushroom is a common fungal fruiting body that produces

basidiospores at the tip of club like structures called basidia, which are arranged along the gills of the mushroom.

Sufficient food supply is a country's most precious asset. Mushroom provides a rich addition to the diet in the form of proteins, carbohydrates, valuable salts and vitamins. As food, the nutritional value of mushrooms lies between meat and vegetables. Mushrooms provide a high protein and low caloric diet. They are the number one diet to be recommended to heart patients.

In *Pleurotus* sp., exposure to light (artificial or sunlight) is necessary for initiation of fruiting body primordia, and thus, light is given for at least 15 minutes per day [10]. The raw materials which can be applied for oyster mushroom cultivation are cheaply available in farmer's yards and easily cultivated in various climatic conditions as a fast-maturing crops.

Because of low-cost production technology and high biological efficiency, *Pleurotus* species are well-known and widely cultivated around the world, mostly in Asia, America, and Europe. The taste, nutritional value, and therapeutic qualities of oyster mushrooms are also contributing significantly to their growing popularity. Species of *Pleurotus* can grow in a wide variety of temperatures and effectively break down agricultural waste. In contrast to other edible mushrooms, *Pleurotus* species grow quickly, and disease attacks on their fruiting bodies are rare.

Proteins, minerals (P, Ca, Fe, K, and Na), and vitamins (thiamine, riboflavin, folic acid, and niacin) are all abundant in *Pleurotus* species. In addition to its nutritional importance, their medical potential in the treatment of cancer and diabetes has been highlighted. A large variety of metabolites with antitumor, antigenotoxic, antioxidant, antihypertensive, antiplatelet-aggregating, antihyperglycemic, antibacterial, and antiviral activity are present in a variety of mushroom species. The use of certain oyster mushroom species in medicine is significant. *Pleurotus ostreatus* also has anticancer potential, while *Pleurotus cystidiosu*s is a potent antioxidant. To thrive in their natural habitat, mushrooms require substances that are antibacterial and antifungal. As a result, a variety of mushroom species may yield antibacterial chemicals that are useful to humans [8].

The present study was conducted to compare the effects of different agro - wastes on the growth, yield, and nutritional composition of oyster mushroom *Pleurotus florida*. The final aim was to find the best substrates for effective cultivation of oyster mushroom with best nutritional composition and cost effective.

2. Materials and methods

2.1 Mushroom shed

The first step in the cultivation technique is the preparation of the mushroom shed. A mushroom shed should be kept free from insects and rodents. The shed should also be almost air tight, allowing only a minimum air to filtrate. The shed floor should be kept wet at all times and the walls sealed with a layer of plastic to conserve moisture and maintain humidity. Spray the mushroom bag with water three times a day. But in an area where water resources are limited, water should be added once in the morning and one in the afternoon as long as the humidity well maintained. After the fruiting bodies have appeared, watering should be continued, but avoid wetting the fruiting body of the mushroom directly as they will absorb water like sponges.

2.2 Sources of mushroom

The oyster mushroom spawns (*Pleurotus florida* Mont. Singer.) were purchased from IRTC (Integrated Rural Technology Centre), Mundur, Palakkad, Kerala.

2.3 Collection of substrates

The various substrates used were sugarcane bagasse, vetiver roots, lemon grass, rubber sawdust and neem cake. These substrates were collected from nearby areas of Manjeri, Malappuram, Kerala.

2.4. Sterilization of substrates

Sterilization of substrates was done by autoclaving. Autoclaving is an easiest method and suggested for minimizing labour and expenditure. In this method, the substrates to be autoclaved are subjected to gradual temperature increase under high pressure at 15 pound per square until 121°C is reached and then steamed for around 15 minutes. Each of the substrates thus autoclaved would be free from microorganisms and other infectants.

2.5 Preparation of bags

The bag preparation was by poly bag method. Here, polythene bag having 1 ft breadth and 2 ft height was sterilized by diluted Dettol and perforated (whole size 0.4 diameter) at a distance of 4 inch all over the body surface. The perforation was done by narrow sterilized needle. Made the polythene bags and the substrates dry. All the substrates were packed on the same day. Substrates

were placed inside the bag in 4-5 layers intermittent with spawn and made tight by pressing with hand palm. The sealed portion of the polythene bag was tied at the end so that the bottom portion of the bed became round in shape and convenient for keeping them on stands or hung them from top. After the completion of one layer, 30-40 grams spawn grains were spread over the substrate layer. Then other layer of substrate is prepared and spawn grains are spread over them similarly. In this way, 4 / 5^{th} portion of the transparent polythene bag is filled up by the spawned substrate. Then the bag was closed tightly by tying with rubber band. About two bags were prepared from a packet of spawn. Then all the bags or beds were kept on ropes that are hanging in chains with a support from the top of the mushroom shed, where humidity has been artificially raised by hanging wet jutes at different place. Sprinkling of water was done regularly at the morning and afternoon to develop fruiting bodies.

Weight of packed bags of substrates

Sl. No.	Substrate	Weight of packed bags in (kg)	
1.	Sugarcane Bagasse	2.49	2.33
2.	Roots of Vetiver	2.11	2.05
3.	Lemongrass	3.10	3.01
4.	Rubber sawdust	3.86	3.8
5.	Neem cake	3.50	3.46

2.6 Fruiting body development

Periodical observation is done to see spawn growth, mycelial development started within 20 days. After the mycelial running was visible, clear markings were done along the substrates and narrow longitudinal cuts were made by using sterile surgical blades. The fruiting body development started within 7-10 days of bag opening. Thereafter, the mushrooms were grown on the same beds for 2-3 times in flushes for about 7-8 days interval.

2.7 Harvesting

Mushroom harvesting was generally done at morning. The maturity of fruiting body was identified by seeing edges of the caps that start to fold or curl upward. Plucking was done by giving gentle twist at the base of fruiting body. The adhering substrate particles are removed by and picking or cutting and the mushrooms are made ready for marketing or food preparation. After harvesting of mushrooms weighing of the harvested mushrooms was done from each bed and it was used for analysing the production rate from each substrate.

Two samples of mushrooms with rubber sawdust and lemongrass as substrates were sent to Signature solutions training and research centre, Perinthalmanna, Malappuram Dist. Kerala, to analyse their nutritional composition.

2.8 Nutritional analysis

2.8.1 Determination of carbohydrates

Weigh accurately 2-3 gm of ground sample and transferred into 1000 ml round bottom flask, Add 200 ml of 2.5% dilute HCl, Connect the flask into a water condenser and reflux for 2.5 hours, Cool and neutralize with 50% sodium hydroxide solution (use a litmus paper), Filter (using ordinary filter paper) into a 250 ml volumetric flask and make up to the mark with distilled water. Fill the solution into a 50 ml burette.

Preliminary Titration:

Pipette 5 ml each of Fehling A and B into 250 ml conical flask, Mix and add about 30 ml water and a few boiling chips or glass beads, Heat the flask to boiling, Add 10 drops of methylene blue indicator, Continue the addition of solution drop wise until the blue colour disappears to a brick-red end point. Note down the titre value.

Final Titration:

Pipette 5 ml each of Fehling A and B. Add sample solution about 2 ml less than titre value of the preliminary titration, Heat the flask to boiling within 3 minutes and complete the titration, Perform the titration duplicate and take the average.

Determination of Fehling Factor

Accurately weigh around 4.75 gms of AR grade sucrose, Transfer to 500 ml volume flask with 50 ml distilled water, Add 6 ml conc. HCl and allow to stand for 24 hours, Neutralize with NaOH solution and make up to volume, Transfer to a burette and perform the titration of Fehling solution following the similar procedure as above.

2.8.2. Determination of protein

Weigh quickly about 1-2 gm of the sample and transfer to a 500 or 800 mL Kjeldahl flask taking care to see that no portion of the sample clings to the neck of the flask, Add 0.7 gm of copper sulphate, 15 gm of Potassium Sulphate and 40 mL of concentrated sulphuric acid, Add two to three glass beads, Place the flask in an inclined position on the stand in the digestion chamber and digest, Heat the flask gently at low flame until the initial frothing ceases and the mixture boils steadily at a moderate rate, During heating rotate the flask several times. Continue heating for about an hour or more until the colour of the digest is pale blue, Cool the digest and add slowly 200 ml of water, Cool, add few glass beads and carefully pour down the side of the flask sufficient Sodium Hydroxide solution (450gm/L) to make the contents strongly alkaline (about 110 mL) before mixing the acid and alkaline layer (colour of the solution should be changed into dark), Connect the flask to a distillation apparatus incorporating an efficient flash head and condenser, Pipette 50/100 ml of 0.1 N sulphuric acid into a beaker/conical flask, To the condenser fit a delivery tube which dips just below the surface of the pipetted volume of standard acid contained in a beaker/conical flask receiver, Mix the contents of the digestion flask and boil until 150 mL have distilled into the receiver, Add 5 drops of methyl red indicator and titrate with standardized 0.1 N Sodium Hydroxide solution, Carry out blank titration simultaneously.

2.8.3 Determination of total fat content

Weigh accurately sufficient amount of ground sample into an extraction thimble and plug it from top with cotton, Take the initial weight of soxhlet flat bottom flask, Put the thimble into the soxhlet extraction chamber, Connect the extraction chamber over the flask, Add solvent into the chamber until it siphoned off into the flask, After siphoning stops, again add the solvent into the half level of the chamber, Connect water condenser over the chamber, Heat the flask (heating mantle, temperature set as 5°C) for about 4-5 hours for complete extraction of fat. If the sample containing high amount of fats, more time usually 8 hours is required, After the extraction completed,

disconnect the flask and evaporated off the solvent in a heating water bath, Remove the traces of the residual solvent by keeping the flask in a hot air oven for about 30 minutes, Cool the flask and weigh.

2.8.4. Determination of energy/calorie

Energy or calorie contained in the mushrooms were identified using the equation, Energy as Kcal = [(carbohydrates x 4) + (protein x 4) + (fat x 9)] x 0.99

2.8.5. Determination of fibre content

Weigh accurately about 2 - 2.5 gm ground sample into a thimble and extract for about 1 hour with petroleum/diethyl ether in a soxhlet extractor, Transfer the material in the thimble to a 1 litre round bottom flask, If the sample is not containing any fat/ oil, skip the oil extraction steps, instead directly transfer the weighed sample into the 1 litre flask, Add 200 ml dilute sulphuric acid into the flask and connect a water condenser, Reflux for 30 minutes (Rotate the flask frequently, taking care to keep the material from remaining on the sides of the flask and out of contact with the acid), Remove the flask and filter through nylon filter bag, Wash the bag with water till acid free, Semi-dry the contents of the bag in a hot air oven and transfer again into the 1 litre flask, Add 200 ml dilute sodium hydroxide solution into the flask and connect a water condenser, Reflux for 30 minutes (Rotate the flask frequently, taking care to keep the material from remaining on the sides of the flask and out of contact with the acid), Remove the flask and filter through nylon filter bag, Wash the bag with water till alkali free, Completely dry the extract in an oven and transfer the extract into a silica crucible, Take the weight of crucible + dried extract. Ash the crucible in a muffle furnace heated to 550°C for 2 hours, Cool in a desiccator and weigh.

2.8.6 Determination of Calcium content

Total calcium content was determined by EDTA Titrimetric method.

Mix the sample pre-treated, if so required and transfer a suitable volume (50 to 100 mL) to 250 mL conical flask or a beaker, Add 5 mL of concentrated nitric acid and evaporate on a hotplate at a slow boil to the lowest volume possible (about 15 to 20 mL) before precipitation or salting occurs, Add 5 mL of concentrated nitric acid, cover with a watch glass and heat to obtain a gentle refluxing action, Continue heating and adding concentrated nitric acid as necessary until digestion is complete as shown by a light coloured clear solution, Do not let sample dry during digestion, Add 1 to 2 mL of concentrated

nitric acid and warm slightly to dissolve any remaining residue, Wash down beaker walls and watch glass with water and then filter, if necessary. Transfer the filtrate to a 100 mL volumetric flask. Cool, dilute to mark and mix thoroughly, Take a portion of this solution for the determination of calcium.

Sample preparation

Because of the high pH used in this procedure, the titration should be performed immediately after the addition of the alkali and indicator, Use 50mL of sample or a smaller portion diluted to 50 mL so that the calcium content is about 5 to 10 mg, Analyse hard waters with alkalinity higher than 300 mg/LCaCO3 by taking a smaller aliquot and diluting to 50 mL or by neutralization of the alkalinity with acid , boiling for one minute and cooling before beginning the titration, Add 2.0 mL of sodium hydroxide solution or a volume sufficient to produce pH of 12 to 13, Stir. Add 0.1 to 0.2 gm of the indicator murexide - sodium chloride mixture selected, Add EDTA titrant slowly with continuous stirring to the proper end point. Check the end point by adding 1 to 2 drops of titrant in excess to make certain that no further colour change occurs.

2.8.7 Determination of Iron content

Total iron content was determined by Phenanthroline method

Pipette out appropriate portions of standard iron solution into 125 ml conical flasks to contain from 10 to 100 µg of Fe. For the reagent blank, pipette out 10 ml of water to a separate conical flask. Dilute the contents of each conical flask to about 50 ml by adding water. To each flask, add 1 ml $NH_2OH.HCl$ solution and 2 ml conc HCI. Add a few boiling chips and boil the solution until the volume is reduced to about 20 ml. Cool to room temperature and quantitatively transfer to 100 ml volumetric flasks. Add 10 ml ammonium acetate buffer solution first and add 10 ml 1, 10-phenanthroline solution to each flask. Dilute to 100 ml with water and mix thoroughly and allow to stand for 10 to 15 min. Measure the absorbance of the iron complexes at 510 nm against the reagent blank. Construct a calibration curve by plotting absorbance values against micrograms of iron in 100 ml of the final solution.

3. Results

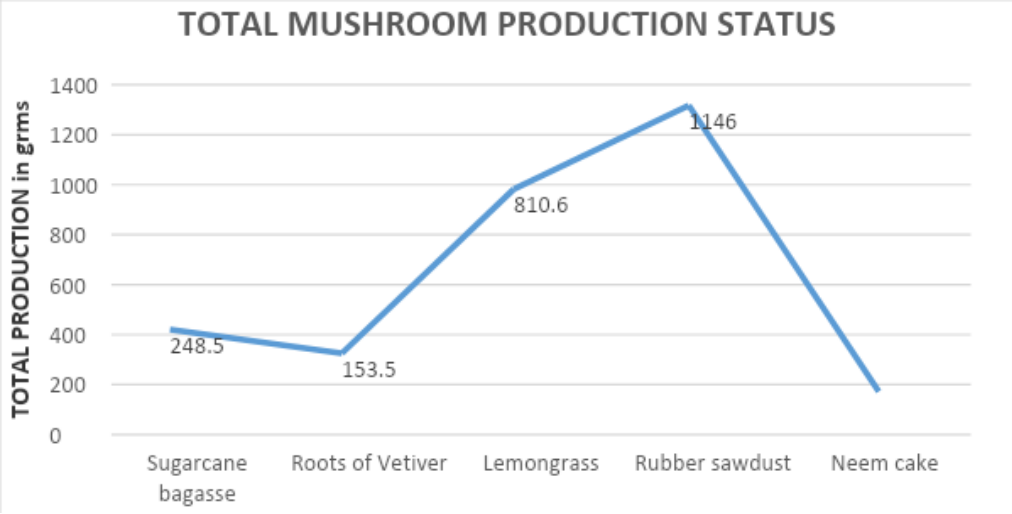
During cultivation, the fruiting body initiation was noticed on 26^{th} day, 28^{th} day, 33^{rd} day and 35^{th} day after bag preparation on lemongrass, roots of vetiver, sugarcane bagasse, rubber-sawdust respectively, and there was no fruiting body development on neem cake.

3.1 Mushroom production status

Among all substrate, rubber sawdust proved the best substrate for the effective cultivation of oyster mushroom [*Pleurotus florida* (Mont.) Singer]. There were no mycelial growth and fruiting body production, when neem cake used as a substrate.

Table 3.1 Total Mushroom Production Status

Sl. N0.	**Substrate used**	**Total production** (in grams)
1.	Sugarcane bagasse	248.5
2.	Roots of vetiver	153.5
3.	Lemongrass	810.6
4.	Rubber sawdust	1146
5.	Neem cake	Nil



4.2 Nutritional analysis

Table 4.2a_Mushroom with Lemongrass as substrate

Sl. N0.	**Parameters**	**Result**
1.	Energy	21 Kcal/100g

2.	Carbohydrates	3.5 g/100g
3.	Protein	3.5 g/100g
4.	Fat	BDL
5.	Fibre	0.7 g/100g
6.	Calcium	4.1 mg/100g
7.	Iron	0.9 mg/100g

Table 4.2b Mushroom with rubber sawdust as substrate

Sl. No.	Parameters	Result
1.	Energy	21 Kcal/100g
2.	Carbohydrates	3.6 g/100g
3.	Protein	3.4 g/100g
4.	Fat	BDL
5.	Fibre	0.8 g/100g
6.	Calcium	3.5 mg/100g
7.	Iron	0.6 mg/100g

BDL = Below Detectable Limit

4. Discussion

For the cultivation of mushrooms, a variety of substrates including Sugarcane bagasse, Vetiver roots, Lemongrass, Rubber sawdust, and Neemcake are used. The findings indicates that rubber sawdust produces the highest output, followed by lemongrass, sugarcane bagasse, and vetiver roots. Neemcake produces no yield, and no mycelial growth is seen in this. On nutrient analysis of mushrooms harvested from lemongrass and rubber sawdust, there were no much differences noticed. While they have roughly identical amounts of carbohydrate, energy and protein, samples from lemongrass were superior to those of rubber sawdust in the amount of calcium and iron.

Considering these results, my research demonstrates that rubber sawdust and lemongrass can both be used to cultivate mushrooms more successfully than other substrates. According to the production status, rubber sawdust yields the most, and the nutritional analysis shows, lemon grass produces results that are comparatively superior. The neem cake produces no yield, and the fruiting body from vetiver roots is comparatively small.

In my investigation, there was no mycelial growth in neem cake and no yield. Aqueous extracts of neem cake inhibit the growth of some fungi's mycelial spores [7]. This finding demonstrated that the antifungal action of neem cake, might be a reason for the inhibition of mycelial growth. Adding neem cake and citrus lemon to the substrate increased the yield of the *Pleurotus florida* and P. *ostreatus* oyster mushroom strains [5]. These results led to the conclusion that lowering the incidence of microbes in compost by using a concentrated form of a certain medicinal plant increases the yield of oyster mushrooms. Using neem extract in low amount in combination with other substrates may resist microbial attack and results in better production.

Oyster mushrooms grew best on sugarcane bagasse among several other substrates; whereas, in my experiment, lemongrass and rubber sawdust produced better results than sugarcane bagasse [6]. In my research, rubber sawdust and lemon grass show roughly equal nutritional qualities in the case of carbohydrate, energy and protein, with the calcium and iron contents being better in lemongrass than rubber sawdust. The effects of various sawdust substrates on *Pleurotus* growth, the best nutritional composition containing mushroom was grown on fig tree sawdust [1]. Results suggests that the nutrient composition of mushroom is dependent on the substrate in which they grow.

In order to determine the best substrate, oyster mushrooms were grown on a variety of substrates, including mustard straw, paddy straw, and sugarcane bagasse, at the Horticulture Demonstration and Training Center (HDTC). The quantity of primordial, fruiting bodies, and fresh weight or production of oyster mushrooms in cylindrical block systems were all significantly impacted by the different substrates. In all flushes, sugarcane bagasse produced the most primordial, fruiting bodies, and fresh weight while mustard straw produced the least of these [2]. In my experiment, less primordial and fruiting bodies are produced from sugarcane bagasse when compared to other substrates I used.

Rice straw gives higher yield when evaluating mushroom cultivation on different substrates [9]; [3] My findings shows that lemon grass is also a good substrate like rice straw, eventhough rubber

sawdust produces better yield. Hard wood sawdust produces mushrooms with more nutritional value whereas soft wood sawdust promotes better development [9]. My study demonstrates that rubber is a hard wood, and that rubber sawdust has a good yield and nutritional value.

The numerous lingo-cellulosic substrates used in mushroom growing can be easily recycled, and the wasted substrate from my work can be composted to make a high-quality fertiliser for outside plants. Some findings shows that the mushrooms, that transform low-quality waste streams into high-quality food, and the spent mushroom substrates has numerous applications, including compost, a growing medium for other fungi that produce mushrooms, animal feed, an improvement in animal health, the production of biofuels, and building and packaging materials [4] . The efficiency and sustainability of agricultural output can both be improved by this variety of uses.

5. Conclusions

The gathered substrates, such as sugarcane bagasse, lemongrass, vetiver roots, rubber sawdust, and neem cake were pasteurised using an autoclave by placing the substrates in a gunny sack. By using diluted dettol, the polythene bag was sterilised. For the spawn running and oyster mushroom production, the spawn and chosen substrates were filled in an organised and sequential fashion. By creating a pathway for watering, the humidity and temperature are maintained, which also helps to keep the environment aseptic. Statistics were applied to daily data on the weight of mushroom production on various substrates. Although rubber sawdust from the three-month project work produced the highest yield. The fact that diverse substrate wastes are recycled into vermicompost after mushroom harvesting has become interesting.

Oyster mushroom cultivation is often carried out by seasonal growers in a regular home without the installation of any environmental control equipment. It may grow on different agricultural waste materials both with and without fermentation. Although the cultivation process is quite straightforward, multiple technologies are employed to prepare the substrate and pasteurise or disinfect it. Additionally, the substrates that are employed in various locations vary. The availability of substrates at affordable prices in the specific location where oyster mushrooms are produced determines their characteristics. The substrates are frequently waste products of nature. As a result, there is no standard way to grow oyster mushrooms across the nation. Because we can use industrial and agricultural wastes such dried leaves, sawdust, sugarcane bagasse, tea powder, and paper scraps

for culture, growing mushrooms is less expensive. The poor farmers might embrace its high labour intensity, short duration, and ability to save land. To meet the demands of a balanced diet, they might step forward to raise edible mushrooms on a commercial scale as well as at home. It is a very nutrient-dense diet that contains different amounts of glucose, protein, fat, fibre, calcium, iron, and other minerals according on the substrate utilised. Due to the simple method of cultivation, low production costs, strong demand for their delectable taste, nutritious value, and improved market price, mushroom production can be a significant source of revenue. So that social entrepreneurs are likewise advised to do it. Nowadays, there is a lot of interest in oyster mushrooms in both academic and business circles. Mushroom cultivation becomes one of the most profitable agri business that may create nutrient-dense food from various substrates and aid in their environmentally friendly disposal.

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