Cultivation of Oyster Mushroom and a Comparative Analysis of its Bioactive Components

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DOI 10.51129/ujpah-2024-37-2(14)

Received – November 18, 2024
Revised – November 20, 2024
Accepted – November 24, 2024
Published – December 07, 2024

Abstract-Mushrooms are one of the most loved foods not only for its exotic taste but also for its benefits for health. It is consumed in various forms like fresh, pickled, dried, powdered, canned etc. Its farming has picked up a fast pace among contemporary entrepreneurs owing to its nutritional and medicinal benefits and low cost. Mushrooms are a fleshy fungus (Basidiomycota & Agaricomycetes) having a stem, cap and gills underneath the cap. They can be edible, wild and some of them can be toxic too. It contains more than 90% water and less than 1% fat, loaded with Vitamin B copper and selenium and low in sodium. Usually vegetables, milk and other food products are fortified with Vitamin D by irradiation or direct addition but mushrooms are unique in this sense because they are naturally a rich source of Vitamin D which otherwise is procured from animals or poultry. The reason being that it contains copious amount of plant sterol "Ergosterol", it is a precursor of Vitamin which when stimulated by sunlight or artificial lightening source converts to Vitamin D.

Key Words: Mushroom, cultivation, spawn, substratum, harvesting, Polyphenols, Flavonoids

Introduction

Mushrooms are a fleshy spore-bearing fruiting body of a fungus having a stem, cap and gills undeneath the cap. Mushrooms are natural source of foods and medicine. Approximately 15,000 species of mushrooms are known worldwide, about 2000 are used for human consumption and more than 700 have medicinal properties[1].

Mushroom or toadstools can be edible or toxic based on their aroma, taste, toxicity and physical appearance. Mushrooms can also be categorized based on their habitats such as temperate, tropical or sub-tropical mushroom. *Pleurotus* consists of about 40 species distributed in a wide range of tropical and temperate regions Twenty-six species, including *Pleurotus eryngii* (*PE*), *Pleurotus citrinopileatus* (*PC*), *Pleurotus flabellatu* (*PFL*), *Pleurotus ostreatus* (*PO*), *Pleurotu djamors* var. *roseus* (*PDR*), and *Pleurotus florida* (*PF*), have been reported to be cultivated using different types of lignocellulosic wastes[2]. *Pleurotus ostreatus* is the second most cultivated edible mushroom worldwide after Agaricus bisporus[3]**.** Mushroom *Pleurotus ostreatus* (Fr.) Kumm., used in cultivation for over 100 years, is now in the third place in the world in terms of production volume, after champignon mushrooms and Lentinula edodes (shiitake)[4]. The most cultivated mushroom worldwide is Agaricus bisporus, followed by Lentinus edodes, Pleurotus spp., and Flammulina velutipes[5]**.** Pleurotus species are widely cultivated throughout the world owing to its excellent flavor and low-cost production technology. It can be cultivated at moderate temperatures, ranging from 20 to 30°C, and at a humidity of 55–70% because of its ability to adapt a variety of factors[6]**.**

The production of oyster mushrooms and the research thereof, dates back to 1917 by Falck, who described the cultivation of Pleurotus on tree tumps and logs[7]**.** It is estimated that the first mushroom was cultivated around 600A.D. This was *Auricularia auricula*. Later, around 800-900 A.D Flammilina *velutipes* was also cultivated in China.*P. ostreatus* is considered as a healthy food because it is rich in a variety of bioactive compounds including polysaccharides, proteins, amino acids, polyphenols, vitamins and fatty acids[8]. *P. ostreatus* are well known for their medicinally beneficial activities such as anticancer activities, immune-modulatory effects, and antiviral, antibiotic anti-inflammatory and cholesterol lowering activities[9].

Literature Review

Global Scenario:

Oyster mushrooms are the third largest cultivated mushroom. China, the world leader in Oyster production, contributes nearly 85% of the total world production of about a million tonnes. The other countries producing oyster mushrooms include Korea, Japan, Italy, Taiwan, Thailand and Phillipines[10].

The global mushroom market size was USD 17.25 million tonnes in 2023 and is projected to grow from USD 18.39 million

tonnes in 2024 to USD 32.04 million tonnes in 2032 at a CAGR of 7.18% during the forecast period (2024-2032)[11]. Majorly six mushrooms dominate the global production and market viz, shiitake mushroom (26%), oyster mushroom (21%), black ear mushroom (21%), button (11%), Flammulina (7%), paddy straw mushroom (1%), and others mushrooms (13%).

Indian Scenario:

India receives a lukewarm response in the production of mushroom though mushroom cultivation, both in east and west started many centuries ago. First systematic attempt in cultivating button mushroom was made in 1961, when a scheme entitles "Development of Mushroom Cultivation in Himachal Pradesh" was started at Solan by H.P. Government in collaboration with ICAR, New Delhi[12].

Oyster mushrooms were analysed using the HPLC-DAD technique by Palacios and co-workers (2011). Several polyphenols were identified in the samples, including p-coumaric acid (11.15 ± 0.85 mg/g), ferulic acid (20.16 ± 0.16 mg/g), gallic acid (290.34 ± 3.61 mg/g), gentisic acid (292. 62 ± 3.42 mg/g), p-hydroxibenzoic acid (4.69 ± 1.59 mg/g), homogentisic acid (629.86 ± 1.54 mg/g), myricetin (21.99 ± 0.89 mg/g) and protocatechuic acid (19.32 ± 0.84 mg/g)[13].

Phenolic compounds such as phenol, flavonoid, and tannins are known to exhibit a significant amount of antioxidant properties. Rahimah et al. conducted a study on white oyster mushroom to evaluate antioxidant properties; it reveals that nearly 5.45 to 8.03 mg GAE (gallic acid equivalent)/g of total phenol were shown in different concentrations[14].

In the study of Fatimah Buba at.al,. Protein content was 28.45±1.15 mg/g, the elemental content (1.56±1.14 µm/g) for calcium Ca2 (5.04±3.58 µm/g) potassium "K" and (0.55±0.53 µm/g) phosphorus "P". Mushroom appears to have a high content of potassium than the other element[15].

Material and Methods

Present Work

Cultivation of oyster mushroom (*Pleurotus ostreatus)* and analysis of its nutritional values. Oyster mushrooms are a rich source of nutraceutical and other bioactive components like phenolic components, flavonoids, alkaloids, tannins, lectins, laccase, vitamins and polysaccharides such as β-glucan as well as other components with high antioxidant activities and therapeutic properties. So the aim for this research project is to cultivate oyster mushroom and analyze nutritional values of oyster mushroom and button mushroom. In my experimental work I did analysis of

polyphenols, flavonoids and alkaloids in button and oyster mushroom among the bioactive components of oyster and button mushroom.

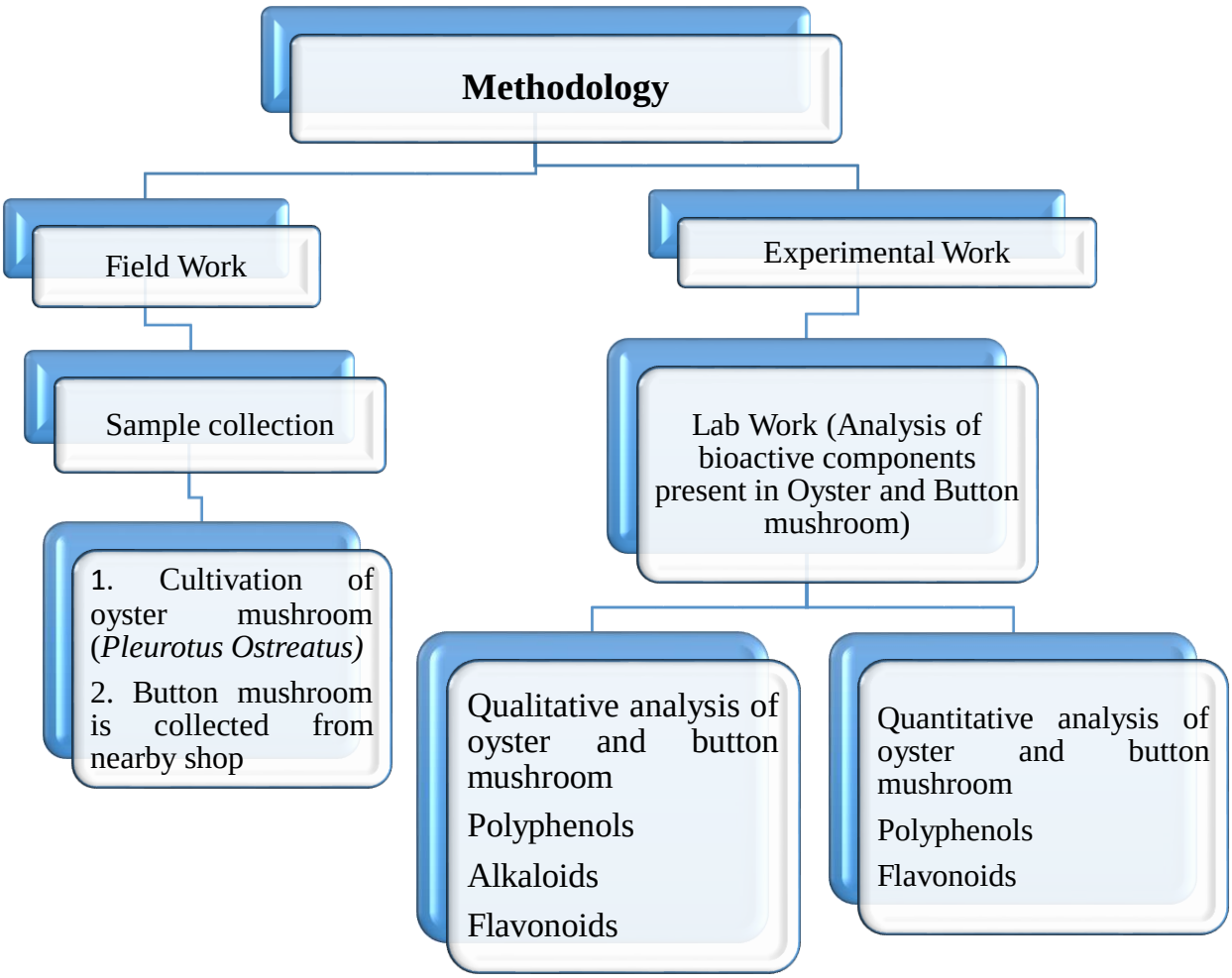


Figure-1 Methdology

Field Work

Samples of Oyster and Button mushrooms were collected and then cultivation procedure of Oyster Mushroom was started including following steps:

Step : 1 Preparation of Culture Media

Make a solution of 7.8gm PDA in 200 ml distil water. Mix it well and put it into the autoclave at 121 °C at 5 psi for 25 min. After autoclave place the jar in laminar airflow under UV light. Now fill the slant and petri plates with the PDA solution and let it solidify. Place one piece of cut mycelia form mother petri plates on each petri plates with PDA solution and place them into the incubators.

PDA (Potato dextrose agar): It is the most basic and widely used medium for growing mycelia of most cultivated mushrooms. It is

available commercially as a ready-to-use powder that can be used to make the medium in the laboratory with a concentration of 20gm/l of distilled water.

Step : 2 Preparation of Spawn or Seed

Wheat seed with fungal mycelium called spawn or fungal seeds**.** Wash the wheat seeds in water. Dry the seeds under shade. Add Rose Bengal in the wheat seeds and boil the seeds in water for 50 minutes. Dry the seeds in air by spreading on polythene sheet. Coat the seeds with 2% calcium carbonate to adjust the pH around 7 and also keeping the seeds in individuals. Fill polythene bags with the grain and close it using cotton plug. Autoclave it for 2 hours at 126°C. After autoclave, add the cut pieces of mycelium into the wheat bags and mix it well so the pieces spread evenly. It should be done in the laminar airflow and after mixing put these bags inside the incubator. After 15-20 days mycelium grows on the grain and afterwards it can be used as spawn.

Step: 3 Preparation of Substratum

Soak the Paddy Straw in Water for 24 Hours. Boil in water for one hour. Dry under the shade the pasteurized substrates. But maintains moisture content up to 65-70% Spray 10% calcium carbonate solution for maintain the pH around 7. Add Gypsum For High Yield. Now, the substrate mixture is ready for mushroom cultivation. Use polypropylene bags for the cultivation of mushroom.

Step: 4 Method of Cultivation

The cylindrical block System is better for Oyster mushroom cultivation. Take the substrate mixture in polypropylene bag about 5 cm height as a first layer. Spread 50 gm of spawn on the substratum. This process called spawning. Make the second layer of substratum about 10 cm height. Spread 50 gm of spawn on the substratum, mostly on the periphery. Repeat this process for several times in the same manner. Finally, the spawn covered with 5 cm height of substratum. Make many holes on the surface of the bag for watering, good aeration and reduce temperature. The inoculated bags then transferred to an incubation chamber and the temperature is kept between 21 and 22 degrees Celsius. The mycelium can move through the substrate and start consuming it at that temperature. In about two weeks, the bags will begin to turn white as the mycelium colonizes them. Afterward, the loads must be transferred from the inoculation room to the fruiting chamber. A small hole must be pierced in the face of each bag. This exposes the mycelium and substrate to fresh air and humidity. The mushroom will then start growing. After one week, each of these

will produce a lovely bouquet of fresh mushrooms. After that, it's harvest time!

Step: 5 Harvesting and Yield

Harvesting is done when the cap has the diameter of 8-10 cm. Picking is done by twisting gently so that it is pulled out without leaving any stalk and also the nearby fruiting bodies are not disturbed. When the base of the stipe is deeply immersed within the straw, cutting the base of stipe with sharp knife can be done. It is possible to get 500-800 g to a kilogram fresh mushrooms per kilogram of the dry substrate. The bags are kept in the growing chamber after the first harvest to allow other mycelium to grow and produce more fruiting bodies, which can then be harvested again.

Experimental Work

A. Qualitative and Quantitative analysis of Bioactive components of Button Mushroom and Oyster Mushroom

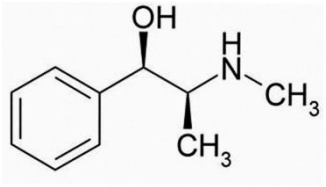
Sample Preparation: Drying of both the mushrooms under shading. After drying make powder of dry mushroom using mortar and pestle. Powdered mushrooms is then mixed with 50 mL distilled water and after mixing put the jars in the rotatory flask shaker for 24 hours. After 24 hours sample is ready for analysis.

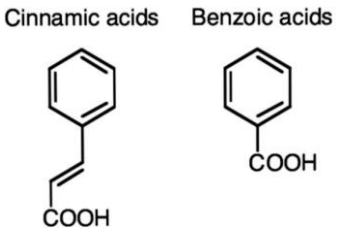
Reagents Required: Sodium hydroxide, Ferric chloride, Wagner's reagent.

Table-1 Qualitative analysis of oyster mushroom and button mushroom

Experiment	Observation	Inference
Test for Flavonoids Alkaline reagent test: Add few drops of sodium hydroxide solution to test solution	Development of intense yellow colour	Presence of Flavonoids
Test for Alkaloids Wagner's test: Two drops of Wagner reagent was added to extract and mixed well	Appearance of a reddish color indicates the presence of alkaloids.	Alkaloids present
Test for Polyphenols This test depends on the variety of changes that happens when polyphenols respond with ferric chloride. A couple of drops of ferric chloride is added to the sample, and the development of variety change, typically a blue, green, or earthy colored tone, demonstrate the presence of polyphenols	Presence of earthy colored tone	Presence of Polyphenols

Structure of Alkaloids **Phenolic Acids** **Structure of Flavonoids**





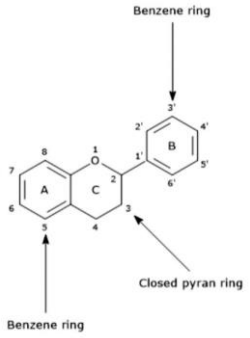


Figure- 2

B. Quantitative Analysis of Oyster and Button Mushroom

Preparation of Standards: Prepared a series of standard solutions of a reference polyphenol compound. Commonly used standards include Gallic acid, catechin, or tannic acid. Concentrations typically range from 40,50,60 µg/mL

Preparation of Sample Extracts: Extracted polyphenols from mushroom samples using an appropriate solvent mainly distilled water.

Folin-Ciocalteu Assay: Took aliquots of appropriately diluted sample extracts and placed them in test cuvettes. Added an appropriate volume of Folin-Ciocalteu reagent to each tube. The Folin-Ciocalteu reagent is usually added in excess to ensure all polyphenols are oxidized. Mixed thoroughly and allow the reaction to proceed in the dark for a specified time (typically 30 minutes to 2 hours) at room temperature. During this time, the Folin-Ciocalteu reagent reacts with the polyphenols to form a blue-colored complex.

Measurement of Absorbance: After the reaction period, measure the absorbance of each sample at a specific wavelength using a spectrophotometer. The wavelength was measured at 765 nm. Recorded the absorbance values for both mushroom sample extracts and the standard solutions.

Calculation of Total Polyphenol Content (TPC): Constructed a calibration curve using the absorbance values of the standard solutions plotted against their known concentrations (µg/mL). Used the equation of the calibration curve to determine the concentration of polyphenols in sample extracts. Express the TPC in µg of Gallic acid equivalents (GAE) per gram or milliliter of sample extract (µg GAE/g or µg GAE/mL).

Table-2 Optical Density of Polyphenols content in button mushroom and oyster mushroom

OD (Optical density) of Button Mushroom	Concentration (µg/mL) of Button Mushroom Absorbance=0.045× Concentration +0.002	OD (Optical density) of Oyster Mushroom	Concentration (µg/mL) of Oyster Mushroom Absorbance=0.045× Concentration+0.002
0.000	0.000	0.000	0.00
1.352	30	1.834	40.71
1.357	30.53	1.836	40.53
1.376	30.31	1.817	40.33

Table-3 Optical Density of Flavonoids Content in button mushroom and oyster mushroom

OD (Optical density) of Button Mushroom	Concentration(µg/mL) of Button mushroom Absorbance =0.037× Concentration+ 0.015	OD (Optical density) of Oyster Mushroom	Concentration (µg/mL) of Oyster Mushroom Absorbance= 0.037+ Concentration+0.015
0.000	0.000	0.00	0.00
0.0722	19.10	1.028	0.35
0.730	19.32	1.048	0.81
0.720	19.05	1.055	1.08

RESULT AND DISCUSSION

In qualitative study we found out the presence of flavonoids, alkaloids and polyphenols in both Oyster mushroom *(Pleurotus ostreatus*) and Button mushroom (*Agarcius Bisporus).*

Table-4 Qualitative Results of analysis of Oyster and Button mushrooms

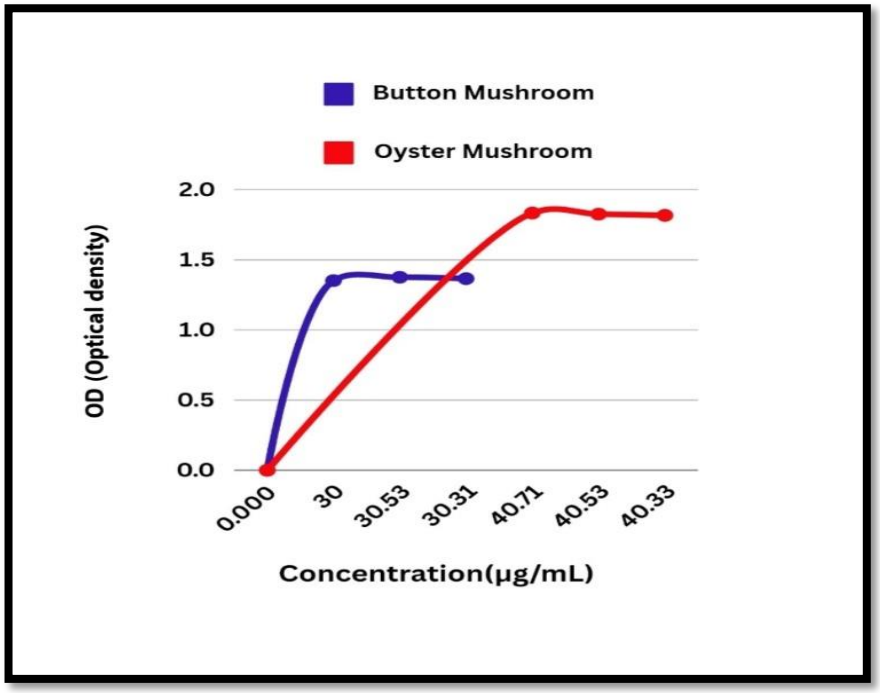
Sr. No.	Qualitative test	Observation	Results
I.	Flavonoids test: Alkaline reagent test	Positive	Presence of flavonoids
II.	Alkaloids test: Wagner's test	Positive	Presence of alkaloids
III.	Test for Polyphenols	Positive	Presence of polyphenols

Quantitative results of Oyster and Button Mushrooms

TPC (Total phenolic content)

In quantitative study the total phenolic content (TPC) of Button Mushroom sample, expressed in µg/mL of gallic acid equivalents (GAE), is approximately 30.28 µg/mL and Oyster Mushroom sample is approximately 40.52µg/mL.

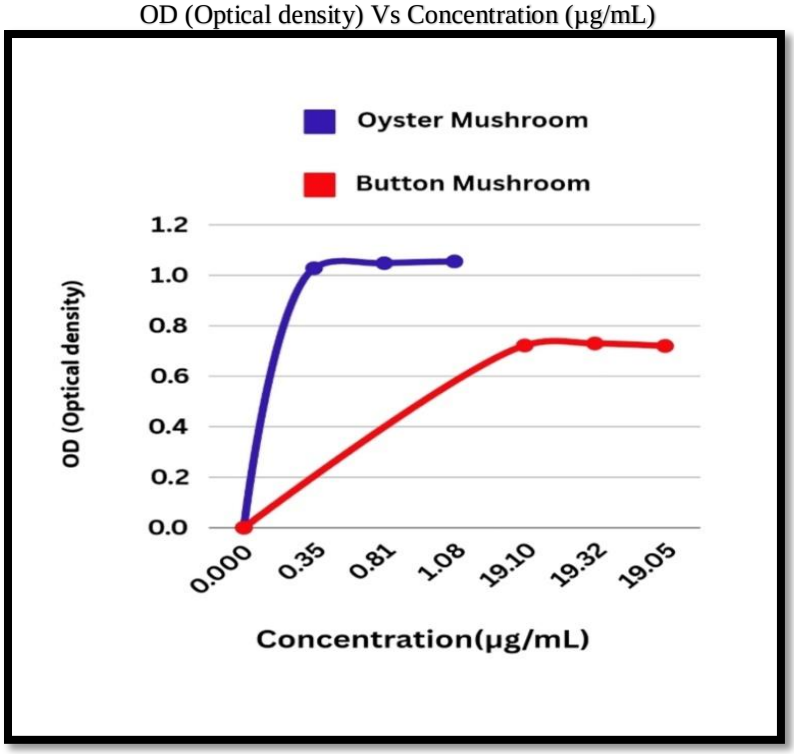
Chart-1 Polyphenols Content
OD (Optical density) Vs Concentration (µg/mL)



TFC (Total Flavonoid Content)

The total flavonoid content (TFC) of the Button Mushroom sample is approximately 19.05 µg/mL and Oyster Mushroom sample is 0.74µg/mL.

Chart-2 Total Flavonoid Content
OD (Optical density) Vs Concentration (µg/mL)



This research aimed to cultivate the Oyster mushroom *(Pleurotus ostreatus) a*nd analyze the bioactive components of Oyster Mushroom *(Pleurotus ostreatus)* and compare them with Button Mushroom *(Agarcius Bisporus)*. Mushrooms are one of the most popular and versatile gifts of nature. It can be mixed into any food preparations or can be processed to give a new product. A lot of mushroom products are currently available in market such as mushroom pickle, seasonings, beverages, extracts, dried and canned mushrooms, mushroom supplements, cosmetics etc. Apart from the mushroom food products many innovative products are emerging in other industries as well such as mushroom based building materials, medicines, myceliabased platforms, biodegradable packaging, mycelium based leather etc.Mushrooms are easy to cultivate, have quick growth and nil carbon emission and waste generation.Oyster mushrooms can grow on a ton of different materials, including hardwood sawdust, supplemented sawdust, straw, the masters mix, coffee grounds, paper, and pretty much any other liginous material. Here we used paddy straw as substrate for oyster mushrooms. For spawn preparation of oyster mushroom we can use different grains like wheat, barley, sorghum and millet grains could be equally used in the production of good quality spawn for the cultivation of oyster mushrooms.

Conclusion

Oyster mushroom can grow at moderate temperature ranging from 20° to 300° C and humidity 55-70% for a period of 6 to 8 months in a year.The fungi is a good source of income generation for the growers and also provides additional benefits through its processing.Mushrooms holds a bright future in every aspect owing to its diverse properties. Mushroom cultivation is highly compatible with a variety of other traditional agricultural and domestic activities, and can make a particularly important contribution to the livelihoods of the disabled, of women and the landless poor who, with appropriate training and access to inputs, can increase their independence and self-esteem through additional income generation. In qualitative analysis we found out the presence of flavonoids, alkaloids and polyphenols in button mushrooms and oyster mushrooms and in quantitative analysis it was observed that Polyphenol contents are found in concentrations are 30.28 µg/mL and 40.52 µg/mL for Button and Oyster mushrooms and the OD are between 1 to 2 as shown in table-2. Study of Optical Density of Flavonoids in different concentrations of oyster Mushroom and button mushroom have shown values from .072 to 1.08 as

given in table-3. So, we can conclude that both the mushrooms are good source of bioactive compounds and considered as super food.

Acknowledgement

Authors sincerely thank to Uttarakhand Science Education & Research Center (USERC) Dehradun for providing help in the cultivation procedure of Oyster mushroom and we are also thankful to Dr Narotam Sharma, Scientist DLCAS, Dehradun for providing laboratory help for our experimental and analytical work.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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