CULTIVATION OF OYSTER MUSHROOMS

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CULTIVATION OF OYSTER MUSHROOMS

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By

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DECLARATION

I hereby declare that this internship report entitled “Cultivation of Oyster Mushrooms” has been prepared by me and has not previously been submitted for the award of any Diploma or Degree in this university or any other university. It is submitted in partial fulfillment of the degree Master of Science in General Biotechnology of Goa University. The present internship was carried out in Goa University, associated with SBSI EOC.

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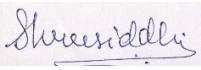

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1. INTRODUCTION

1. INTRODUCTION

Mushrooms are fleshy, spore-bearing, multicellular saprophytic fungi growing on dead organic matters of vegetative origin and can utilize almost all forest and agricultural residues as substrates. The consumption of mushrooms by humans is an age-old practice. However, mushrooms have become a part of every continental dish because of its good taste, flavor and nutritional value. It contains large amount of proteins, vitamins, minerals, and fiber, hence considered as pool of nutrients. The discovery of bio-active compounds, including antitumor properties has sparked an interest in such mushrooms from industries, the media and the scientific community. (Pokhrel, 2016)

Pleurotus spp, commonly known as Oyster mushroom, are decomposer of wood and vegetable residues. Oyster mushroom are commonly referred as 'Dhingri' in India. They are basidiomycetes and belong to the genus *Pleurotus*. The name *Pleurotus* originated from Greek word, 'Pleuro' which means shaped laterally or lateral position of the stalk or stem. There are several species of *Pleurotus* which are available for cultivation. These are *P. florida*, *P. sapidus*, *P. eryngii*, *P. sajor-caju*, *P. columbinus*, *P. cornucopiae*, and *P. flabellatus*. However, *Pleurotus sajor-caju* species is widely used for growing. (Maurya & John, 2020) Oyster mushrooms have been widely cultivated and commercialized next to *Agaricus bisporus*, known as Button mushrooms.

The raw materials which can be applied for Oyster mushroom cultivation are easily and cheaply in accessible from farmers' yards. Oyster mushroom is a fast-maturing crop and has abilities to grow at a wide range of temperatures utilizing various

lignocellulosic sources. Oyster mushrooms produce extensive enzymes and utilize complex organic compounds which occur in agricultural wastes and industrial by-products. Thus, most organic matters containing cellulose, hemicellulose and lignin can be used as mushroom substrates. Examples include rice and wheat straw, cottonseed hulls, corncob, paddy straw sugarcane bagasse, sawdust, waste paper, and leaves. However, an ideal substrate should contain nitrogen which can be added as a supplement, and carbohydrates for rapid mushroom growth. The spawn running, pinhead formation and fruiting bodies formation are three important phases in the cultivation of mushroom, require proper humidity and temperature. (Shah, Ashraf, & Ch., 2004)

Mushroom cultivation is an environment friendly agricultural activity. Mushrooms can be grown on agricultural and industrial waste. The by-products of farming remain unused as waste in the form of straws, leaves, stems, roots etc. These wastes can be recycled into production of food. Mushroom cultivation should be welcomed by the poor farmers as it is highly labor intensive, short duration crop and land saving. (Shah, Ashraf, & Ch., 2004) Oyster mushroom can be cultivated with agricultural residues, such as rice and wheat straw. It is a value-added process to convert the waste agriculture materials into human food. It epitomizes one of the most efficient biological ways by which these residues can be recycled. Mushroom cultivation helps to reduce the protein deficiency especially in developing countries. Moreover, mushroom cultivation can increase income of the rural poor people through rural development programs for farmers if they are made aware of its process and its importance. Likewise, livelihood can be improved because the demand of mushroom has been increasing due to increasing population, market expansions and changing of consumer behavior. (Pokhrel, 2016)

2. AIMS AND OBJECTIVES

2.1 AIM

Production of Oyster mushrooms using agricultural by-products as substrate

2.2 OBJECTIVES

The internship was undertaken with the following objectives:

A. Recycling agriculture waste to obtain a suitable substrate for the production of mushrooms.

B. Developing a cultivation center in the university campus for growing the mushrooms.

C. Growing at least two different species of Oyster mushrooms. (*P. florida* and *P. sajor caju*)

D. Encouraging local farmers to take up mushroom cultivation as an additional source of income.

D. Conducting awareness programs and workshops to promote mushroom farming.

3. METHODOLOGY

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3.1 Setting up the Cultivation Center

3.1.1. Cleaning the center

Cleaning is necessary before disinfection to keep the contaminants at bay. The room should be cleaned at a regular basis in order to manage contamination and maintain sterility. The mushrooms are sensitive to airborne contaminants; therefore, the growing room should always be hygienic. The incubation center and the environment were thoroughly cleaned, swept and mopped using clean water and disinfectant. Furthermore, the sterility of the mushroom cultivation center was sustained by washing hands and feet with soap and water; sanitizing with ethanol or hand sanitizers, before entering the room.

Fig. 1. Cleaning the cultivation room and surroundings

3.1.2. Removing mold from ceilings

The ideal growing conditions for mushrooms source the growth of mold which produce spores and when exposed to the growing mushrooms, lead to contamination. The patches of mold from the ceilings were removed using

sandpaper and towel to prevent the growth of undesired fungi on growing mycelium.

Fig. 2. Removing molds from ceilings

3.1.3. Preparing racks for incubation

Old, unused and rusted racks were brought from the University library to the growing center. The rust was removed by meticulously scraping with sandpaper. The racks were given three coats of white paint. Paints can inhibit corrosion and prevents rust from occurring on the metal surface of the racks. It forms a protective barrier between the metal and, water and air.

Fig. 3. Shifting of racks from university library to the cultivation center

Fig. 4.a. Removing rust from the racks

Fig. 4.b. Painting of the racks

3.1.4. Carpeting of the walls and floor

Due to warm temperatures and humidity, the walls absorb moisture and the chances of mold growth increases which can lead to contamination of the developing spawn. Therefore, the walls were covered with plastic sheets to ensure effective insulation and make them damp-proof. Spawn running and harvesting should not take place in the same room. The dark room and the fruiting room were physically separated by plastic walls to keep the different stages of cultivation apart. The paint stains were removed from the floor, and carpeted with 3.5x4.5m plastic. This is done because the humidifiers spray the water in all directions, making the walls and floor damp, inviting growth of fungus on their surfaces, leading to contamination and foul smell.

Fig. 5. Wall carpeting

Fig. 6.a Scraping of paint from floor

Fig. 6.b Stithcing two sheets together

Fig. 6.c Floor carpeting

3.1.5. Installation of the irrigation system

All *Pleurotus* species require high relative humidity (80-90%) during fruiting. Frequent spraying of water is required in the cropping room depending upon the atmospheric humidity. Fruit body produced under humid conditions is larger with less dry matter while those grown at low humidity tend to be small with high dry matter. In the fruiting chamber, foggers were installed that were connected to the water tank in the storage facility through pipelines. HTC 1 hygrometers (Fig.7)

were installed to monitor the humidity as well as temperature of the room during fruiting.

Fig. 7. Installation of the irrigation system and hygrometer

3.1.6. Fumigation of the room

Disinfection of mushroom cultivation center is done by fumigating the inoculation room, incubation room, and fruiting body induction room, before the spawning activity. The cultivation room was fumigated with 2% formaldehyde 24-48 hours prior to spawning of the substrates. Fumigation was done installing formaldehyde solution in petri dishes and allowing the fumes occupy the entire room for 24 hours.

3.2 Mushroom Cultivation Process

3.2.1. Substrate preparation

A mushroom substrate is a material that provides mushrooms with the nutrients, moisture and energy they need to grow and fruit. The mushroom mycelium grows and establishes itself in the substrate. Oyster mushrooms can grow at a wide range

of temperatures utilizing various lignocelluloses as they produce extensive enzymes and utilize complex organic compounds which occur as agricultural wastes and industrial by-products. Thus, most organic matters containing cellulose, hemicellulose and lignin can be used as mushroom substrate. Examples include rice and wheat straw, cottonseed hulls, corncob, paddy straw sugarcane bagasse, sawdust, waste paper, and leaves. (Tesfay, Godifey, Kalayu, & Girmay, 2020)

The substrate should be rich in cellulose, lignin and hemicellulose, as they contain large amounts of carbon that serves as the main food source to the growing mycelium. Additionally, the substrate needs to contain trace amounts of minerals such as magnesium, calcium, sulfur, and phosphorous; and a good structure that enables air exchange. This is vital for the mycelium to colonize well. (Patar, 2018) The substrate needs to lack competing organisms which can be eliminated by sterilization. The substrates can be sterilized by various approaches to ensure minimal risk of contamination. The methods for sterilization are:

a. Hot water treatment

The substrate, kadba kutti or dry hay was soaked in water overnight. It was boiled in water for up to one hour. However, in case of paddy straw, hot water treatment is given at 70-80˚C for 45 minutes whereas for wheat straw, it is boiled at 65-70˚C for 60 minutes. After boiling, excessive water was drained and cooled down. The substrate is then ready for spawning.

Fig. 8. Hot water treatment of sterilizing substrate

b. Autoclaving

The chopped substrates were tightly packed heat resistant biohazard bags after washing and soaking. This was followed by autoclaving the substrates at 121˚C for 15-20 minutes. The substrate was cooled and excess water was drained out after autoclaving, as spawn must not be added to the substrate while the temperature is above 30˚C. The purpose of autoclaving is to kill any microorganisms as well as dormant spores in the substrate.

Fig. 9.Autoclaving of the substrate

Fig. 10.a Draining excess water after autoclaving

Fig. 10.b Substrate ready for spawning

3.2.2. Spawning of substrate

- Spawn

A pure culture of *Pleurotus* sp. is needed for inoculation on a sterilized substrate. Spawn is homologous to “seeds” and is used for growing mushrooms *in vitro*.

The spawn production process involves placing mycelium from a mushroom culture onto a steam sterilized grain, which completely grows through the grain. This grain and mycelium mixture is called spawn. However, the spawn used in the cultivation center was ordered from Vana Shrubs, Mapusa. Spawn can be ordered from various distributors such as the Directorate of Mushroom Research which provides spawn services. They supply spawn of various commercially grown mushrooms such as button mushrooms, various species of oyster mushrooms, milky mushrooms, shiitake mushrooms and paddy straw mushrooms.

Grain spawn which is 20-30 days old is best for spawning. The spawn should be mixed at 2 to 3% of the wet weight of the substrate. The spawn can be mixed thoroughly or mixed in layers wise, because in mixing method the quantity of spawn increased. The perforated bags give superior and early crop (4-6 days) than non-perforated bags because of accumulation of high CO_2 which inhibits fruiting. The old spawn approximately 3-6 months stored at room temperature (20-30˚C) forms very dense mat like structures due to mycelium aggregation and sometimes young pinheads and fruit bodies start developing in the spawn packet itself. (Maurya & John, 2020)

Fig. 11. Spawn grain for *P. sajor caju*

- Inoculation

The spawning was done in pre-fumigated room. This was done with 2% formaldehyde 48 hours prior to spawning. The surfaces were cleaned with isopropyl alcohol during inoculation. Two species of Oyster mushrooms, *P. florida* and *P. sajor caju* were inoculated. Spawned substrate was filled in polythene bags. Hands were cleaned using antibacterial soap and water before filling the bags. The polythene bags were filled with 3-4 layers of substrate and spawn. Grains of the spawn were thoroughly separated from each other and must be evenly inoculated on each layer of the substrate on its periphery, leaving the last layer as substrate. The bags were sealed by twisting the end and folding it inward and tying with rubber bands. 10-15 small holes of 0.5 to 1cm diameter were made on the bags using sterile needles for the purpose of aeration. These holes were plugged with cotton to avoid infestation by insects. The bags were labelled with names of species, variety, and the date of inoculation to know the age and type of the spawn, and kept on the racks in the dark room.

Fig. 12. Filling of bags

Fig. 13. Inoculated substrate in polythene bags

3.2.3. Incubation

The spawned bags were incubated in the dark room for 15 days with the least amount of oxygen and a high amount of carbon dioxide until mycelium run was observed. Temperature was maintained less 30˚C as increased temperature inhibits growth. Furthermore, the concentration of carbon dioxide was maintained less than 1.5 to 2%. The bags are not opened during mycelial growth and contamination was checked regularly.

Fig. 14. Spawned bag in dark room

3.2.4. Fruiting

When the mycelium was fully colonized the spawned substrate and forms thick mycelial mat, the bags were transferred to the fruiting chamber. In the fruiting chamber, the mushrooms require higher humidity which causes the mushroom pin heads to develop. The humidity was increased by regularly spraying of water using a humidifier. Oxygen is essential for mushroom during fruiting stage and the concentration of carbon dioxide should be less than 0.06%. For this purpose, exhaust fans were installed for the exhaust of gases from mushroom growing room. Once pinning started, cotton plugs were removed from the holes to induce fruiting. As the mushroom began fruiting, the bags were constantly monitored for pests like flies and mice. The humidity and temperature were monitored twice a day using hygrometer. The mushrooms were watered for 15 minutes using the auto-irrigation system. The following measures were taken to maintain precise humidity in the fruiting room:

- The mushrooms were irrigated twice a day using foggers.
- Humidity was checked every morning and evening using the HTC-1 hygrometer.
- Two fans were used in alternate mode to provide uniform humid levels.
- Buckets were kept underneath the sprayers to collect the leaking water and to maintain humidity in the room after spraying.
- Water from tank emptied and replaced with fresh water every two weeks.

Fig. 15. Fruiting chamber with arrangements for humidity

Fig. 16.a Mushrooms being watered using foggers

Fig. 16.b HTC-1 hygrometer showing temperature and humidity level

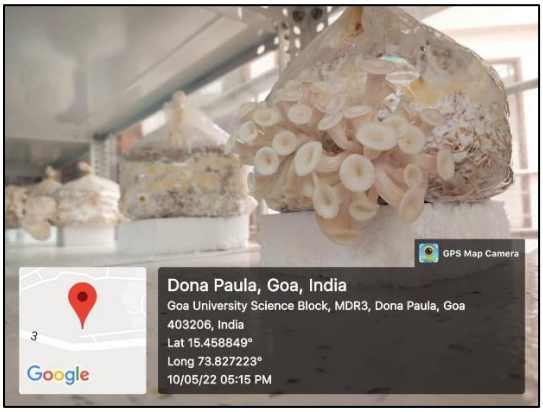

Fig. 17. Fruiting mushrooms

3.2.5. Harvesting

The mushrooms were harvested when they reached their full size by removing them completely by twisting the base firmly. The right stage for picking can be judged by the shape and size of fruiting body. In young mushrooms the edge of the cap is thick and cap margin is enrolled while the caps of mature mushroom become flat and inward curling starts. Picking was done by twisting the mushroom gently so that it was pulled out without leaving any stub, and also the surrounding fruiting bodies were not distressed.

Fig. 18. Harvesting the first flush

- Good Harvest Practices

The mushrooms should be harvested before water spray and preferably early morning. They must be harvested before the cap starts curling and everything at once to give space for other buds to grow. Contaminated bags with molds were

discarded while bags with patchy mycelial growth were left for few more days to complete mycelial growth.

- Post-Harvest Practices

After harvesting, the lower portion of the stalk with adhering debris must be cut using a knife. Stipe is kept short because it could be hard. Fresh mushrooms should be filled in perforated polythene bags for selling in market. They can also be sun dried by spreading thinly on a cotton cloth in bright sunlight or diffused light. The dried produce with 2-4% moisture can be stored for 3 to 4 months after sealing well. (Pokhrel, 2016)

Fig. 19. Dried mushrooms

Precautions

- The room was cleaned every week with IPA and water.
- Position of exhaust fan was installed near to the ground as carbon dioxide is heavier than air and is settled down.
- Doors and windows were opened to give embryo breathing space.
- Water pH was checked before spraying. The pH should be 7 to 8.

- If the fruiting bodies are showing trumpet growth, it means the concentration of carbon dioxide is more than required.

➢ Additional activities

1. Visit to Zonal Agriculture Office

The interns were introduced to the process of mushroom cultivation at the Zonal Agriculture Office, Mapusa. The resource person, Miss Gauri Prabhudesai shared her knowledge and experience in mushroom farming and focused on the importance of growing mushrooms. The conditions and process for cultivating Oyster mushrooms was imparted. Additionally, the statistics on mushroom growing countries was illustrated and the economics involved in production of Oyster mushrooms was explained. It was brought to our knowledge that the EOC, SES-REC will work closely in association with Agriculture Technology Management Agency (ATMA), Directorate of Agriculture, Government of Goa to conduct workshops and awareness programs on Oyster Mushroom Cultivation for local farmers, villagers and SES-REC interns of the university. Moreover, a resource person from Vana Shrubs Mr. Volvoikar displayed mushroom bags explaining the different stages of mushroom growth.

Fig. 20 Workshop at Zonal Agriculture Office

2. Making poster on "Oyster Mushrooms: Science of Cultivation and Commercialization"

Fig. 21. Making of the poster

3. Workshop at the University

A mushroom cultivation workshop was organized by the SBSI interns and mentors at the university for GU faculties, local public and famers from adopted villages. Talks by various guests were given and the audience were enlightened and motivated for growing mushrooms on their own. The interns and mentors demonstrated the spawning techniques to the participants. Moreover, e-certificates of participation were emailed to the registered participants before concluding the half-day workshop.

Fig. 22.a. Meeting regarding the workshop

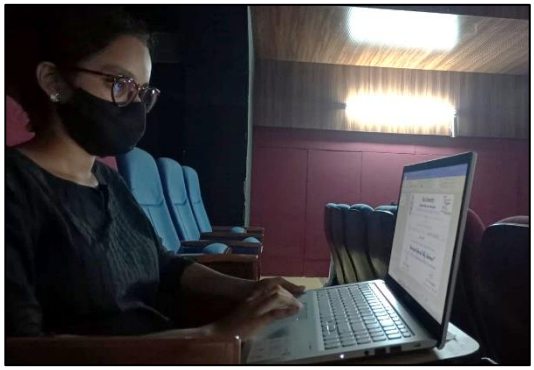

Fig. 22.b. Making of e-certificates

Talks on Mushroom Cultivation for general public, students and teachers on 30-04-2022

Fig. 23. Talks at the workshop

4. Awareness programs

The interns visited GU adopted villages of Goa to spread awareness about the science of Oyster mushroom cultivation and commercialization, and its benefits. During these visits, the techniques involved in various stages of growing mushroom were demonstrated to the villagers. It was explained that mushroom cultivation is very simple and economical in rural areas because raw materials and facilities required are easily accessible. Additionally, the importance of mushroom as crop was elaborated since it provides wide range of nutrients for the growing population. The farmers were encouraged to cultivate mushrooms as it generates income for the farmer besides helping in recycling of agriculture by-products.

Fig. 24. Awareness program for villagers

4. OBSERVATIONS

4. OBSERVATIONS

The following phases were observed during mushroom cultivation:

a) Spawn running

The amount of time taken for the spawn to fully colonize the substrate in the dark room is called spawn run period. It took 15-20 days for the mycelium to spread entirely through the bag.

b) Pinning stage

The stage where mushrooms initiate fruiting. It was induced by increasing the humidity to 80-90%. The initiation of pin heads appeared within the third week of inoculation.

c) Fruiting

The fruiting of mushrooms started 2-3 days later pinning.

d) First flush

The first flush was harvested within 30 days of spawning.

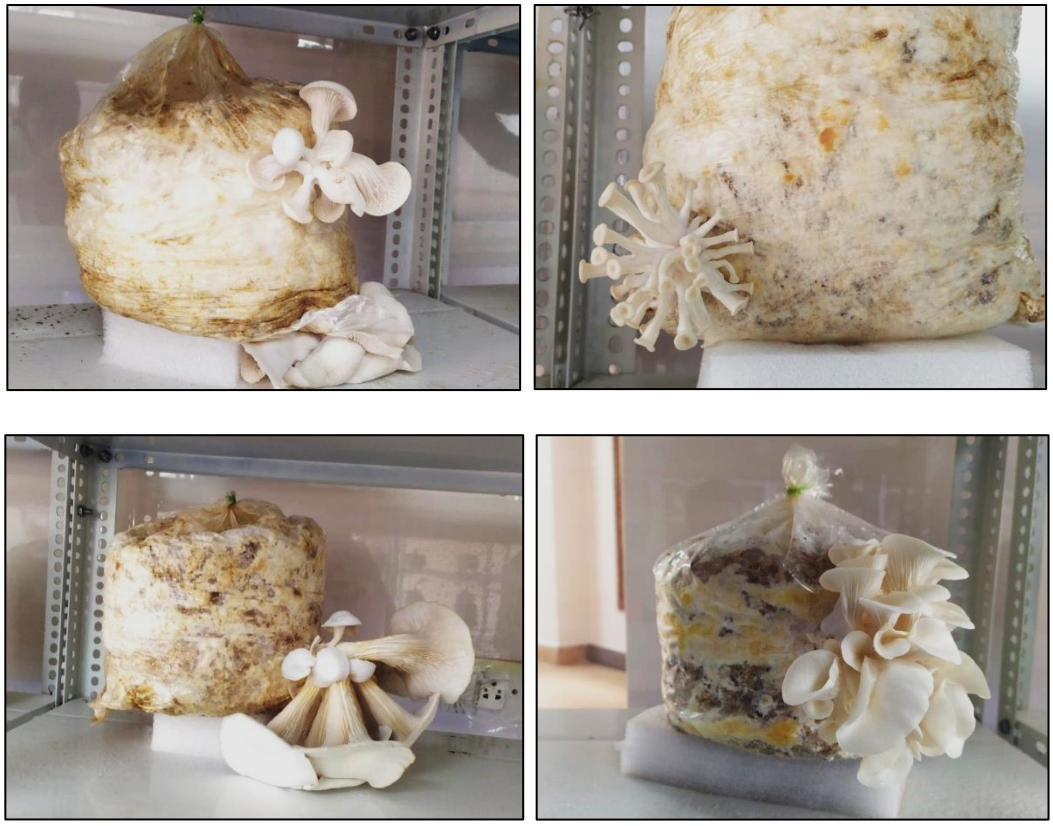

Fig. 25. Stages of growing mushrooms

Fig. 26. Fully grown Oyster mushrooms

5. RESULTS

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Two species of Oyster mushrooms were cultivated in the incubation center. Two flushes were harvested withing the period of 45 days, with decreasing amount of crop. The total cropping period of *P. sajor caju* is 60 days. Out of 100 bags, a few bags were contaminated and were discarded to avoid contamination of other bags. The biological efficiency was not calculated. Since the mushrooms were grown during the summer season, a good yield was obtained which could be due to high atmospheric humidity.

6. CONCLUSION

6. CONCLUSION

Oyster mushrooms contain high nutritional as well as medicinal value. They are a rich source of protein and hence could be more effective in decreasing protein deficiency as well as malnutrition problem in rural areas. *Pleurotus* genus of Oyster mushrooms have a complete lignocellulolytic enzyme system which can utilize a wide range of agricultural and industrial wastes for growth and fruiting. This gives them the most significant advantage of mushroom cultivation that is, transforming agricultural and other organic wastes into nourishing and marketable products. Therefore, establishing mushroom production across the country could be the best alternative agriculture business and employment opportunities in the rural areas, and best way of income generation for disadvantageous groups and small family farms.

7. REFERENCES

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