Comparative study of oyster mushroom (*Pleurotus ostreatus*) cultivation by physical and chemical method of sterilization using two different substrates

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Abstract - Mushroom is a crop which is cultivated in many countries using different agricultural wastes. Oyster mushroom can be cultivated by using two types of substrates such as sawdust, straw. It can be used in both physical and chemical sterilization. The physical method of sterilization on the substrate straw yields a product average of 416g (w/v) whereas the substrate sawdust yields a product average of 360g (w/v). In chemical sterilization method, the substrate straw yields a product average of 371g (w/v) whereas in the substrate sawdust yields a product average of 310g (w/v).

Index Terms- Autoclaving, Chemical Sterilization, Incubation, Mushroom, *Pleurotus ostreatus*, sawdust, straw

1 INTRODUCTION

Mushroom cultivation is a profitable agribusiness. Oyster mushroom (*Plerotous ostreatus*) is an edible mushroom having an excellent taste and flavour. It belongs to the class Basidomycetes. It grows wild in the forest and is cultivated in the temperate and sub tropical regions of the world.

Fungi lack the most important feature of plants - the ability to use energy from the sun directly through chlorophyll. They lack chlorophyll and cannot synthesize their own food. Thus, fungi depend on other organisms for food, absorbing nutrients from the organic material in which they live. The living body of the fungus is mycelium made out of a tiny web of threads (or filaments) called hyphae. Under specific conditions, sexually compatible hyphae will fuse and start to form spores. The larger spore producing structures (bigger than about 1 mm) are called mushrooms [1].

Mushrooms depend on dead organic matter as saprophytes, on living plants as parasites or they co-exist with other living organisms as symbionts. They grow on grassy ground, rotten wood, leaf litter, dung, cellars and mines. 'Mushroom' is the fleshy spore-bearing organ or fruiting body. Usually, the fruiting bodies are umbrella shaped structures, which produce spores in large numbers. These spores are minute, microscopic and are dispersed through wind. When they happen to fall on suitable substrates (like dead wood, straw, manure, litter or any other cellulose material), the spores germinate and develop into mycelia. As long as the condition is favourable for mycelial development and growth, the mycelia continue to grow, ramify and absorb food from the substrate until they develop many fruiting bodies [2].

1.1 Oyster mushroom

Oyster mushrooms are one of the most popular edible mushrooms and belong to the genus *Pleurotus* and the family Pleurotaceae. They were was first cultivated in Germany as a subsistence measure during World War I and is now grown commercially around the world for food. Oyster mushroom is one of the more commonly sought wild mushrooms, though it can also be cultivated on straw and other media. It often has the scent of anise due to the presence of benzaldehyde (which smells more like almonds).

Like oyster mushroom (*Pleurotus ostreatus*), many of *Pleurotus* mushrooms are primary decomposers of hardwood trees and are found worldwide. This mushroom has basidia with four basidiospores and a tetra polar mating system. Its hyphae have clamp connections and most members of the genus, excepting a small minority, have amonomitichyphal system. Fruiting bodies as well as active mycelia of Pleurotus species also possesses a number of therapeutic properties like anti-inflammatory, immunostimulator and anticancer activity, immunomodulatory, ribonuclease activity and many other activities [3].

2 MATERIALS AND METHODS

2.1 Sterilization

Sterilization is the process which is involved in killing of micro-organisms. Sterilization requires a minimum of 121°C steam at 15Psi (1 atm pressure) for 15-20 minutes.

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2.2 Preparation of agar media

Agar media is prepared by dissolving 23g of agar-agar in 1000 ml of distilled water. 1% agar is prepared by boiling it for about 10 minutes. The jars are kept in the autoclave and sterilized at 121℃ for 45 minutes. After autoclaving of the agar media, the bottles are then taken into the laminar airflow chamber in order to avoid contamination. The laminar airflow chamber must be wiped thoroughly with cotton cloth dipped in 70% alcohol. The autoclaved agar is a liquid but it becomes solid when it cools down. So the prepared agar media is then poured into the sterile petri plates at equal volumes.

After the agar is poured into the sterile petri plate, it is allowed to cool down, then it is wrapped and then kept in the incubator.

2.3 Preparation of the mother culture

The fresh culture of mushroom was collected from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The outer layer of the fresh culture is removed with the help of scalpel and forceps. The middle whitest portion of the culture is taken and inoculated into the prepared agar plates. The agar plate is then entirely covered tightly with the help of the wrapper. The culture plate is then incubated in the incubator at 25-27°C for 7-14 days. After the incubation period, fungus had grown on the entire agar plate.

Fig.1.Oyster mushroom first generation

A fully grown fungus agar plate is taken into the laminar airflow chamber. With the help of scalpel and forceps, it is cut in a criss-cross manner. A small piece of the culture from the fully grown fungus agar plate is taken and inoculated into another sterile agar plate. This is called second generation. Likewise, many pieces of culture are taken and inoculated into the agar plate. It is then wrapped in order to avoid contamination. Later it is incubated for 7-14 days at 25-27℃. Temperature is highly important as it affects the growth and adaptability as well as quantity and quality of fruiting bodies produced [4].

2.4 Preparation of spawn bags

Milo (grain sorghum) is commonly used for making spawn as it shows very good mycelium growth [5]. Millet grains were thoroughly washed and soaked for 24 hours in water, and then sieved [2]. After overnight soaking, 10 kg of grain is taken in a vessel with 15 L of water. It is boiled for about 15 minutes and allowed to cool for 15 minutes, water is drained and the spawn is dried in cotton cloth. 120g of gypsum ($CaSO_4.2H_2O$) is added with 30g of ground limestone ($CaCO_3$) and mixed well. The grain is packed into the polypropylene bags. One bag contains 300-350g of the prepared grain and is packed tightly [5]. The packed bags are then autoclaved at 121℃ [1].

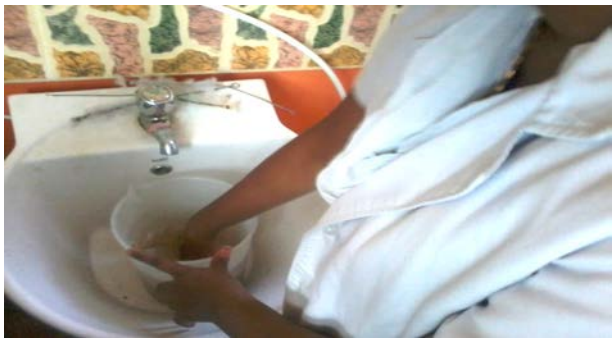

Fig.2. Washing

Fig.3. Drying

Fig.4. Packing

Fig.5. Mushroom bags

2.5 Inoculum of the mycelium

The fully grown mycelium plates and the prepared spawn bags are taken into the laminar airflow chamber. After autoclaving the sterilized bags, they are allowed to cool for 24 hours. The bags were immediately inoculated with mycelial culture of *P.ostreatus* [6]. The sealed spawn bags are opened and meanwhile the mycelium plate is also opened. The mycelia plates are cut into pieces in a criss-cross manner with the help of the sterile scalpel and forceps. The pieces are then carefully taken out of the plate with forceps. The culture of mycelium is inoculated into the prepared spawn bags in front of the flame (aseptically) in order to prevent contamination [7]. Once inoculated, the spawn bags should be incubated for mycelium of pleurotus at 27±2°C for 10-15 days until the mycelium fully cover the grains [8]. After about one week, they should all be shaken to spread the mycelium evenly through the bags. Shaking will speed growth and make the spawn a more even product.

Fig.6. Fully grown oyster mushroom bag

2.6 Preparation of the mushroom bed

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 dried well [9]. The mushroom bed is prepared by using two kinds of substrates such as wheat straw and sawdust. The mushroom bed is done by using two methods. Viz.,

1. Chemical sterilization
2. Organic sterilization

2.6.1 Chemical sterilization

In chemical sterilization, two kinds of substrates are used such as hay or husk or wheat straw and sawdust. Take 10L of water and add 10ml of formaldehyde. Then soak the substrates in water overnight separately. After overnight soaking dry the substrate in air for few minutes [10]. Take the polythene bags and (45×30cm) spread the substrate evenly in the bags. Then add the fully grown spawn to the bags and once again spread the substrate evenly. Pasteurized straw is mixed with 2% spawn and filled in bags. Likewise prepare many beds and close the bags tightly. Then it was gently pressed, and the bags were sealed for spawn running (development). Then small holes were made in the bags for respiration of the mycelium. Spawned bags were stacked on racks in neat and clean places, in closed position. Temperature (25-35°C) and humidity (70-85%) was maintained by spraying water twice a day on walls and floor. It took 15- 20 days when the bags were fully covered with white mycelium [11].

2.6.2 Organic sterilization

In organic sterilization, substrates such as straw and sawdust are packed in the polythene bags for autoclaving at 121°C. The polythene bags (45×30cm) are taken and the substrate is evenly spread in the bags. Then the fully grown spawn is added to the bags and once again the substrate is spread evenly. The pasteurized straw or sawdust was mixed with 2% spawn and filled in bags. Likewise prepare many beds and close the bags tightly. Later it was gently pressed, and the bags were sealed for spawn running (development). Then small holes are made in the bags for the respiration of the mycelium. Spawned bags were stacked on racks in a neat and clean place, in closed position. Temperature (25-35°C) and humidity (70-85%) were maintained by spraying water twice a day on walls and floor. It took 15- 20 days when the bags were fully covered with white mycelium [12]. The inoculated media is incubated in damp and dark conditions. Maintaining adequate moisture content of growing media is vital for mushroom growth [13].

2.7 Harvesting of the mushroom

After completion of the spawn run, the polythene bags were removed by cutting with a sterilized blade [14]. Before harvesting of the mushroom, on the 10thday, each bag was cut open and holes cut in the sides to initiate formation of primordial. The first yield of the mushroom was obtained on the 22ndday from both the substrate and by two sterilization methods. The maximum days for the appearance of first flush was recorded in controlled condition (18 days) [15]. The

obtained mushroom was harvested with a sterile knife and they are weighed and tabulated. The mushroom yield is obtained till the 45th day.

Fig.7. Primary stage

Fig.8. After incubation period

3. RESULTS

3.1 Oyster mushroom cultivation by autoclaving

Oyster mushroom (*Pleurotus ostreatus)* cultivation by one of the physical methods of sterilization (autoclaving sterilization) in the substrate straw, yields a product maximum of 433g(w/v) and the minimum product obtained is 398g(w/v) whereas the substrate sawdust yields a product maximum of 403g(w/v) and the minimum product obtained is 339g(w/v). The yield is obtained from the 20th day to the 45th day. The average weight of mushroom cultivated by autoclaving method of sterilization in the substrate straw is calculated as 416g(w/v) whereas in the substrate sawdust, it is calculated as 360g(w/v).

TABLE 1

Calculation of average yield using physical method

Yield	Replicates	Weight of mushroom on sawdust	Weight of mushroom on straw
Yield obtained	Replicate 1	345 g	415 g
	Replicate 2	367 g	398 g
	Replicate 3	339 g	427 g
	Replicate 4	403 g	408 g
	Replicate 5	347 g	433 g
Average		360 g	416g

3.2 Oyster mushroom cultivation by chemical sterilization

Here the yield is obtained a little later from the 22nd to the 45th day. Oyster mushroom (*Pleurotus ostreatus)* is cultivated by adding formaldehyde (chemical sterilization) in the substrate straw, which yields a maximum product of 401g(w/v) and the minimum product obtained is 371g(w/v) whereas the substrate sawdust yields 348g(w/v) as maximum product and the minimum product obtained is 289g(w/v). The average weight of mushroom cultivated by chemical sterilization in the substrate straw is calculated as 371g(w/v) whereas in the substrate sawdust, it is calculated as 310g(w/v).

TABLE 2

Calculation of average yield using chemical method

Yield	Replicates	Weight of mushroom on sawdust	Weight of mushroom on straw
Yield obtained	Replicate 1	319 g	389 g
	Replicate 2	295 g	391 g
	Replicate 3	348 g	377 g
	Replicate 4	301g	401 g
	Replicate 5	289 g	297 g
Average		310g	371 g

4. DISCUSSION

On comparing both the methods of sterilization, it is concluded that the physical method of sterilization [i.e] autoclaving method is more efficient than the chemical method of sterilization (Table 1, 2). The average yield obtained on the autoclaving method of sterilization in straw is 416g whereas in sawdust, it is 360g (Table 1). Therefore, in autoclaving method of sterilization, the substrate straw yields more maximum product than sawdust. The average yield of oyster mushroom obtained in chemical sterilization in straw is 371g whereas in sawdust, the average yield is 310g (Table 2). So, the substrate straw yields maximum product than the substrate sawdust. Hence, in both the methods of sterilization, straw has more efficiency than sawdust. Finally, it has been concluded that autoclaving method of physical sterilization yields a better yield in both the substrates (sawdust and straw) than the chemical method of sterilization.

5. CONCLUSION

Pleurotus ostreatus is an edible mushroom which is prepared by the various agro-based products such as sawdust, cotton waste, wheat straw, etc… [16].In this study, sawdust and straw has been used as a substrate. Oyster mushroom is grown on non-sterilized substrate in bag cultivation [17]. Here the oyster is produced by two methods of sterilization (physical and chemical methods). Of the two methods of sterilization, the autoclaving method (physical method of sterilization) is more efficient than the chemical sterilization method. This finding on the yield contrasted with the results [18] and seems relatively, the cultivation of oyster mushroom on sawdust is low compared to commercial production. Further it is concluded that the yield obtained on straw is more than on sawdust in both the methods of sterilization.

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