



P-ISSN: 2349-8528

E-ISSN: 2321-4902

www.chemijournal.com

IJCS 2020; 8(2): 2580-2583

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Received: 21-01-2020

Accepted: 23-02-2020

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Comparative study of oyster mushroom cultivation by physical and chemical method of sterilization

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DOI: <https://doi.org/10.22271/chemi.2020.v8.i2am.9138>

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. Effect of different sterilization techniques of substrate on yield of *P. sajor caju*, an experiment was laid out by treating paddy straw substrate with hot water, steam and chemicals. No treatment was subjected to the control. The experiments were laid out in completely randomized block design with six replication and four treatments viz. T₁ = In hot water treatment-substrate after presoaking for four hours, was immersed in hot water (80 °C +5 °C) for one hour, T₂ = In steam sterilization treatment, presoaked substrate was rapped in gunny bag and autoclaved at 15 lbs p.s.i. for one hour., T₃ = In chemical sterilization, substrate was immersed in 75ppm carbaendazim (50 w.p.) + 500 ppm formaline (40%) solution for 18 hours and T₄ = In control treatment – substrate steeping was done in plane water for 18 hours. During the both year experiment results are indicated that the treatment of chemical sterilization of substrate enhanced the spawn run which was completed in 21 days as compared to control (23 days) during both the years. It was followed by hot water substrate treatment (22 days) and steam sterilization of substrate (23 days) during both the years. The first harvest was also hastened 2 days by chemical sterilization followed by steam sterilization. As regards yield, all the treatments produced significantly higher yield than control. Chemical treated substrated produced maximum and significantly higher yield (420.00g and 418.0g) than other treatments during both the years.

Keywords: Chemical sterilization, yield, hot water and water substrate

Introduction

Mushroom cultivation is a profitable agribusiness. Oyster mushroom is an edible mushroom having an excellent taste and flavour. It belongs to the class Basidiomycetes. 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 (J.N.Buah, *et al.* 2010) [7]. 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 favourable for mycelial development and growth, the mycelia continue to grow, ramify and absorb food from the substrate until they develop many fruiting bodies (E.H. Amuneke, *et al.* 2011) [4]. Oyster mushrooms are one of the most popular edible mushrooms and belong to the genus *Pleurotus* and the family Pleurotaceae. They were first cultivated in Germany as a subsistence measure during World War I and is now grown commercially around the world

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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 a monomitic hyphal system. Fruiting bodies as well as active mycelia of *Pleurotus* species also possess a number of therapeutic properties like anti-inflammatory, immunostimulator and anticancer activity, immunomodulatory, ribonuclease activity and many other activities (Yashvant Patel *et al.* 2012) [15]. The market for mushrooms continues to grow due to the interest in their culinary, nutritional and health benefits (Vostrovsky and Jablonska, 2007) [13]. The production of mushrooms has increased in response to high customer demand and awareness on these health benefits (Aida, 2009) [1]. However, the government of Malaysia still has to import large quantities of mushrooms due to the inability of local mushroom growers to produce a sufficient amount to accommodate demand. Generally, mushrooms contain more protein, calcium, potassium, sodium, phosphorus, vitamins and a niacin than other vegetables (Jonathan, 2012). They contain protein (30.4%), fat (2.2%), carbohydrate (57.6%), fibre (8.7%) and ash (9.8%) (Bhatti, 2007) [2]. In Asian countries, mushrooms such as lingzhi (*Ganoderma lucidum*), shiitake (*Lentinus edodes*), and yiner (*Tremella fuciformis*) are considered edible and medicinal resources (Wasser, 2002; Zhang, 2007) [14, 16]. Oyster mushrooms are one of the most highly valued mushrooms due to their significant nutritional value, very good taste and medicinal properties.

Prior to spawning, the mushroom substrate has to undergo a disinfection process to prevent different pathogens (bacteria, moulds or pests) affecting the mushroom development and yield. Killing competitive fungi will permit faster, better and more uniform spawning and will assure better resistance to future infections (Ficior, 2006) [5]. Therefore, the mushroom yield will be higher and qualitatively superior. Several disinfection methods have been implemented, including pasteurisation (Royse, 2003) [9]. In pasteurisation, the substrate is pasteurised by C for 1 hour and using electrical passing an air-steam mixture through the substrate at 650 stimulation to reduce unwanted fungi (Takaki, 2007) [12]. The conventional method uses a steam oven (autoclave). The steam oven inactivates all fungi, bacteria, viruses and bacteria spores in the mushroom substrate as shown in Figure 1. High-temperature steam must be constantly F) or 0.5 bar at average cycle times of 6–8 hours to effectively ° C (250° produced at around 121 sanitise the mushroom substrate and to ensure complete biological inactivation. Subsequently, the steamed substrate is allowed to cool down for approximately 6 hours before spawning. The total time consumed is about 12–14 hours. To complete the process, a large amount of heat must be generated, and large quantities of energy are needed. Energy produced from natural gas, diesel and electricity is essential for the mushroom substrate disinfection process. Rapid increases in energy costs and the time-consuming nature of the sanitisation process system have major implications for operating margins and costs of mushroom farms.

Materials and Methods

Experiments were conducted at Department of Plant Pathology, T.D.P.G. College, Jaunpur in the two consecutive years 2010-2011 and 2011-12. To effect of different sterilization techniques of substrate on yield of *P. sajor caju*, an experiment was laid out by treating paddy straw substrate with hot water, steam and chemicals. No treatment was subjected to the control. The experiments were laid out in completely randomized block design with six replication.

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.

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 °C 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.

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.

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.

Preparation of spawn bags

Milo (grain sorghum) is commonly used for making spawn as it shows very good mycelium growth (S.R. Mondal 2008). Millet grains were thoroughly washed and soaked for 24 hours in water, and then sieved (E.H. Amuneke *et al.* 2011). 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 ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) 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 (S.R. Mondal *et al.* 2010) [10]. The packed bags are then autoclaved at 121 °C.

Inoculum of the mycelium

The inoculum of *P. sajor caju* was grown PDA for 5 days at 25 °C. Small discs (5mm in diameter) were transferred to Petridishes containing the test medium. A set of 5 petridishes was used for each treatment. Linear growth of measured in mm and recorded when the mycelium reached the edges of the Petridishes.

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.

Sterilization

Study the effect of different sterilization techniques of substrate on yield of *P. sajor caju*, an experiment was laid out by treating paddy straw substrate with hot water, steam and chemicals. No treatment was subjected to the control. These treatments were replicated six times. Details of the treatments were replicated eight times. Details of the treatments were as under: T₁ = In hot water treatment-substrate after presoaking for four hours, was immersed in hot water (80 °C +5 °C) for one hour, T₂= In steam sterilization treatment, presoaked substrate was rapped in gunny bag and autoclaved at 15 lbs p.s.i. for one hour., T₃ =In chemical sterilization, substrate was immersed in 75ppm carbaendazim (50 w.p.) + 500 ppm formaline (40%) solution for 18 hours and T₄= In control treatment – substrate steeping was done in plane water for 18 hours. In each treatment substrate was spawned by using techniques described in previous experiments with fixed quantity (1 kg dry straw) of substrate and spawn (120 g/bag). All requirements for cropping of mushroom were accomplished as usual. All the observation were recorded for every treatment.

Harvesting of the mushroom

After completion of the spawn run, the polythene bags were removed by cutting with a sterilized blade (Shubhra Shukla *et al.* 2011) [11]. 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) (Gopinath Lakshmipathy *et al.* 2011). The obtained mushroom was harvested with a sterile knife and they are weighed and tabulated. The mushroom yield is obtained till the 45th day

Results and Discussion

The result presented in the Table-1 indicated that moisture content of the substrate at the time of spawning ranged from 70-75 percent in 2011 and 69-74 percent in 2012. It was observed that chemical sterilization of substrate enhanced the spawn run which was completed in 21 days as compared to control (23 days) during both the years. It was followed by hot water substrate treatment (22 days) and steam sterilization of substrate (23 days) during both the years. The first harvest was also hastened 2 days by chemical sterilization followed by steam sterilization. As regards yield, all the treatments produced significantly higher yield than control. Chemical treated substrated produced maximum and significantly higher yield (420g & 418.0g) than other treatments during both the years. Steam sterilization substrate and hot water treated substrated produced respectively 300.0 g and 385.0g

yield during 2011 during 2012 and were significantly different. 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. 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. Oyster mushroom is an edible mushroom which is prepared by the various agro-based products such as sawdust, cotton waste, wheat straw, etc. (P.Dinesh *et al.* 2010) [8]. In this study, sawdust and straw has been used as a substrate. Oyster mushroom is grown on non-sterilized substrate in bag cultivation (Deepika Kumari *et al.* 2008) [3]. 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 (Gopinath Lakshmipathy *et al.* 2012) [6], 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.

Table 1: Effect of different Sterilization techniques of substrate on production of oyster mushroom

Weekly average max. and min. temperature and R.H. during 2011 24.0 ⁰ C – 12.4 ⁰ C and 89.0-58.10%										
Weekly average max. and min. temperature and R.H. during 2012 22.50 ⁰ C – 13.0 ⁰ C and 90.80-55.40%										
Sterilization techniques	Moisture content of the substrate at spawning %		Days taken for spawn run		Days taken for first harvest		Mushroom yield (g/kg dry substrate) in 30 days		Biological efficiency %	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Hot water treatment	72	71	22	22	27	28	300.00	385.00	30.00	28.50
Steam sterilization	70	69	23	23	28	28	345.00	342.80	34.50	34.28
Chemical sterilization	75	74	21	21	25	25	420.00	418.00	42.40	41.80
Control (No sterilization)	75	74	23	23	27	27	274.00	263.00	27.40	26.30
CD at 5%	-	-	-	-	-	-	15.30	16.85	-	-

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