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Optimization of oyster (*Pleurotus ostreatus*) mushroom cultivation using locally available substrates and materials in Debre Berhan, Ethiopia

Asmamaw Tesfaw[1], Abebe Tadesse[1], Gebre Kiros[2]

[1]Department of Biology, Debre Berhan University, Debre Birhan, Ethiopia.
[2]Department of Plant Science, Debre Berhan University, Debre Birhan, Ethiopia.

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ABSTRACT

Techniques to grow oyster mushrooms from culture to harvest were evaluated using locally available materials. Oyster was successfully grown in Potato Dextrose Agar (PDA). Spawn for *Pleurotus ostreatus* was prepared from sorghum and wheat without showing significance preference on them. Wheat straw, barley straw, sinar straw, waste paper, and *gabi* wastes were used as substrates; waste paper and gabi wastes alone or in mixture with saw dust yielded more oyster than wheat straw, barley straw, sinar straw. Substrates were reused and the yield was slower when it is compared to original substrates and they were found contaminated during pasteurization. Effects of pore size, temperature, and relative humidity on growth of mushrooms were evaluated. Pin hole size, high temperature (25^{o}C) and high relative humidity were optimal for oyster growth. This temperature is optimal for spawn running both in cultivation and spawn production.

1. INTRODUCTION

Pleurotus species, commonly known as oyster mushrooms, are edible fungi cultivated worldwide especially in south east Asia, India, Europe and Africa [1]. China produces 64 % of all edible mushrooms in the world and 85% of all oyster mushrooms all over the world (*Pleurotus* spp.) is also produced in China [2]. Oyster mushrooms is the third largest [3] commercially produced mushroom in the world; however, Sánchez [4] reported that *P. ostreatus* is the second largest next to *Agaricus bisporus* in the world market. Mushroom cultivation is the fifth largest agricultural sector in China with 24 billion USD value and 10% growth rate every year for the last 30 years [5]. Oysters are naturally found on rotten wood material. The growing and consumption interest of oyster mushroom is increasing largely due to its taste, medicinal and nutritional properties [6]. Large volumes of unused lignocellulosic by-products are available in tropical and sub tropical areas. These by-products are left to rot in the field or are disposed off through burning. Utilizing these by-products for mushroom cultivation using locally available technologies may be one of the solutions to transforming these inedible wastes into accepted edible biomass of high market value.

** Corresponding Author*
Asmamaw Tesfaw, Email: astesfa@yahoo.com
Cell phone: +251911956817, P.O. Box 445 Debre Berhan, Ethiopia

The spent substrates from mushroom cultivation can also potentially be used as an animal feed supplement, possibly providing additional animal feed resources [3]. A shorter growth time is required to *P. ostreatus* in comparison to other edible mushrooms. The substrate used for their cultivation does not require sterilization, only pasteurization, which is less expensive. High percentage of the substrates can be converted to fruiting bodies by oyster mushroom and hence it increases profitability.

P. ostreatus demands few environmental controls, and their fruiting bodies are not often attacked by diseases and pests, and they can be cultivated in a simple and cheap way [4]. All this makes *P. ostreatus* cultivation an excellent alternative for production of mushrooms when compared to other mushrooms. Therefore, it is better for unskilled farmers than other mushrooms. Mushroom cultivation provides an alternative employment and it contributes food security to rural disadvantaged groups especially women and old people in Tanzania hence improve their livelihood [7]. The expansions of mushroom cropping decline the price of mushrooms and hence it safe guard food insecurity [5].

Debre Berhan is located in North Shewa Administrative Zone, Amhara Region and it is 130 Km North to the Capital City, Addis Ababa. Debre Berhan is the coldest and windy town in the country with an elevation of 2,850 meters above sea level. According to World Bank [8], North Shewa is under high risk for drought and hence the zone needs an alternative agricultural practice that can't be easily affected by climate change.

Oyster mushroom cultivation is best in this regard since it doesn't require fertile soil and natural rain unlike other crops in Ethiopia. The aim of this research is to evaluate optimum conditions for oyster (*Pleurotus ostreatus*) cultivation using locally available substrates, materials and technologies.

2. MATERIALS AND METHODS

2.1 Potato dextrose agar (PDA)

PDA was prepared from locally available fresh potato and industrially produced agar and glucose. About 200 g of potato was washed and sliced and placed in 1 liter of boiling water in a flask like brass locally available and boiled for 15 minutes. Potato broth was filtered using a piece of cloth. Glucose (20g) and 20g agar was added and the volume was adjusted to one liter by adding water. The flask was plugged with absorbent cotton. The agar mixture and petridishes were sterilized in pressure cooker for 30 minutes. The agar (25ml) was poured carefully and aseptically in to petridishes around flame and for comparison, the laminar air flow hood was also used. After cooling, the PDA was used for *P. ostreatus* inoculation and incubated at 25^{o}C and at ambient temperature.

2.2 Spawn production

The grains, such as sorghum, wheat and dried malt wheat, were cleaned manually to remove inert matter, stubble and debris. The cleaned grains were soaked in tap water overnight. In addition, the grain was boiled soon after soaking for urgent spawn preparation. Thereafter, the soaked grains were drained and the excess water was removed and the following additives were added. Wheat bran at the rate of 10%, and chalk ($CaCO_3$) at the rate of 2% were added on dry weight basis of the grains. The additives were thoroughly and evenly mixed with the grains. The grain medium was filled in to bottles (made of brass) locally found. The bottles were covered using cotton wool plugged conduit. The bottles with spawn materials were autoclaved using pressure cooker and the supporting materials in bottles were allowed to cool for over six hours. The media in bottles were immediately inoculated with mycelial culture of *P. ostreatus* maintained on PDA. For successive production of spawn, the media in the bottles were inoculated with *P. ostreatus*. Then, it was incubated at 25^{o}C for mycelial growth without any light for 10-15 days until the mycelium fully covered the grains. It was shaken every four days to distribute mycelium throughout the grain till the end of the growing day. Alternatively, the grains were put on bench at varying ambient temperature (12-19^{o}C).

2.3 Substrate preparation

Wheat straw, barely straw, saw dust and sinar straw were collected from Debre Birhan surroundings and 90% of each substrate on dry basis were measured and soaked in tap water overnight to moisten and make the substrate soft for colonization to oyster mushroom hyphae. Alternatively 3% gypsum, chalk or $CaCO_3$ was added to 10% wheat bran. The buffer and wheat bran were mixed separately and then they were added to the substrates. In addition to different substrates, different supplements were also evaluated to observe their impact on oyster growth. Oil seed cake, wheat bran and cow dung (10% on dry basis) were added to 90% wheat straw and saw dust separately to ensure the even distribution of materials. Then the substrates and supplements were well mixed after the supplements and buffers were separately mixed. The relative humidity after mixing was measured as

$$\text{Relative Humidity} = \frac{weight\ of\ moistened\ substrate - weight\ of\ dry\ substrate}{weight\ of\ moistened\ substarate} \times 100\%$$

2.4 The growth of oyster on organic wastes

Many waste papers are released and burnt every month in Debre Berhan University. Hence an attempt to use waste paper as a medium for oyster cultivation was evaluated. Different amount of waste papers was mixed with saw dust. Saw dust (100%) was used as a control. In addition, in all 10% wheat bran was added after it was mixed with 3% gypsum separately. Finally the growth and the yield were measured using pileus diameter and biological efficiency. The *P. ostreatus* was inoculated and placed at ambient temperature. *Gabi* is, the Ethiopian traditional clothe and made of woven cotton, commonly dressed in the country. No clothe as frequently used as *gabi* by the majority of the people in the country and hence too much gabi wastes can be released every year in the country. Trials were made to cultivate the *P. ostreatus* on *gabi* wastes. Here 100%, 75%, 50%, 25%, and 0% saw dust were added to 0%, 25%, 50%, 75% and 100% gabi wastes respectively. In addition, 10% wheat bran and 3% gypsum were added. The percentage was calculated on dry basis. The substrates were pasteurized using oil drum. After cooling for an hour, the substrates was inoculated with *P. ostreatus* and placed at ambient temperature.

2.5 Reuse of the Substrates

The substrates that were used for cultivation of oyster for 2 flushes were used again for other cultivation. Here substrates were utilized in two ways. On the first hand, substrates without adding anything on used substrates were taken to grow mushrooms. On the other hand, 10% wheat bran and 2% gypsum was added to the used substrates. In addition, they were pasteurized in steam and sterilized using autoclave to evaluate the effect of contaminants that were presented in used substrates.

2.6 Spawning

The wooden hood and hands were disinfected with 70% alcohol. The mixer rod was sterilized by flame locally made. Then, the pasteurized substrates were inoculated with 4-6% *P. ostreatus* seed aseptically from mother spawn and they were incubated at 25^{o}C and ambient temperature.

2.7 Effect of pore size

After substrates were filled to plastic bags, different size holes were made to evaluate effect of aeration, contamination and moisture loss. Pine holes, 16.18mm^2 and 28.16mm^2 holes were made using circular cutter with different diameter. Here pine holes

were made after substrates were filled in the bags. However 16.18mm^2 and 28.16mm^2 holes were made before substrate were placed to the containers. The one with pin hole was arranged in two ways. First it was tied at top and second cotton plug was attached to plastic at the top to allow more aeration.

2.8 Effect of temperature

Since Debre Birhan is relatively cold especially from end of September to January, effect of ambient temperature on yield of oyster was evaluated since farmers in rural area are not able to buy incubators. Here, the bottle method was used due to its easiness to handle in the incubator. Equal amounts of substrates were put into two bottles. After inoculation, one was placed in the incubator at 25^{o}C and the other was put on bench. The bench temperature was varying from 3^{o}C to 19^{o}C. Then every week the growth and development of oyster mycelium was physically observed and assessed.

2.9 Pinhead initiation

Light and temperature were used to initiate the formation of pinheads after the mycelium was fully grown on the substrate i.e. after the substrate was fully turned into white color due to mycelium mass. The growing mycelium that was placed in dark was brought to a fully illuminated region; on the other hand, the bottles and plastic bags that were put in the incubator at 25^{o}C were placed at ambient temperature. The ambient temperature, when the experiment was conducting, was varying from 3^{o}C to 19^{o}C.

2.10 Fruiting

When the substrate was completely covered by the mycelium, the cotton plug was removed . Top of the plastic bag was partially removed. In addition, the part of the plastic that covered the primordia was also removed without harming the mycelium. The plastic bags that contain primordia was sprayed with water three times a day to increase the relative humidity [9].

2.11 Biological efficiency (yield)

Biological efficiency is a measureable tool used to know the growth potential of the oyster mushroom. It is calculated based on the following formula.

$$\text{Biological efficency} = \frac{\text{weight of freish mushroom}}{\text{dry weight of substrate}} \times 100\%$$

3. RESULTS AND DISCUSSIONS

3.1 Oyster culture on PDA

PDA is the simplest and the most popular medium for growing mycelia of most cultivated mushrooms [2]. *P. ostreatus* was successfully grown on PDA. The oyster completely covered the petridishes in 9 days and its color and appearance looks like pure cotton (fig 1). The mycelium should be white and grow out from the tissue. If yellow, blue, green or grey mycelia form on other places on the surface, then these are fungal contaminants [10]. A creamy, shiny growth often indicates bacterial contamination [10]. *P. ostreatus* is a slow grower when it is compared to molds and other fungi.

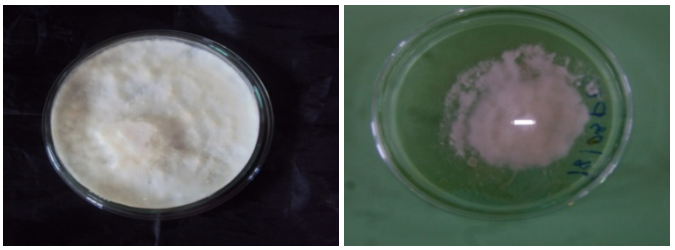

Fig. 1: *P. ostreatus* culture on PDA 5 days old (right) and 11 days old (left).

3.2 Oyster spawn

Once a pure culture of mushroom is obtained, the spawn could be made from it. The spawn is the mycelium of the mushroom on a solid material. Functionally, it is the starter inoculums of mushroom cultivation. Solid materials such as saw dust, whole grain of cereals, shredded maize and corn cobs etc are used for spawn preparation [9].

P. ostreatus was grown on sorghum wheat, and dried malt wheat based spawn without showing significant preference on them. Sorghum and wheat are cheap in Debre Birhan market hence it is economical to use them to produce spawns. The choice of grain is made after considering several factors such as its prevailing price, easy supply and grain size. In some areas in Africa, sorghum is better available and cheaper than maize [9].

Besides, the space between wheat or sorghums is quite enough for air circulation. However if teff was used, there would be no good aeration and teff seeds also get clamped together during sterilization [9]. That is why oyster didn't grow very well in spawn that contain too much wheat bran especially at the bottom of the flask (data not shown).

The whole inoculated cereals put on incubator at 25^{o}C were covered by mycelium of *P. ostreatus* within 14 days. However, those placed at bench took 22 days to colonize the cereal grains. Therefore, it is possible to prepare spawn without incubator, by simply placing on bench even if it takes longer period relatively.

Sorghum needs longer time to imbibe the same amount of water than wheat [9]. That is why sorghum was boiled but the sorghum should not be burst because the burst sorghum will be clamped and then it reduces the air circulation due to starch exposure during bursting.

Oei [11] compared the advantage and disadvantage of grains over sawdust or wooden stick. The main advantage of grains is that it is very nutritious for fungi and form kernels easily. The kernels can easily be distributed to the substrate. The main disadvantage is that it provides optimal substrate for other organisms too. The chances of contaminations are therefore much higher compared to sawdust or wooden stick spawns.

The moisture content of the grain should be around 50% [11]. However, Dawit [9] stated that water content of 40-60% is optimal for spawn making. In our case, the water content was

52%. If the water content is higher, mycelia growth may be faster but the danger of wet spot bacteria is also greater. If it is drier than 35%, mycelia growth will be slow [11]. To get good quality of spawn, what is most important is the quality of the inoculums. The inoculums should be fresh and pure. If the inoculums are preserved in refrigerator, they should be activated before they are used for spawn production.

3.3 Substrates

Mushroom cultivation is different from conventional agriculture in one major point. The soil in the ground is the substrate for production of crops but the mushrooms grow on lignocellulosic agro industrial wastes. Oyster can grow on wide agricultural wastes what matter is which agricultural residues are found around us [9].

Wheat straw, barely straw, sinar straw and saw dust were used as substrates for *P. ostreatus.* There was no significant biological efficiency difference on the substrates (table 1). It was successfully grown on them. Dawit [9] and Oei [11] pointed out that *Pleurotus* can grow on wheat and barley straws.

The moisture content of the substrates was varying from 69.8% to 74.5%. Significantly the highest run rate was recorded at 70% moisture level [12]. pH of the straw before adding chalk and gypsum was 5.8 .This might be because of the acids produced by microbes in substrates during soaking of substrates in water since it was soaked overnight. However, the pH rose to 6.9 when chalk was added. Here, washing is essential to neutralize the pH of the substrate. In addition, the chalk neutralizes the lowered pH. Furthermore, chalk and gypsums act as buffer. Besides, the Ca^{2t} obtained from gypsum ($CaSO_4$) and chalk ($CaCO_3$) neutralize oxalic acid produced during mycelial growth [9]. Highest yield is observed at pH 7 even though there is no significant yield difference at the pH 6-8 [13, 14]. However, the biological efficiency, biological yield and economic yield were increased with the increase of pH levels up to 5.04 and then decreased [12]. On contrary, Nwokoye *et al.* [15] showed that *P. ostreatus* was able to grow optimally at pH of 9.

Oyster productivity difference when different buffer such as gypsum, chalk and pure $CaCO_3$ were optimized; they showed no difference on oyster yield. However, there was pH difference after they were added (table 2).

Supplements are additives which increase the yields by providing specific nutrients for the growth of the mycelium [9, 11]. However, supplements increase the risk of contamination at least by 25% [16] since supplements also provide good nutrients to other microorganisms. Dawit [9] also stated that supplements change physical conditions of substrates more suitable for cultivation of mushrooms. Addition of supplements (rice husk) to waste paper significantly increase spawn running, pin head formation, fruit body formation and mushroom yield [17]. Similarly, cultivation of oyster mushroom on wheat straw and bagasse amended with distillery effluent yield better result than substrates without distillery [18] effluent. The effects of wheat bran, oil seed cake (fig 2) and cow dung on growth were evaluated and it was found that oil seed cake gave maximum yield (table 1 and fig 2). Likewise, Ruiz-Rodriguez *et al.* [19] found that oyster yield increased on substrates with supplementation of oil mill waste without affecting cultivation parameters. The yield reduction in cow dung might be due to contamination since pasteurized substrates containing caw dung was partly contaminated by green mold.. Similarly, Baysal *et al.* [17] proposed that the yield reduction in oyster production during supplimenattion of peat and chicken manure might be due to high nitrogen content of the substrate.

Fig. 2: Supplements: wheat bran (left) and oilseed cake of niger seed (right)

The substrates used for cultivation of oyster were used again for other cultivation. The pasteurized substrates were found to be contaminated. However, *P. ostreatus* was grown in non-contaminated parts. As it is indicated in table 1, reused substrates that contained additional supplements exhibited better growth on both pasteurized and sterilized substrates. The yield decrease in reused substrate is because *P. ostreatus* is primary decomposer. The reused substrates were very soft and made into small pieces easily. Therefore, the aeration in reused substrates will be relatively lower than original substrates. Furthermore, biological efficiency of oyster was evaluated using waste paper and gabi wastes and it was found that waste paper and gabi were better than wheat straw (fig 3).

Fig. 3: Oyster in gabi waste alone (left) and saw dust with *gabi* waste (right).

In order to find why gabi and waste paper gave maximum yield, moisture holding capacity of the substrates were evaluated and the waste paper and gabi had high water holding capacity. Hence one reason for high yield of oyster in gabi and waste paper might be its high water holding capacity. Oyster cultivation on shredded office paper and cardboard yielded more edible sporophore biomass than other lignocellulosic residues [1].

3.4 Effect of pore size

Pin holes, 16.18 $mm^2$and 28.16mm^2 holes were made on plastic bags to evaluate the effect of aeration, contamination and

moisture loss. Substrate in plastic with pin holes and 16.18 mm^2 holes were well colonized by *P. ostreatus*. However, the yield was lower in bags with 16.18 mm^2 (table 1). On plastic bag with 28.16mm^2 areas around the holes were not colonized and it was contaminated by molds. Inability to colonize might be due to great loss of moisture since the hole is large enough.

3.5 Effect of temperature

The substrates in the bottle placed in the incubator at 25^oC were colonized by oyster within 15 days where as the one put at beach with temperature varying from 3 to 19^oC was colonized within 21 days. At the temperature below 14^oC, the growth of the oyster was found to be so slow that they couldn't give primordia within 28 days. In stead 46 days were required for pinhead formation at temperature below 14^oC and the yield was also very small (table 1). To increase the temperature, the room was heated using wood charcoal. For most mushrooms, mycelial invasion is favored at higher temperature and fruit body formation at relatively lower [9, 11]. On the other hand, Alam *et al.* [14] investigated that the maximum growth is recorded at 25 ^oC and the lowest at 15 ^oC and no significance difference is observed between 25 ^oC and 30 ^oC. Therefore, the temperature ranging between 25-30 ^oC is best for mycelial growth of *P. ostreatus*. However, edible mushrooms cultivated between 15-20 ^oC present better quality and durability than those at 25 ^oC [20]. In addition, *P. ostreatus* grew faster at 30 °C [20]. In general, oyster yield decreases when the temperature decreases in different climatic zones of Pakistan [21].

3.6 Pin head initiation

Light and temperature changes were used to facilitate pinhead formation. Humidity and CO_2 concentration adjustment is also essential. All oyster mushrooms need light for the proper development of cap. Usually there is enough light for oyster [11] if it is possible to read a news paper in the growing room. Pin heads were developed in 26-31 days. *P. ostreatus* completed spawn running in 17-20 days on different substrates and time for pinheads formation was noted as 23-27 days [22]. In addition, temperature plays very important role for pin head formation. That is why pinhead evolvements were failed at a temperature lower than 11^oC. Furthermore, humidity should be very high during primordia formation. To achieve it, the mycelium was sprayed with water 3 times a day. Oei [11] stated that humidity at time of induction must be ≥ 90%. In some countries like Philippines and Nepal growers take off all or almost all the plastic from the bags. This leads to fast drying out of the substrate and lower the yields and hence it should be discouraged [11]. Another disadvantage of removing plastic is that more but smaller fruit bodies are formed, which increases the picking cost considerably.

3.7 Harvesting

At the first trial, only the first flush was harvested and 2nd and other flush were failed. These motivated us to see different environmental conditions and when the mycelium were covered by plastic after spraying with water, pinheads were developed and then 2nd, 3rd and 4th flushes were also successfully cultivated. Hence, what is most important for 2nd, 3rd and 4th flushes cultivation is humidity. Furthermore, temperature also affected our 2nd, 3rd, and 4th flushes (data not shown).

Unlike many other mushrooms by which the yield continuously decrease in consequent flushes, the pattern of *P. ostreatus* is different. More yields were found in second flushes (table 1). The highest crop of the mushroom is obtained during the second and third flush [9]. However, the maximum yield was obtained in first flush than the second and third flush [22]. In general, one crop of mushroom with four flushes is most economical. It is unwise to keep spawn running of substrate for longer than 90 days as the yield significantly decrease.

Good control of the humidity during cropping is very important for all types of mushroom. The moisture content of growing mushroom media is a very important factor; hence, the proper moisture content value encourages the growth, while higher or lower ones had a negative effect on growth [23]. It is good keep the humidity high (80 - 90%) by spraying water several times per day [10]. However, no water should be sprayed directly onto mushrooms that are ready for picking; their shelf life will decrease drastically if they become too wet [9].

Table 1: Oyster yield with varying substrates, supplements, pore sizes, temperature, reused substrate and flushes.

Experimentation	BE%	Experimentation		BE%
Substrates		Effect of temperature		
Wheat straw	68.16	Temperature	Primordia days	BE%
Sinar straw	66.02	3 to 19^oC (ambient)	46 days	65.44
Barely	65.52	25^oC	28 days	71.59
Saw dust	66.31	Effect of reused substrate with different heat treatment		
Supplements		Reused substrates	Heat treatment	BE
Wheat bran	64.21	Used substrate	Pasteurization	21.54*
Oil seed cake (Niger seed)	71.56	Used substrate and supplements	Pasteurization	28.18*
Cow dung	12.32*	Used substrate	Sterilization	39.39
Pore size		Used substrate and supplements	Sterilization	54.02
Pin holes	62.31			
16.18mm^2	60.02			
28.16mm^2	28.79*			
Flushes				
First flushes	18.24			
Second flushes	24.67			
Third flushes	16.92			
Fourth flushes	10.22			

*Was found to be contaminated

Table 2: Effect of buffer on wheat straw substrate.

Buffer	Initial pH	pH after addition of buffer	Biological efficiency
Gypsum	5.8	6.87	59.23
Chalk	5.8	6.93	60.19
Pure Na_2CO_3	5.8	7.02	58.77

4. CONCLUSIONS

Relative humidity, aeration, temperature, and contaminations are most important factors during oyster cultivation in Debre Berhan using locally available substrates, materials and technologies. Even though oyster spawning grow best at 25^{o}C, it is possible to cultivate it at lower temperature. Drying the substrates greatly reduce the yield hence spraying it with water is mandatory. To activate 2nd, 3rd, and 4th flush, covering the substrates with plastic is very important to make the substrate moist. Gabi and paper wastes can be used as a substrate separately or in mixture with saw dust. Generally, oyster can be cultivated in Debre Berhan with locally available materials without using sophisticated lab equipment for spawn production.

5. ACKNOWLEDGMENT

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