

Growth and Yield Performance of Three Oyster Mushroom (Pleurotus Spp.) Species Using Fresh and Tissue Grain Spawns

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Abstract: *Oyster mushroom cultivation has great potential since it requires limited space, low initial investment and the raw materials used are cheap and easy to acquire. The objectives of the study was to compare the growth performance of three oyster mushroom species using fresh and tissue grain spawns; determine the yield of fresh and tissue grain spawns; determine the interaction effect of the three oyster mushroom species using fresh and tissue grain spawns and determine the cost and return analysis of three oyster mushroom species using fresh and tissue grain spawn. It was conducted at Research Center for Hillyland Development (RCHD), Lanise, Claveria, Misamis Oriental. It was laid-out in 2 x 3 Factorial in CRD with two kinds of different grain spawn as Factor A and three different oyster mushroom species as Factor B. Results revealed that Treatment 1 (combination of Tissue Grain Spawn and Dae Pung species) gave countless results compared to others with 6 days to pinhead formation, biggest diameter cap of 27.79mm, heaviest weight of each flush and three major flush with 15.02 and 38.32 grams respectively. The results also showed that this combination got the highest yield and net income of 287.63 grams and P1, 094.45 respectively.*

Keywords: Pleurotus ostreatus (Jacq:Fries) Kummer, Florida Pleurotus florida, Pleurotus sajor caju, Research Center for Hillyland Development, Yellow cracked corn grain spawn

1. Introduction

Oyster mushroom is an edible mushroom which was originated from Germany during the World War I. Pleurotus ostreatus is commonly known as Oyster Shelf, Tree Oyster, Tamogitake and Straw Mushroom, is a mushroom very similar to Pleurotus pulmonarius with some differences. Over 200 species of mushrooms have long been used as functional foods around the world (Kalac, 2013), but only about 35 species have been commercially cultivated (Aida et al., 2009; Xu et al., 2011)(Bellettini et al., 2015). The mushrooms of the genus Pleurotus rank second in the world mushroom market and is the most popular mushroom in China. The Pleurotus spp. of the class basidiomycetes belongs to a group known as “white rot fungi” (Tsujiyama and Ueno, 2013) as they produce a white mycelium and are generally cultivated on non-composted lignocellulosic substrates (Savoie et al., 2007) in which various kinds of Pleurotus are commercially cultivated and have considerable economic value, including P. ostreatus (oyster mushroom), P. eryngii (king oyster or Cardoncello), P. pulmonarius (phenix mushroom), P. djamor (pink oyster mushroom),

P. sajor-caju (indian oyster), *P. cystidiosus* (abalone oyster), *P. citrinopiea*-tus (golden oyster mushroom) and *P. cornucopiae* (Pe´ rez- Marti´ nez et al., 2015; Knop et al., 2015; Zhang et al., 2016). *Pleurotus* species require a short growth time, compared to other mushrooms. Its fruiting body is not often attacked by diseases and pests and it can be grown in a simple and cheap way, with high yield, wider substrate utilization, sporelessness, wide temperature and chemical tolerance, as well as environ- mental bioremediation.

Mushrooms are biota characterized by wonder. They rise up from lignocellulosic wastes, yet they become so bountiful and nourishing. Mushroom cultivation technology is friendly to the environment (Training Manual on Mushroom Cultivation Technology,2009).

The increasing population and the decreasing land size for crop cultivation throughout the world poses a serious problem to sufficient food production. With the prevailing poverty affecting rural areas in the Philippines, there is a need to develop technologies of oyster mushroom cultivation that may be used by less privileged to help themselves earn their livelihood.

Oyster mushroom cultivation has great potential since it requires limited space, low initial investment and the raw materials used are cheap and easy to acquire. The cultivation of *Pleurotus* mushrooms requires less elaborate technologies. The *Pleurotus* mushroom can adapt easily in rural and urban areas since it can utilize farm wastes (Khare et.al, 2006). Thus, its cultivation is the most affordable to small scale farmers in order to generate extra income.

Cultivation of the oyster mushroom, *Pleurotus* spp., has increased greatly throughout the world during the last few decades (Royse, 2002); in 1997 it accounted for 14.2 % of the total world edible mushroom production (Chang, 1999). Its popularity has been increasing due to its ease of cultivation, high yield potential and high nutritional value (Banik et. al., 2004).

For successful mushroom production it is necessary to ensure that production is economical and efficient so that higher yield can be obtained. Qualities of spawn play an important role in the successful production of any mushroom species (Pani, 2011). The quality spawn may be achieved by selection of a suitable spawn carrier which influences the growth habit of the mycelium and subsequent yield. As a consequence, escaping one of the procedure in making grain spawn could greatly affect its yield. Therefore, it is on this ground that these studies was conducted.

2. Literature Review

Growing Oyster Mushroom

Oyster mushrooms are one of the most popular edible mushrooms and belong to the genus *Pleurotus* and the family Pleurotaceae. Like oyster mushroom (*Pleurotus ostreatus*), many of *Pleurotus* mushrooms are primary decomposers of hardwood trees and are found worldwide. The type species of the genus *Pleurotus* (Fr.) Quel. is *P. ostreatus* (Jacq. et Fr.) Kummer. This mushroom has basidia with four basidiospores and a tetrapolar mating system. Its hyphae have clamp connections and most members of the genus, excepting a small minority, have a monomitic hyphal system. To date approximately 70 species of *Pleurotus* have been recorded and new species are discovered more or less frequently although some of these are considered identical with previously recognized species. Determination of a species is difficult because of the morphological

similarities and possible environmental effects. The materials are generally not composted previous to inoculation. The wood sawdust may be aged or the plant fibers hydrated for several days. It is important to select proper strains for the cultivation method of a particular grower's choice (Gharehagaji et al.2007).

Characteristics of Commercially Important (*Pleurotus* Mushrooms)

Pleurotus florida belongs to family Pleurotaceae and it is commonly called as Dhingri in India. This mushroom is an edible mushroom having excellent flavor and taste. Its productivity is maximum in a short time providing more protein per unit area than any other area. *Pleurotus florida* produces metabolites of medicinal and pharmacological interest, such antioxidant antimicrobials, immune stimulants and antitumor activities (Nayana and Janardhanan, 2000; Manpreet et al., 2004; Elmastas, 2007). Among the numerous species of mushroom, oyster mushrooms (*Pleurotus florida*) are more advantages in terms of easiness in cultivation, role in biodegradation and bio-remediation, production of extracellular enzymes and nutraceuticals (Rashad et al., 2009).

Oyster mushroom *Pleurotus sajor caju* (Fr.) Singer have great potential as an integral part of a sustainable agricultural system (Gupta & Sharma, 2014).

The mushroom grows wild in subtropical and tropical regions like India. It is known to be compatible with *P. sapidus* but they are different in appearance. With its optimal temperature range for fruitbody development relatively high, it is suitable for growing in subtropical and tropical areas (Kong, 2004).

Pleurotus ostreatus (Jacq.: Fr.) Kummer, a wood-destroying fungus, is widespread in the temperate zones and forms fruitbodies in relatively cool temperatures in comparison with other *Pleurotus* species. This is the most frequently cultivated species among the genus *Pleurotus*. One of the features of this species is it requires a low temperature treatment called “cold shock” to initiate primordia formation. Growing temperatures for the production of fruiting bodies is rather low at 10-2°C (Kong, 2004). *Pleurotus ostreatus* (Jacq: Fries) kummer is commercially important edible mushroom commonly known as the oyster mushroom. This fungus is industrially produced as human food and it accounts for nearly a quarter of the world mushroom production (Abdellah et al., 2000).

Spawn production

The mushroom “seed” (propagation material) is generally referred to as spawn. Availability of good quality spawn is the limiting factor for mushroom cultivation in many developing countries. Customs' bureaucracy, high shipping costs and the difficulty to keep the spawn cooled during transport, often hinders imports. It might therefore be necessary for the mushroom grower to produce his own spawn. The complete procedure of spawn production involves preparation of the medium, filling the test tubes or Petri dishes and sterilizing them, and the process of inoculating larger containers with this culture. Basically, spawn production is nothing more than putting mycelium of the desired mushroom in suitable sterilized substrates under aseptic conditions. In practice, however, producing spawn is not that simple. Suitable strains from the required mushroom species have to be maintained under strict conditions to avoid degeneration. If this is not possible tissue culture from a fresh and healthy mushroom should be used for spawn

production. In addition, the spawn production room has to be kept meticulously clean to avoid any contamination (Oei, 2005).

Spawn, i.e. seed required for growing mushroom, is the vegetative mycelium from a selected mushroom cultured on a convenient medium like wheat, pearl millet, sorghum grains, etc. In simple words spawn is grains covered with mushroom mycelium. It essentially involves preparation of pure culture of mushroom from tissue/spores, evaluation of selected cultures for yield, quality and other desirable traits, maintenance of selected cultures on suitable agar medium, followed by culturing on sterilized grains and further multiplication on grains (www.Mushroom-lit/05Mush_spawn_Prod.html).

Oyster Mushroom Health Benefits

Antioxidant Effects

Oyster mushrooms contain ergothioneine, a unique antioxidant exclusively produced by fungi, according to a 2010 study led by Penn State food scientist Joy Dubost. The study found that oyster mushrooms have significant antioxidant properties that protect cells in the body. A 3 oz. serving of oyster mushrooms contains 13 milligrams of ergothioneine, and cooking the mushrooms does not reduce this level (<https://www.healthbenefitstimes.com/oyster-mushroom/>).

Anti-Bacterial Effects

Oyster mushrooms have significant antibacterial activity, according to a 1997 study published in the "Journal of Agricultural and Food Chemistry." The study found that the active compound benzaldehyde reduces bacterial levels. It may form on the mushroom as a reaction to stress (<https://www.healthbenefitstimes.com/oyster-mushroom/>).

Nutritional Value

There are 42 calories in one cup of oyster mushrooms, making them a low-calorie addition to any meal. Oyster mushrooms are also high in nutrients. According to a study published in "Food Chemistry," oyster mushrooms contain significant levels of zinc, iron, potassium, calcium, phosphorus, vitamin C, folic acid, niacin, and vitamins B-1 and B-2. The study concluded that consuming oyster mushrooms as part of a healthy diet contributes to recommended nutritional requirements. Major Nutrients: Vitamin D (166.67%), Vitamin B3 (26.64%), Copper (23.33%), Vitamin B2 (23.08%), Vitamin B5 (22.26%) (<https://www.healthbenefitstimes.com/oyster-mushroom/>).

3. Methodology

Time and Place of the Study

This study was conducted at the Research Center for Hillyland Development (RCHD), Lanise, Claveria, Misamis Oriental for 4 months starting January 2018 to April 2018.

Media Culture Preparation for Tissue Spawn

A. Pure Culture Media Preparation for 1 Liter Using Potato Dextrose Agar

- 1) The 39 grams of PDA and 1 liter of purified water were prepared.
- 2) This was then be placed in a casserole and placed in a working stove.
- 3) It was then be stirred until it boiled.

- 4) The liquid PDA were poured with 30ml solution to each clean bottle.
- 5) The hole of bottle was covered with cotton and foil.
- 6) The bottles were sterilized for 15 minutes at 121°C and arranged in standing position.
- 7) It was then be rested for a while lying down until it was cooled down and had a form.

B. Inoculation

The mother culture making were done in the inoculation room with the following procedures:

- 1) The fruit was prepared on its vegetative stage and other materials needed.
- 2) Fruits were cleaned prior to inoculation
- 3) Soak method: Fruits were soaked into a 3% chlorine solution.
- 4) Alcohol method: Fruits was cleaned with cotton and 70% alcohol.
- 5) Stems and fruits were cut lengthwise.
- 6) The inner most part of the fruit was taken without touching the fruit with hands to avoid contamination.
- 7) The pure culture media were inoculated.

C. Grain Spawning

The pure culture media was observed five to seven days after inoculation to check if there were contamination.

D. Treatment Preparation

To determine the best spawn on the production of three oyster mushroom species, tissue grain spawn and fresh grain spawns were prepared to be used to raise mushroom crop. Treatments to be used are the three oyster mushroom species using Fresh Grain Spawn and Tissue Grain Spawn. Ten (10) bags per treatment were replicated three (3) times arranged in 2 x 3 Factorial Complete Randomized Design (CRD).

Factor A: (Different Grain Spawn)

A₁- Tissue Grain Spawn (F1)

A₂- Fresh Mushroom Grain spawn

Factor B: Three Oyster Mushroom Species

B₁- *Pleurotus ostreatus* (Jacq:Fries) Kummer (Dae pung)

B₂- *Pleurotus florida* (Florida)

B₃- *Pleurotus sajor caju* (Sambok) (Fr.) Singer

E. Inoculation of Substrate and Incubation

The substrates were inoculated based on the treatment combination. After inoculation, incubation was done for 30 days or until it was fully white.

Table 1: Treatments and Treatments Combinations

TYPES OF GRAIN SPAWN (Factor A)	THREE OYSTER SPECIES (Factor B)	TREATMENT COMBINATIONS/ CODES	TREATMENTS
A ₁ - Tissue Grain Spawn Farmers Practice	B1- <i>Pleurotus ostreatus</i> (Jacq:Fries) Kummer (Dae pung)	A ₁ B ₁	T ₁
	B2- <i>Pleurotus florida</i> (Florida)	A ₁ B ₂	T ₂
	B3- <i>Pleurotus sajor caju</i> (Sambok) (Fr.) Singer	A ₁ B ₃	T ₃
A ₂ - Fresh Mushroom Grain spawn	B1- <i>Pleurotus ostreatus</i> (Jacq:Fries) Kummer (Dae pung)	A ₂ B ₁	T ₄
	B2- <i>Pleurotus florida</i> (Florida)	A ₂ B ₂	T ₅
	B3- <i>Pleurotus sajor caju</i> (Sambok) (Fr.) Singer	A ₂ B ₃	T ₆

T ₆ R ₂	T ₁ R ₂	T ₃ R ₂
T ₂ R ₃	T ₄ R ₃	T ₄ R ₂
T ₃ R ₃	T ₅ R ₁	T ₂ R ₂
T ₂ R ₁	T ₄ R ₁	T ₁ R ₃
T ₅ R ₂	T ₃ R ₁	T ₅ R ₃
T ₆ R ₁	T ₆ R ₃	T ₁ R ₁

Appendix Figure 1: Experimental Lay-out

Data Gathered

- Number of days to pinhead formation-** Each treatment was determined by counting the days wherein pinheads formed
- Average Diameter of Mushroom cap (cm)**
Five samples of mushroom fruits in each treatment per bag were measured using a vernier caliper to get the diameter of the mushroom cap and were recorded.
- Total number of fruit bodies:** Total numbers of fruit bodies of each treatment were counted and calculated in each treatment per bag.
- Yield of mushroom (g):** Total yield of mushroom in grams were calculated for each treatment
 - Weight of each flushes. This was obtained by counting the number of flushes harvested per bag.
 - Average weight in three major flushes. This was determined by weighing the 3 major flushed and obtained their over-all average.
 - Yield. This was obtained by weighing the harvested mushroom per bag in grams.
- Cost and Return Analysis-** The cost and return analysis were computed based on the actual records of the cost and the gross sales on the prevailing price of the mushroom in the market.

Statistical Analysis

Data were consolidated and analyzed using the analysis of variance in 3 x 2 Factorial Arrangement in Completely Randomized Design (CRD). Observed significant differences among treatment means were compared using Tukey test.

4. Conclusion

This study was conducted at Research Center for Hillyland Development (RCHD) Lanise, Claveria Misamis Oriental from CY January- April 2018. This study was laid- out in 2 x 3 Factorial in Complete Randomized Design (CRD) with 2 Different Grain Spawn as Factor A and Three Oyster Mushroom Specie as Factor B, with 10 fruiting bags per treatment.

The different objectives of this study were: (1) compare the growth performance of three oyster mushroom species using Fresh Grain Spawn and Tissue Grain Spawn; (2) determine the yield of Fresh Grain Spawn and Tissue Grain Spawn for mushroom production on three oyster mushroom species; (3) determine the interaction effect of yield of the three oyster mushroom species using Fresh Grain Spawn and Tissue Grain Spawn. and (4) to determine the cost and return analysis of three oyster mushroom species using Fresh Grain Spawn and Tissue Grain Spawn.

Results revealed that Tissue Grain Spawn and Dae Pung Specie alone gave significant production in terms of diameter of the cap, weight of each flush, weight of three major flushes, yield and cost and return analysis. The earliest days to pinhead formation was found in the combination of Tissue Grain Spawn and Dae Pung Specie with 36 number of days and the latest to pinhead formation was found in Fresh Grain Spawn and Sambok Specie with 48 number of days. Tissue Grain Spawn and Dae Pung Specie consistently gave the highest results in terms of weight of each flush, weight of three major flushes, yield and cost and return analysis with 15.02 grams, 38.32 grams, 287.63 grams, P 1, 584.50 net income and ROI of 58.04%, respectively. Fresh Grain Spawn and Sambok Specie constantly gave the lowest yield of weight of each flush, weight of three major flush and average yield with 11.65 grams, 30.27 grams and 242.77 grams, correspondingly.

Basically, spawn production is nothing more than putting mycelium of the desired mushroom in suitable sterilized substrates under aseptic conditions. *Pleurotus sajor caju* (Sambok) its optimal temperature range for fruitbody development relatively high, it is suitable for growing in subtropical and tropical areas (Kong, 2004). *Pleurotus ostreatus* (Jacq.: Fr.) Kummer, a wood-destroying fungus, is widespread in the temperate zones and forms fruitbodies in relatively cool temperatures in comparison with other *Pleurotus* species (Kong, 2004). *Pleurotus florida*, oyster mushroom variety is a thermophilic species (loves heat) suitable to be cultivated in warm and moist weather.

On the other hand, there is no significant effect on the interaction between Different Grain Spawn and Oyster Mushroom Specie in terms of all the data gathered. It is clearly evident from the results that fresh grain spawn and tissue grains spawn are suitable for spawn and mushroom production purposes. Hence, it is concluded that farmers who wants to venture mushroom production could use any of the mushroom specie either with the use of Fresh Grain Spawn and Tissue Grain Spawn depending on the availability of the Spawn and Mushroom Specie.

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