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## Multiplication of Oyster Mushroom Mother Spawn (*Pleurotus Ostreatus* L.) Using Different Boiling Periods of Sorghum

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### ABSTRACT

The study was focused on the multiplication of oyster mushroom mother spawn using different boiling periods of sorghum. It was conducted at the Mushroom Laboratory of the Department of Plant Science – College of Agriculture, Mindanao State University Main Campus, Marawi City, from March 31, 2022, to April 21, 2022. The length of mycelia of each treatment is measured every five (5) days for the expansion of mycelia inside the bottles of sorghum. The ANOVA was the statistical tool that was used in analyzing the data. The results revealed that (1) there is a significant difference in the number of days from inoculation to colonization of substrate in every treatment; (2) Treatment 1 has the slowest mycelial growth in the bottle; (3) Treatment 2 has the fastest mycelial growth in the bottle; (4) there is a level of 10% significant difference in the length of mycelial growth in every treatment on the third data gathering; (5) Treatment 1 (fifteen-minute boiling period) has the slowest colonization of the sorghum as mother spawn substrate in the bottle, (6) Treatment 2 (thirty-minute boiling period) has the fastest colonization of the sorghum as mother spawn substrate in the bottle, and (7) Treatment 2 (thirty-minute boiling period) has the best boiling period of the substrate for mother spawn multiplication.

### INTRODUCTION

Mushrooms are an attractive crop to cultivate in developing countries for many reasons. One of the most appealing aspects of mushroom cultivation is that it is the only food on the market that is pesticide-free. Mushrooms are high in protein but have no bad cholesterol. It improves the blood circulation of our bodies, and they are grown on agricultural wastes (Heleno *et al.*, 2010).

Nowadays, farmers are looking for other methods to earn extra income. The method of recycling farm waste materials is used in mushroom cultivation, and it provides extra income to farmers since it enables them to acquire substrates or materials at low prices or even for free. It is an efficient means for the conversion of agricultural wastes into valuable protein and has a huge potential for generating additional income and employment.

The first thing to be prepared in mushroom cultivation is the mushroom mother spawn. One of the most important aspects of mushroom cultivation is represented by sterilization. It is recommended to sterilize the substrates before spawning to eliminate or kill different pathogens (bacteria, molds, or pests) in the substrate where the mushrooms will grow, develop, and produce a yield. Prior to sterilization, the length of time to boil sorghum seeds will be done in preparation of the substrate, the material on which the mycelium of the mushroom grows.

The substrate for the multiplication of mother spawn is sorghum seeds; it is the best recommended substrate among other substrates like barley seeds, corn cracks, and other substrates to be inoculated into the production bags for mushroom production. The length of time to boil the sorghum seeds is very important for the expansion of mycelia and the colonization of the boiled sorghum

seeds inside the bottle. Oyster mushrooms are relatively easy to cultivate and are one of the most profitable agribusinesses that requires low investment and less space. That is why farmers' interest in oyster mushrooms has rapidly increased in the last few years due to its special taste, medicinal, and nutritional value (Kumla *et al.*, 2020). Although their desire to grow oyster mushrooms for extra income has increased, most of them still do not know the proper process of producing the oyster mushroom's mother spawn as the planting material in mushroom production (Chang & Wasser, 2017).

Compared to other edible mushrooms, *Pleurotus ostreatus* has the advantage of being able to rapidly colonize a wide range of substrates, converting a high proportion of them to fruiting bodies and hence increasing profitability (Sanchez, 2010). *Pleurotus ostreatus*, or commonly known as Oyster mushroom or as Oyster fungus and hiratake, is an edible type of mushroom that is primarily consumed for its nutritive value. It is one of the most sought wild mushrooms and is now grown commercially around the world for nourishment and consumption. Apart from its food value, *Pleurotus ostreatus* or Oyster mushrooms are rich sources of protein, minerals (calcium, phosphorus, iron), and vitamins (thiamine, pyridoxine, biotin, riboflavin, and niacin).

Its medicinal value for diabetics and in cancer therapy has also been emphasized. Most of all, Oyster mushrooms can utilize various kinds of agricultural waste materials than any other types of mushrooms. Sorghum seeds which have low economic value, may be used instead of expensive products like wheat bran as suitable supplement for the cultivation of Oyster mushrooms (Gréta *et al.*, 2022).

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According to Yang *et al.* (2013), Fan *et al.* (2011), Li *et al.* (2011), based on their study on the performance of oyster mushrooms (*Pleurotus ostreatus* L.) using different ratios of rice straw and sawdust as a substrate, with five treatments replicated three times, which revealed the results of T1 (100%) rice straw, grits, T2 (3:1) rice straw and sawdust, T3 (3:1) rice straw and sawdust, T4 (1:1) rice straw and sawdust, and T5 (100%) sawdust. The experimental design was laid out in a Complete Randomized Design (CRD) and the results on the length of mycelia every fifteen (15) days, the gathering of data from ten (10) data bags per replication from 15-90 days, showed that it has high significance in all its treatments. The yield at forty-five (45) days of fruiting revealed that the first and second flushing were not significant in each treatment, but the third, fourth, and fifth flushing were highly significant at the 5% level. The return of investment showed that T1 has the highest return of investment with 239.70% and the lowest return of investment is with -82.94% in 30 bags per treatment and 10 data bags per replication. Portillo *et al.* (2018) and Longman *et al.* (2010) attempted to demonstrate that, in addition to sorghum seeds, which are an accepted spawn substrate for *P. ostreatus*, other grains suitable for the production of spawns, such as job's tears, corn, and rice, were tested.

The study used a completely randomized design (CRD) with three (3) replicates. The parameters evaluated were the number of days since inoculation, mycelial length, diameter, density, and return on investment. The rice and sorghum had the fastest mycelial growth at 14 days, with a slight difference to the corn and job's tears at 15 days. These did not differ significantly. During the incubation period, the diameter and mycelial length of each grain increased steadily but did not differ significantly.

Most of the grains were uniformly white in terms of mycelial density, and sorghum and corn had the densest mycelia of any treatment, while job's tears and rice were denser. The return on investment for most grains was similar, but the job's tears provided the highest value, followed by rice. As a result, this study was carried out to determine the mycelial growth of oyster mushrooms in sorghum seeds as substrate with varying boiling times. The researchers specifically aimed to (1) determine the number of days from inoculation to colonization of the oyster mushroom mother spawn based on the research questions listed below. (2) to determine which of the three treatments performed the best. (3) to advise mushroom growers on the best outcome. Most farmers are still attempting to determine how long the boiling period of sorghum seeds should be in order to maximize the productive multiplication of the mother spawn. As a result of this scenario, the researchers wanted to determine the optimal boiling period for oyster mushroom mother spawn multiplication.

## MATERIALS AND METHODS

### Location and Duration of the Study

The study was conducted at the Mushroom Laboratory

of the Department of Plant Science – College of Agriculture, Mindanao State University Main Campus, Marawi City, from March 31, 2022, to April 21, 2022.

### Experimental Design and Treatments

This study used Oyster mushroom's mother spawn as explants, empty bottles that served as containers of the growing media, pressure cooker and gas stove for sterilization, rice cooker for boiling of sorghum, basin for washing of sorghum, strainer for draining the excess water of cooked sorghum, denatured alcohol and alcohol lamp for sterilization of stirrer, stirrer for loosening of the mother spawn, cotton for covering the opening of the bottle, scratch paper for covering the opening of the bottled sorghum, and rubber band for tying the paper around the bottle. There were three treatments used in this study. In the first treatment, sorghum seeds were boiled for fifteen minutes in the rice cooker. In the second treatment, sorghum seeds were boiled for thirty minutes in the rice cooker, and lastly, in the third treatment, sorghum seeds were boiled for forty-five minutes in the rice cooker.

### Procedures

In preparation of sorghum seeds, the seeds were washed thoroughly using water and were rinsed three times to ensure dust and other foreign materials were removed. The sorghum seeds were boiled in the rice cooker following the procedures of three different treatments. The first treatment was boiled for fifteen minutes in the rice cooker. After the first treatment, it was followed by the second treatment with thirty minutes boiling period, and the last treatment was forty-five minutes boiling period. Afterwards, the sorghum seeds were drained and placed to the clean empty bottles. The bottles were then filled with cotton and covered with scratch paper. Following these steps, the bottled sorghum was secured with rubber bands.

The bottles containing sorghum seeds were then sterilized in a pressure cooker and immediately cooled. For 12 to 24 hours, the bottled substrates were turned upside down to allow excess water to drain into the cotton. Finally, after drying the bottled substrates with a sterilized inoculating loop, each bottle was aseptically inoculated with *Pleurotus ostreatus* spawn obtained from the Mushroom Laboratory of Mindanao State University's Main Campus.

Thereafter, the sterilized bottles of sorghum seeds were incubated without sunlight in the incubation room. The data gathered from inoculation to colonization of sterilized sorghum seeds inside the bottles were the number of days to colonization of mycelia and the length of mycelial growth inside the boiled and sterilized bottles of sorghum. The number of days to colonization of mycelia inside the bottles was done by counting the number of days from incubation to colonization of the substrate inside the bottles, while the length of mycelial growth was done in every treatment by measuring the expansion and length of mycelia inside the bottles of

sorghum every five (5) day interval until the bottles of sorghum were colonized by the mycelia in eighteen to twenty-one days.

**Length of Mycelial Growth (cm)**

The length of the mycelial growth of oyster mushroom mother spawn as affected by the three treatments of different boiling periods as shown in Table 1 above shows the result of mycelial growth of oyster mushroom

in three different treatments in every five-day interval within the first fifteen (15) days of observation (Column 1 to 3) and the daily observation after the fifteen days that lasted for four (4) days (Column 4 to 7) for a total of nineteen (19) days of colonization. The results were not significantly different from each other. The mean length of mycelial growth in five days after inoculating the bottles of spawns: Treatment 3, which had a boiling period of forty-five minutes, had the highest length of

**Table 1:** Growth length of mycelia in different boiling periods with 5 days interval

Treatments	5 Days	10 Days	15 Days	16 Days	17 Days	18 Days	19 Days
T <sub>1</sub> (15mins)	2.6	6.0	10.9	11.3	11.4	11.47	11.5
T <sub>2</sub> (30mins)	2.6	6.4	11.4	11.5	11.5	11.5	11.5
T <sub>3</sub> (45mins)	2.8	6.3	11.3	11.4	11.5	11.5	11.5

mycelia with a mean of 2.8 cm, followed by Treatments 1 and 2, which had the boiling periods of fifteen minutes and thirty minutes, with the same mean length of mycelia at 2.6 cm (Column 1).

In the mean length of mycelial growth at five (5) days after inoculating the bottles of the oyster mushroom mother spawns, Treatment 3 (forty-five minute boiling period) had the highest length of mycelia with a mean of 2.8 cm, followed by Treatments 1 (fifteen minute boiling period) and Treatment 2 (thirty minute boiling period), with the same mean length of mycelia at 2.6 cm At ten (10) days after inoculation, Treatment 2 (thirty minute boiling period) had the highest length of mycelia with a mean of 6.4 cm, followed by Treatment 3 (forty-five minute boiling period) with a mean length of 6.3 cm, and Treatment 1 (fifteen minute boiling period) with the lowest mean length of 6.0 cm. At fifteen (15) days after inoculation, Treatment 2 (thirty minute boiling period) had the highest length of mycelia with a mean of 11.4 cm, followed by Treatment 3 (forty-five minute boiling period) with a mean length of 11.3 cm, and Treatment 1 (fifteen minute boiling period) with the lowest mean length of 10.9 cm. Treatment 1, with a boiling period of fifteen (15) minutes, had the slowest colonization period as it took nineteen (19) days for mycelia to colonize the whole bottle.

Girmay *et al.* (2016) and Dissasa (2022) revealed that in spawn production, there are strict standards that are to be met, which are the spawn’s biological purity and vigor. It was observed that all Treatment 1 had thin and weak mycelia, which lacks vigor, while both Treatment 2 and Treatment 3 had thick and vigorous mycelia, indicating

that Treatment 2 and 3 have met the strict standard of Linaac in spawn production, leading to more productive and long-lasting spawn. Mycelial growth was slowest in Treatment 1, and both Treatments 2 and 3 had fast mycelial growth. T<sub>1</sub>R<sub>1</sub> was first noticed to be contaminated on the 14th day after the inoculation of mother spawn in the bottles of sorghum seeds. The contamination is caused by the remaining microorganisms that were not killed during the sterilization period.

These microorganisms are what we call thermophilic bacteria or thermophile. According to Mehta *et al.* (2016) thermophiles live in hot environment which explains why some of these microorganisms were not extinguished during the sterilization process. DEFRA (2011) revealed that there are several fungi that are not easily killed during sterilization. The contaminants appear, usually in black or green, during incubation. The treatment, which appeared to be contaminated, was noticed to have green mold.

In Treatment 3, only replications 2 and 3 had shrunk, because the sorghum seeds were overcooked. Furthermore, Albarracín (2019), grains or seeds should not be overcooked or too soft. The overcooked, burst or overly wet grains are more prone to contamination since their outer protective layers are broken. The shrunken state of the mycelia is an indication that the sorghum seeds were overcooked. Overcooked sorghum seeds as substrates for Oyster mushroom mother spawn are fast and easy to rot and expire (Jongman *et al.*, 2013).

For the number of days from inoculation to complete colonization of the bottled substrate, Treatment 2 had the fastest colonization of mycelial growth in 16.3 days where the sorghum seeds had a boiling period of thirty

**Table 2:** Number of days to colonize the sorghum seeds by the mycelia in different boiling periods.

Treatments	Replications			Total	Mean Total
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>		
T <sub>1</sub> (15mins)	19	18	17	54	18
T <sub>2</sub> (30mins)	16	17	16	49	16.3
T <sub>3</sub> (45mins)	17	16	18	51	17
Grand Total				154	
Grand Mean					17.1

minutes. This result showed a weak significant result on the number of days of mycelial growth in the ANOVA approach (Appendix Table 4.0). It is then followed by Treatment 3 with the slight difference of 17 days where sorghum seeds had a boiling period of forty-five minutes, and lastly, Treatment 1 with an 18-day boiling period where sorghum seeds had a boiling period of fifteen minutes.

### Number of Days to Colonize

Table 2 above shows the number of days of complete colonization of mycelia on all replications of the three different treatments and their mean total. Udayasimha *et al.* (2012) and Kamthan & Tiwari (2017) assert that the fully colonized grain (spawn) is used as a source of seed for the prepared substrates for the agricultural wastes for mushroom cultivation. The results were not significantly different from each other. T<sub>2</sub>R<sub>1</sub>, T<sub>2</sub>R<sub>3</sub>, and T<sub>3</sub>R<sub>2</sub> took sixteen (16) days for the mycelia to colonize the bottles of sorghum seeds. Followed by T<sub>1</sub>R<sub>3</sub>, T<sub>2</sub>R<sub>2</sub>, and T<sub>3</sub>R<sub>1</sub> that took seventeen (17) days for the bottles of sorghum seeds to be colonized, T<sub>1</sub>R<sub>2</sub> and T<sub>3</sub>R<sub>3</sub> for eighteen (18) days; and lastly, T<sub>1</sub>R<sub>1</sub> took the longest for the mycelia to colonize its bottle for nineteen (19) days.

During the days of observation, the researchers observed that all replications of Treatment 1 with a boiling period of fifteen (15) minutes showed the slowest growth of mycelia in the bottles of sorghum seeds. It took a mean total of 18 days for mycelia to colonize the bottles of Treatment 1. Most of the spawns have shown little difference in the mycelial growth.

The fastest mycelial growth was Treatment 2 with 16.3 days to colonize where the boiling period of sorghum seeds was 30 minutes, followed by Treatment 3 with a slight difference of 17 days to colonize where the boiling period of sorghum seeds was 45 minutes, and the last was Treatment 1, with 18 days to colonize, where sorghum seeds were boiled for 15 minutes. The analysis of variance showed a weak significant result on the number of days of mycelial growth. The number of days from inoculation to colonization is related to its mycelial growth rate. Hoa and Wang (2015) and Sánchez (2010) ascertain that fast growth rates resulted in a corresponding reduction in the days required to complete colonization of the grains by the mycelia. The days of total grain colonization varied across treatments, as shown in the table above.

### CONCLUSIONS

According to the findings, (1) Treatment 1 (a fifteen-minute boiling period) had the slowest mycelial growth in the bottle of sorghum seeds, and (2) Treatment 2 (a thirty-minute boiling period) had the fastest mycelial growth. (2) The length of mycelial growth that was measured from every five (5) days to daily data gathering for the expansion of mycelia inside the bottles of sorghum has been shown to have non-significant results. (3) Treatment 1 (fifteen-minute boiling period) had the slowest colonization of the sorghum as a substrate of mother

spawn in the bottle, while Treatment 2 (thirty-minute boiling period) had the fastest. Four (4) Treatment 2 (thirty-minute boiling period) is the best substrate boiling period for mother spawn multiplication. This study's findings will benefit and provide the best ideas for oyster mushroom mother spawn multiplication for mushroom cultivators, agriculturists, farmers, and future researchers. The collected data from this study gives awareness of the exact time to boil the sorghum seeds in preparation for the substrate to be used in the process of multiplying the oyster mushroom mother spawn. It will also serve as a guide for further knowledge and references, and it will also significantly contribute to significant inputs for mother spawn multiplication management and the development of mushroom production in Lanao del Sur.

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