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# Production of white oyster mushroom spawn using different grain-based substrates

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## Abstract

Spawn production is an important step in mushroom cultivation. This study investigates the use of five different sterilized grain-based substrates (rice, barley, wheat, maize and bird seed) for producing *Pleurotus ostreatus* spawn. The aim is to determine which grain-based substrate is most favorable for spawn production. Favourability decision was made after comparing experimental results for mean diameter size of the mycelium spread, time taken for the substrate to be completely colonized and the difference between fresh and dry weights of the spawn. Although all of the tested substrates supported mycelium growth and spawn production, the substrate made with corn grains performed significantly better in terms of the three identified parameters. The larger grain size of corn and probably its higher nutritional content, was most likely responsible for stimulating greater mycelium growth, which was reflected in the greater diameter of the colony, less time taken to colonise the substrate and greater dry weight of the spawn.

**Keywords:** Mushroom; Spawn; Grains; Substrate; Mycelium growth

## 1. Introduction

Under natural conditions mushrooms, saprophytic decomposers, grow on dead organic matter [2, 12] and digest the lignocellulosic content of their growth medium before absorbing the digested substances [2, 12, 23]. Mushrooms are successfully grown under controlled and semi-controlled conditions [2, 12] and the choice of substrate directly influences the growth, yield, and quality of the mushrooms produced [2, 9]. Among the various substrates used for growing mushrooms, grains are commonly used for spawning [4, 7, 23, 32] mainly because of their nutritional composition and ease of accessibility.

When cultivating edible mushrooms, three key factors are needed; reliable spawn, good substrate and a conducive environment [2, 19, 29]. While much research has already been reported on suitable substrates for spawn production and the cultivation of edible mushrooms [13, 19,28], choosing a substrate is often based on the availability of a particular substrate [5]. Among the desired characteristics of a good substrate for the cultivation of edible mushrooms is that the substrate must be sterile and rich in essential nutrients [2, 16, 30].

Spawn preparation is one of the important steps when cultivating mushrooms [20] and inoculation of mycelium into an agar medium, followed by propagation in a grain-based substrate are important follow-on steps [3]. Producing mushroom spawn requires that a sterile, grain-based medium be prepared and inoculated with mushroom mycelium [22] and the inoculated grain-based medium is then used to introduce the fungus to a larger substrate for mushroom cultivation [25, 26]. It is important that attention is placed on producing spawn of high quality that in not contaminated because the quality of the spawn can ultimately affect the yield and quality of the mushroom [1, 11, 33].

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Hoa & Wang (2015) [10] suggested that the main factors influencing the production of spawn, include culture media, temperature, carbon and nitrogen sources, grain sources, and lignocellulosic substrate sources. In this study, spawn production was evaluated on five sterile grain-based substrates (rice, barley, wheat, maize and bird seed) with the main objective being to determine which one will produce better yields of spawn of *Pleurotus ostreatus* in a controlled environment.

The questions that guided this research are:

- How long will it take for the mushroom spawn to completely colonize each substrate?
- Does the diameter or size of colonies of white oyster spawn differ in each substrate?
- Will the fresh and dry weight differ at the end of the maturation of the spawn run?

## 2. Methodology

### 2.1. Study location

This research was conducted at the University of Guyana, John's Science Campus, Berbice and all laboratory work were assessed within the confines of the laboratory under aseptic conditions that were suitable for mushroom inoculation and growth.

### 2.2. Substrate preference and preparation

In this study, rice, barley, wheat, corn and bird seed were used as the primary grain substrates for *Pleurotus ostreatus* and the preparation of the spawn was done according to a method described by Lalithakumari (2006) [17], and with modification for the number of hours the grains were soaked (24hrs). All other procedures for mother culture preparation on potato dextrose agar (PDA), and substrate preparation were followed according to Lalithakumari & Subramanian (2004) [18] for the cultivation of oyster mushroom in Guyana. The same species of white oyster mushroom (*Pleurotus ostreatus)* was used as the control variable and the manipulative variables were the different grain-based substrates made from wheat, barley, maize, rice and bird seed.

### 2.3. Parameters of interest

The parameters analysed were diameter (mm) of colony extensions in four (4) stages at day 4, 8,12 & day 16 respectively, time taken for complete colonization of spawn run in each substrate and fresh and dry weight (after complete maturation of spawn run after 16 days.

### 2.4. Statistical analyses

Results were analysed using the SPSS version 26 and the data were subjected to one-way ANOVA and descriptive statistics analysis. Interpretation of the results was based on comparison of the mean diameter size for the different substrates, comparison of the time taken for the substrate to be completely colonized and comparison of the difference between fresh and dry weights of the spawn.

## 3. Result and Discussion

While spawn production was successful with all five grain-based substrates, the corn grain infused substrate produced the best results for all three parameters that were investigated and bird seed infused substrate was the least performing substrate in terms of time taken to fully colonize the substrate, the diameter of colony and the dry weight (Table 1).

**Table 1** Summary of data collected on the investigated parameters

| Treatments | Number of trials | Time taken to colonise substrate (days) | Weight (g) | | Diameter of colony (mm) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Fresh | Dry | Day 4 | Day 8 | Day 12 | Day 14 |
| Corn | T 1 | 15 | 200 | 125 | 12 | 49 | 116 | 186 |
| | T 2 | 15 | 200 | 123 | 19 | 74 | 136 | 200 |
| | T 3 | 15 | 200 | 124 | 19 | 68 | 121 | 190 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Average | | 15.00 | 200.00 | 124.00 | 16.67 | 63.67 | 124.33 | 192.00 |
| Barley | T 1 | 16 | 200 | 112 | 15 | 44 | 100 | 154 |
| | T 2 | 15 | 200 | 111 | 17 | 54 | 109 | 172 |
| | T 3 | 16 | 200 | 114 | 15 | 41 | 97 | 161 |
| Average | | 15.67 | 200.00 | 112.33 | 15.67 | 46.33 | 102.00 | 162.33 |
| Rice | T 1 | 17 | 200 | 110 | 17 | 48 | 85 | 159 |
| | T 2 | 16 | 200 | 108 | 16 | 39 | 99 | 162 |
| | T 3 | 16 | 200 | 107 | 17 | 49 | 113 | 154 |
| Average | | 16.33 | 200.00 | 108.33 | 16.67 | 45.33 | 99.00 | 158.33 |
| Wheat | T 1 | 16 | 200 | 119 | 15 | 49 | 109 | 164 |
| | T 2 | 15 | 200 | 120 | 16 | 50 | 115 | 177 |
| | T 3 | 15 | 200 | 118 | 15 | 46 | 109 | 160 |
| Average | | 15.33 | 200.00 | 119.00 | 15.33 | 48.33 | 111.00 | 167.00 |
| Bird Seed | T 1 | 17 | 200 | 105 | 14 | 43 | 80 | 149 |
| | T 2 | 17 | 200 | 110 | 19 | 50 | 103 | 152 |
| | T 3 | 16 | 200 | 102 | 15 | 45 | 99 | 160 |
| Average | | 16.50 | 200.00 | 103.50 | 14.50 | 44.00 | 89.50 | 154.50 |

### 3.1. Mean size in diameter of mycelial growth on different test substrates

There was significant variation in the diameter of mycelium growth, depending on the substrate used and the time allowed for growth. The growth rate of the spawn run in this experiment was determined by measuring in millimetres (mm), the diameter of the extension of mycelium growth of the colony on the substrate. Based on the results, the substrate made using corn grains had the best colonisation by mycelium followed by the substrate made from grains of wheat, barley, rice and bird seed in that order (Figure 1).

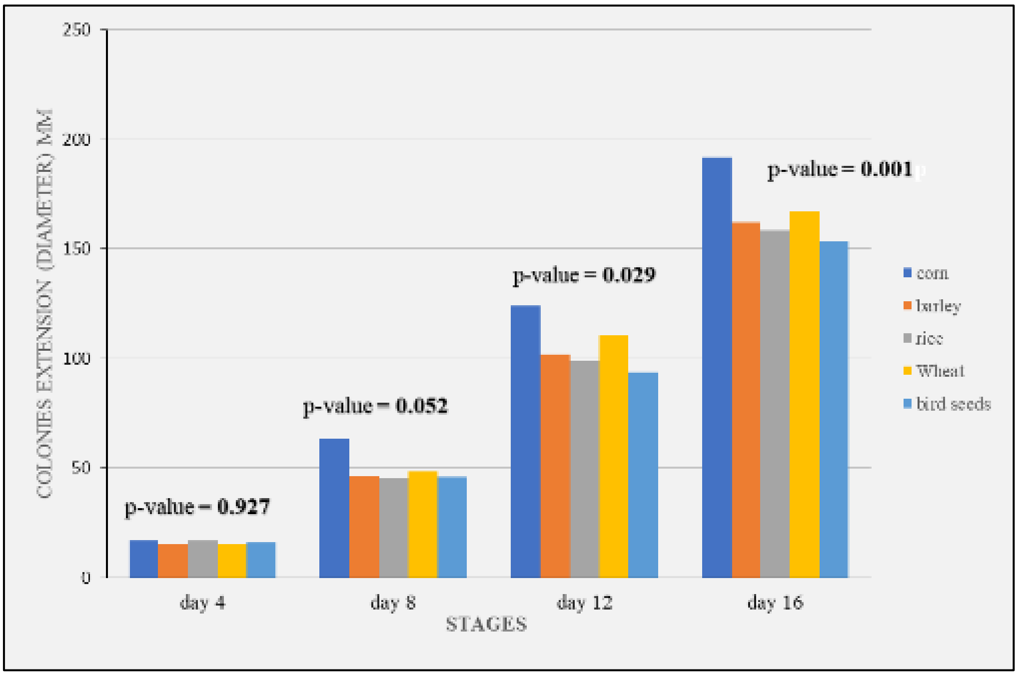


**Figure 1** Mean size in diameter of mycelium growth after 4, 8, 12 and 16 days for different substrates

On the different grain infused agar media, the average diameter of mycelium growth ranged from 14.5 mm to 16.67 mm after 4 days, 44 mm to 63.67 mm after 8 days, 89.5 mm to 124.33 mm after 12 days, and 154.5 mm to 192 mm after 16 days (Table 1), with the highest growth observed on corn substrate, and the lowest on birdseed infused substrate.

There were significant differences observed for the diameter of mycelium growth after day 8 ($p = 0.052$), 12 days ($p = 0.029$) and 16 days ($p = 0.001$), while there was no significant difference in diameter for mycelium growth when the different substrates were compared at day 4 ($p = 0.927$).

These differences in the diameter of mycelium growth were likely associated with the size of the grain used to prepare the grain-infused substrate (Elhami & Ansari (2008) [7], since larger sized grains were reported to have more nutrients available for mycelium growth [34].

Tinoco *et al*. (2000) [34] also suggested that substrates with a larger surface area and pore may support a higher mycelium growth rate and Elhami & Ansari (2008) [7] also noted that larger seeds may contain more nutrients to support mycelium growth. This may have accounted for the greater colony extension and the variance seen in the width of colony extension of spawn grown on different substrates [7].

Narh *et al*. (2011) [21] reported that large grained substrates have larger air spaces which may increase ventilation, thus improving aerobic respiration for the mycelium, since respiration is directly related to the oxygen concentration of the substrate. This too might have accounted for the higher growth rate in the spawn and faster rate of complete colonization observed for the corn grain-infused substrate treatment when compared to the other substrates.

### 3.2. Comparison of the time taken for complete colonization of substrate

Generally, factors such as temperature, humidity, pH, substrate composition, presence of specific nutrients, light, inoculum size and air quality have been reported to influence the rate of mycelium growth or spawn run, the time taken mycelium to colonize a substrate [24, 34]. The spawn run can therefore be taken as being indicative of the health and vigor of the spawn and substrate [7, 10, 34].

Grains provide a nutrient-rich substrate for mycelium growth [27]. The results (Figure 2) show that the corn-infused substrate was the first to be fully colonised in the least number of days. This was followed by barley, rice and bird seeds in that order. This may likely be attributed to the fact that the grains of corn were the largest and according to Tinoco *et al.* (2000) [34] large grains should have a higher availability of nutrients to facilitate mycelium growth [27]. Bird seed-infused substrate, being the smallest of the grains used in this research, took the longest time to be fully colonized.

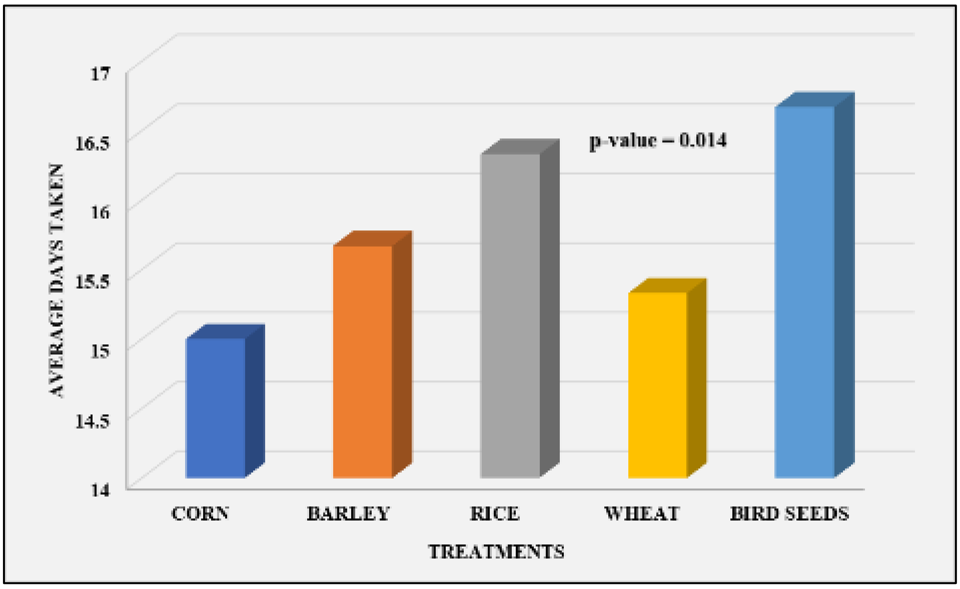


**Figure 2** Time taken for grain-infused substrates to be fully colonised

While this current research did not investigate environmental factors that could have affected the rate of mycelial growth, this could be an area for further investigation in Guyana, because different local environmental conditions may have different influences on the outcome of the experiment.

### 3.3. Comparison of fresh and dry weights of spawn

Mycelium growth is indicative of successful spawn production [6, 31] and spawn production is the very important first step for cultivating mushrooms [31].

Measuring the dry weight of the mycelium is a good indicator to evaluate mycelium growth and spawn viability when comparing the effectiveness of substrates and growth conditions during spawn production. The dry weight reflects the amount of fungal biomass produced and is also a good measure of how well the substrate is utilised by the mycelium [6, 31].

Substrate composition, spawn type and environmental conditions influence the dry weight of mycelium [8, 14, 15] and some substrates tend to support faster mycelium growth and higher biomass production, resulting in different substrates yielding different dry weights of mycelium [8].

In this study, mycelium growth was observed on all of the grain infused substrates. There was a significant difference (p-value = 0.00) between the fresh weight and the dry weight of the spawn obtained from the different test substrates. The results (Figure 3) shows that starting with a similar fresh weight (200 gm) for all substrates, spawn from corn infused substrate had the highest dry weight (124.00g), followed by wheat (119.00g), barley (112.33g), rice (108.33g) and the least dry weight was measured for bird seed (105.67g). This suggests that of the substrates used, the corn infused substrate was the most successful in terms of supporting spawn production for *Pleurotus ostreatus*.

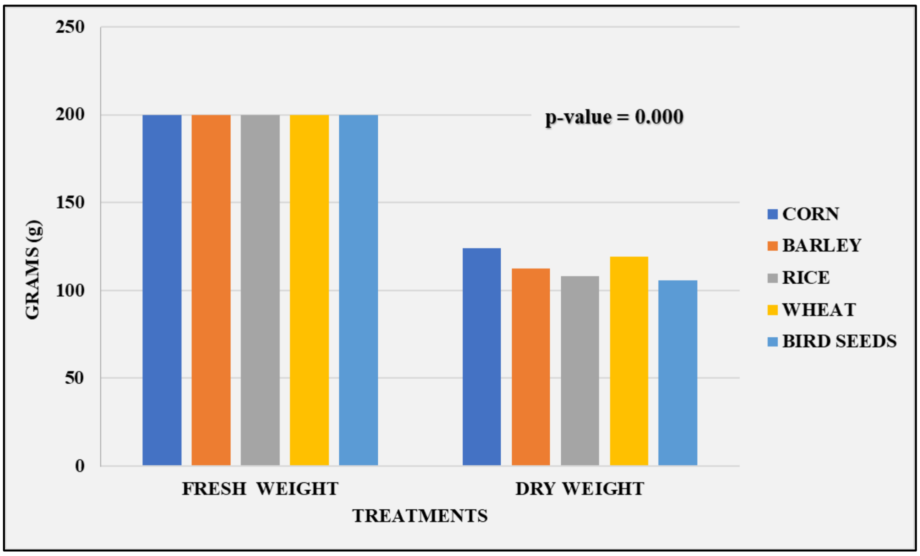


**Figure 3** Fresh weight and dry weight of prepared spawn

The differences may likely have been attributed to the type of substrate used for each treatment. Tinoco *et al*., (2001) [34] suggested that larger grains would normally have higher nutritional content when compared with smaller grains, and this could probably contribute to greater spawn growth from substrates with larger grains, accounting for the higher fresh biomass weight of spawn from corn grain infused substrate.

After complete maturation, the spawn was oven dried to constant dry weight. Comparison of the dry weight of the spawn from the different test substrates revealed that corn grain substrate produced spawn with the highest dry weight followed by wheat, barley, rice and bird seeds in that order. This may be attributed to the fact that as reported by Elhami *et al.* (2008) [7] & Tinoco *et al.* (2001) [34], larger grains have more nutritional content than smaller grains to support greater yield of spawn production.

## 4. Conclusion and Recommendations

All four substrates were successful in growing spawn for *P. ostreatus,* however, the corn grain infused substrate appeared to be the best medium, out of those used in this study, for spawn run. A comparison of results of mean size in diameter of the mycelium growth for the different substrates, the time taken for the substrate to be completely colonized and the difference between fresh and dry weights of the spawn produced all indicated that corn grain infused substrate produced the best results.

The sequence of substrate suitability determined in this study was consistent with the proposed theoretical assumption that larger grains, with more nutrient availability, would be better as a substrate for spawn production, once other environmental conditions were favorable.

The effect of different factors on mycelium growth and spawn run should be undertaken. It will be beneficial to explore other locally available materials for use as substrates for spawn production of *P. ostreatus*. Future studies should also investigate the use of different combinations of grains and nutrient fortification to boost spawn production.

## Compliance with ethical standards


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### *Disclosure of conflict of interest*

The authors certify that this submission is original work and is not under review at any other publication. The authors hereby declare that this manuscript does not have any conflict of interest.

### *Declaration of competing interest*

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### *Data availability*

Data will be made available on request.

### *Statement of informed consent*

The authors declare that informed consent was obtained from all individual participants included in the study. All work utilized in this study was fully cited and referenced, so authors of prior researches are given their due credentials for their work.


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