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Short Communication

Oyster Mushroom Spawn Production Technique

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ABSTRACT: Oyster mushrooms are rich in nutrients and have various therapeutic properties. In this work, grains of millet (*Pennisetum americanum*) were used to produce spawn of four oyster mushroom species, including *Pleurotus ostreatus* (grey strain), *P. ostreatus* (white strain), *P. cornucopiae* var. *citrinopileatus* (yellow strain), and *P. salmoneostramineus* (pink strain). Serial mycological work requires a reliable source of cultures. The time of spawn production is essential for economic sides. The superiority of *P. ostreatus* (white and grey) in the production duration of its spawn is significant (p<0.05) after 8 days. *P. salmoneostramineus* and *P. cornucopiae* have relatively long production times, approximately 10 and 11 days for spawn production, respectively. Finally, this work presents a modified procedure for producing oyster mushroom spawn easily and inexpensively for local mushroom farms.

Keywords: Millet grains, Mushroom farms, Mushroom inoculum, *Pleurotus* sp., Spawn.

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1. INTRODUCTION

Generally, *Pleurotus* sp. (Oyster mushroom) belongs to the kingdom Fungi and the phylum Basidiomycota. It supports saprophytic life by decomposing cellulosic matter and growing various agricultural residues (Stamets & Chilton, 1983). The importance of *Pleurotus* sp. cultivation increased because it possessed significant nutritional value and a wide medicinal value, viz, antimicrobial (Carvalho et al., 2007), anticancer, antioxidant, antityrosinase (Alam et al., 2011), anti-inflammatory (Kanagasabapathy et al., 2012), and antiparasitic activities (David et al., 2012). These mushrooms are essential for bio-recycling organic materials in a rich protein food (Patel et al., 2012). These mushrooms were cultivated on various agro-substrates, including wheat straw, palm date waste, and white sawdust (Atila et al., 2017).

Spawn is the vegetative mycelium from a selected mushroom grown on a convenient medium like wheat, pearl millet, sorghum, etc, for raising a mushroom crop. The first spawn was produced in France in 1894 as a pure culture (manure spawn) using horse manure compost. (Sharma & Kumar, 2011). Many studies have reported the efficacy of various grain species, including kurakkan (*Eleusine coracana*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), paddy (*Oryza sativa*) (Pathmashini et al., 2010), and wheat (*Triticum vulgare*) on oyster mushroom production. The Pennsylvania State University held two patents on the spawn production in 1932 and 1962 (Sharma & Kumar, 2011). Five types of spawn, including sawdust spawn, grain spawn, liquid spawn, stick spawn, and block spawn, are commonly available for oyster mushroom cultivation (Zhang et al., 2019).

However, producing spawn in liquid culture is suitable for *Pleurotus osteratus*. Agricultural residues (corn stalks) were converted into stick-shaped chips to grow. *ostreatus* in liquid culture (Liu et al., 2018). Recently, mother spawn, planting spawn, and commercial spawn of *Pleurotus pulmonarius* were produced to cultivate this mushroom and shorten production time (Bin Ali et al., 2026). Mushroom cultivation is a profitable agribusiness, and the oyster mushroom (*P.*

ostreatus) is an edible mushroom with an excellent flavour and taste (Shah et al., 2004). Thus, this paper seeks to produce inexpensive spawn using simple methods, which will encourage anyone to take up a mushroom cultivation hobby.

2. MATERIALS AND METHODS

2.1. Mushroom Strains

Four strains of *Pleurotus*, *P. ostreatus* (grey strain), *P. ostreatus* (white strain), *P. cornucopiae* var. *citrinopileatus* (yellow strain), and *P. salmoneostramineus* (pink strain) were obtained from Mushroom Box Co., UK, and subcultured on potato dextrose agar (PDA) medium at 25 °C for this test and stored at 4 °C for future studies.

2.2. Preparation of Potato Dextrose Agar medium

Potato dextrose agar (PDA) was prepared by adding 250 g of potatoes, 15 g of base agar, and 10 g of dextrose to 1 L of distilled water. It was sterilized in an Autoclave at 121 ºC and 15 lbs of pressure, and then poured into Petri dishes (Jodon & Royse, 1985). The medium was then used for inoculation. Petri dishes containing PDA medium were inoculated with a mycelial disk taken from the mother culture and incubated at 25 °C for 7 days. Afterwards, the plates were stored at 4 °C in a refrigerator till use (Stamets & Chilton, 1983).

2.3. Preparing spawn

Spawn was prepared on healthy millet (Pennisetum americanum) grains, as described by Stamets and Chilton (1983), with several modifications shown in Fig. 1.

Figure 1. Processes of preparing millet grains as a substrate for spawn.

Legend: 1: adding water to millet grains, 2: heating until boiling, 3: draining off excess water, 4: $CaSO_4$ and $CaCO_3$, 5: adding chemical matters in the last point, 6: mixing, 7: filling bags, 8: make small crater, 9: plugged using cotton, 10: adding foils, 11: sterilization, 12: cooled bags at room temperature.

Millet grains were obtained from the local market. Plant grains were cleaned using tap water, heated till boiling for 15 min after adding enough water. Excess water was drained, then mixed with $CaCO_3$ 1% (to adjust pH, around 6.5-6.7) based on dry weight, and $CaSO_4$ 4% (to adsorb unnecessary humidity and prevent agglomeration of plant grains). When the humidity of grain is 50% and not more than 55%, dispense 450 g into each polypropylene bag (30×50 cm), then seal with cotton and aluminium foil. Sterilization was achieved using an Autoclave at 121 ºC and 15 psi. After cooling at room temperature, they were inoculated with a 2 cm^2 disc of pure culture and thoroughly mixed with millet grains. The bags were incubated at 25±1 °C in the dark, then reversed for mixing grains after 2 days, until completion of overgrowth, and stored at 2-4 °C until use.

2.4. Growth Intensity of Mycelia

Mycelial Growth Intensity (MGI) was evaluated based on growth development and assigned a scale from 1 to 5, including: **Scale 1: Very weak growth:** Mycelium is very light, sparse, and poorly developed, with minimal radial extension. **Scale 2: Weak to moderate growth:** Mycelium is present but uneven and shows irregular density, with incomplete coverage. **Scale 3: Moderate growth:** Mycelium shows good surface coverage but with limited thickness; density is average and not fully compact. **Scale 4: Strong growth:** Mycelium is dense, uniform, and well-developed across most of the plate, indicating vigorous radial expansion. And **Scale 5: Very strong growth:** Mycelium is highly dense and compact, completely covering the plate with a thick, uniform layer.

2.5. Statistical Analysis

Statistical analysis was carried out using a completely randomized design (CRD) with a one-way ANOVA to evaluate differences among treatments. Tukey's HSD test was then applied using the GenStat program (VSN International Ltd., UK) to identify which means differed significantly. The significant difference at $p<0.05$ was considered. The experiment was conducted in three replicates.

3. RESULTS AND DISCUSSION

Fig. 2 presents the daily mycelial growth rate (mm/day) of four oyster mushroom strains (grey, white, yellow, and pink) measured throughout a nine-day incubation period. The results showed apparent differences in the mycelial growth among the studied strains. The yellow isolate (*P. cornucopiae var. citrinopileatus*) exhibited a relatively low growth rate, reaching 4.8 mm/day (moderate growth), which later increased to 7.3 mm/day. Compared to the other strains, *P. salmoneostramineus* (pink oyster mushroom) showed the slowest initial mycelial growth, starting at 5.6 mm/day and exceeding 9 mm/day by the ninth day (Fig. 2). However, its radial expansion increased steadily, indicating a delayed yet consistent growth pattern. These variations in mycelial growth rates among the strains highlight their distinct colonization capabilities and adaptive physiological responses under the same conditions.

The grey oyster mushroom (*Pleurotus ostreatus*) recorded the highest growth rate, reaching 8 mm/day on the second day and exceeding 10 mm/day on the eighth day. This performance reflects rapid, aggressive colonization in the PDA medium. Additionally, the white strain (*P. ostreatus*) showed strong growth, reaching nearly 10 mm/day on the eighth day, following a similar upward trend, though slightly lower than the grey strain.

These differences in Fig. 2 are due to genetic factors, which have a significant effect on phenotypic variation in the time required to complete spawn production among oyster mushroom species (Shukla & Jaitly, 2011). These results reflected variations in the physiological vigor and radial expansion of fungal mycelia.

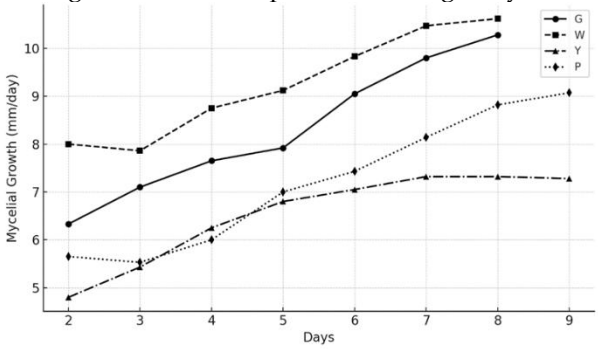

Figure 2. Daily mycelial growth rate (MGR) of oyster mushrooms

Legend: G: *Pleurotus ostreatus* (grey), W: *P. ostreatus* (white), Y: *P. cornucopiae*, P: *P. salmoneostramineus.*

Mycelial growth intensity (MGI) was evaluated. From Fig. 3, which shows the form and density of mycelial growth for the four oyster mushroom strains under study, we find that the grey isolate had the highest mycelial growth intensity. Generally, strains of oyster mushroom showed apparent variation in their MGI when cultured on plates of PDA. This value was assessed using a 1–5 scoring system based on some properties, including colony density, uniformity, and radial expansion.

The grey oyster mushroom (*Pleurotus ostreatus*) exhibited the highest vigor at score 5 (forming a dense, compact, and uniformly expanded fungal mycelial mat that thoroughly covered the PDA medium). Thus, this strain is considered the best one. Moreover, the white strain (*P. ostreatus*) demonstrated similarly vigorous mycelial growth intensity at score 4, due to extensive radial expansion and near-complete surface coverage (Fig. 3W). On the other hand, the yellow oyster mushroom (*P. cornucopiae* var. *citrinopileatus*) displayed moderate MGI at score 3. It was a less compact fungal mycelial structure and lighter.

As for the pink mushroom, it showed the lowest expected value (score 2). *P. salmoneostramineus* produced thin, weakly developed, and non-uniform fungal mycelia with limited radial spread (Fig. 3P). These observations show apparent differences in the natural growth ability of the four *Pleurotus* strains. Such variation in mycelial density is expected, as each strain has its own genetic characteristics that influence its growth behavior (Shukla & Jaitly, 2011).

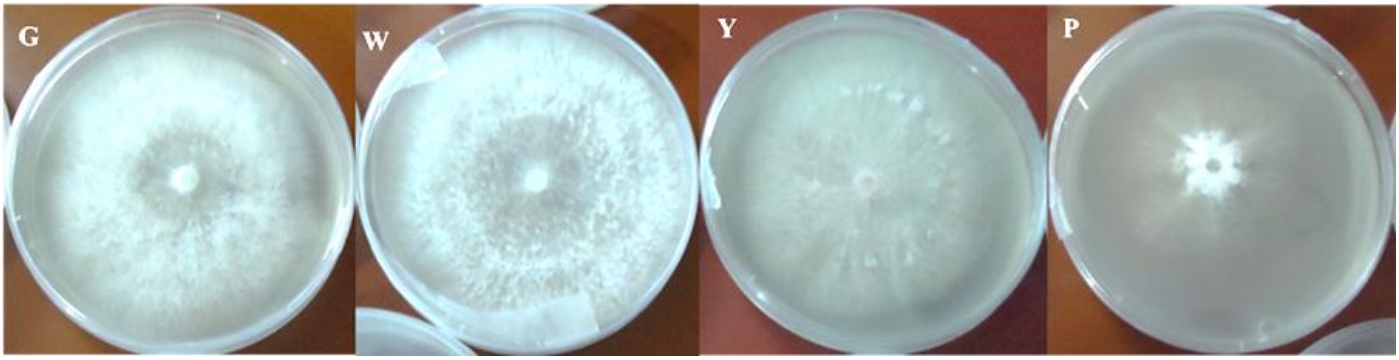

Figure 3. The mycelial growth of oyster mushrooms on PDA plates

Legend: G: *P. ostreatus* (grey), W: *P. ostreatus* (white), Y: *P. cornucopiae* (yellow), and P: *P. salmoneostramineus* (pink)

The mycelial growth performance of all oyster mushroom strains in this study showed distinct variation across all evaluated parameters. The white and grey mushroom strains (*Pleurotus ostreatus*) demonstrated the earliest and most rapid initiation of fungal mycelial development, with visible mycelial emergence on the first day of incubation. Whereas, the yellow oyster mushroom (*P. cornucopiae* var. *citrinopileatus*) initiated growth only on the second day, indicating a delayed onset.

Early growth dynamics further highlighted the superiority of the white mushroom strain, which recorded the highest radial growth rate after 2 days (11.83 ± 0.34 mm/day) and maintained this lead after 4 days (11.40 ± 0.17 mm/day). Meanwhile, *P. salmoneostramineus* (pink) and *P. cornucopiae* var. *citrinopileatus* (yellow) showed noticeably slower early expansion, with the yellow strain consistently ranking last.

The mycelial growth rate (MGR) followed the same direction. The white oyster mushroom (*P. ostreatus*) achieved the highest average MGR, reaching 10.60 ± 0.00 mm/day, reflecting strong and stable colonization potential. The grey mushroom strain (*P. ostreatus*) showed moderate and uniform growth (9.40 ± 0.00 mm/day), while the pink mushroom strain showed slightly lower yet more variable performance (9.10 ± 0.35 mm/day). Once again, the yellow strain (*P. cornucopiae* var. *citrinopileatus*) demonstrated the weakest mycelial growth, recording the lowest MGR (7.00 ± 0.00 mm/day).

These differences were further evident in the time required for complete colonization of a 9-cm Petri dish. The white strain completed colonization the fastest (8 days), followed by the grey and pink strains (9–10 days), while the yellow strain required the longest duration (12 days). These measurements (Figs. 2 and 3, Table 1) helped identify the overall strength of the oyster mushroom and facilitated choosing the fastest-growing strain for future spawn production.

The process of spawn production was summarized in Fig. 4. Varieties of oyster mushrooms were different in the period of mycelial completion on millet grains, as shown in Figs. 2 and 3. The superiority of *Pleurotus ostreatus* (white and grey) in the duration of spawn production is significant ($p < 0.05$), as observed after 8 days (Figs. 5 and 6). This time prevents the growth of undesirable fungi, such as *Trichoderma* sp. (Oh et al., 2003), and other contaminants. At the same

time, *Pleurotus salmoneostramineus* and *Pleurotus cornucopiae* had extended periods, approximately 10 and 11 days for spawn production, respectively. However, the period of spawn production extended from 8 to 11 days, as shown in Fig. 2.

Table 1. Mycelial growth rates, mycelia appearance, and time of full plate coverages of oyster mushrooms

Oyster mushroom strains	Mycelium Appearance Day	Growth Rate After 2 Days (mm/day)	Growth Rate After 4 Days (mm/day)	MGR–Overall Growth Rate (mm/day)	Days to Full Plate Coverage (9 cm)
Grey	1.00 ± 0.00a	10.67 ± 0.29a	9.87 ± 0.29b	9.40 ± 0.00b	9.00 ± 0.00a
White	1.00 ± 0.00a	11.83 ± 0.34a	11.40 ± 0.17a	10.60 ± 0.00a	8.00 ± 0.00b
Pink	1.33 ± 0.23b	8.33 ± 0.39b	8.73 ± 0.33c	9.10 ± 0.35b	9.67 ± 0.44a
Yellow	2.00 ± 0.00b	8.17 ± 0.42b	8.47 ± 0.41c	7.00 ± 0.00c	12.00 ± 0.00c

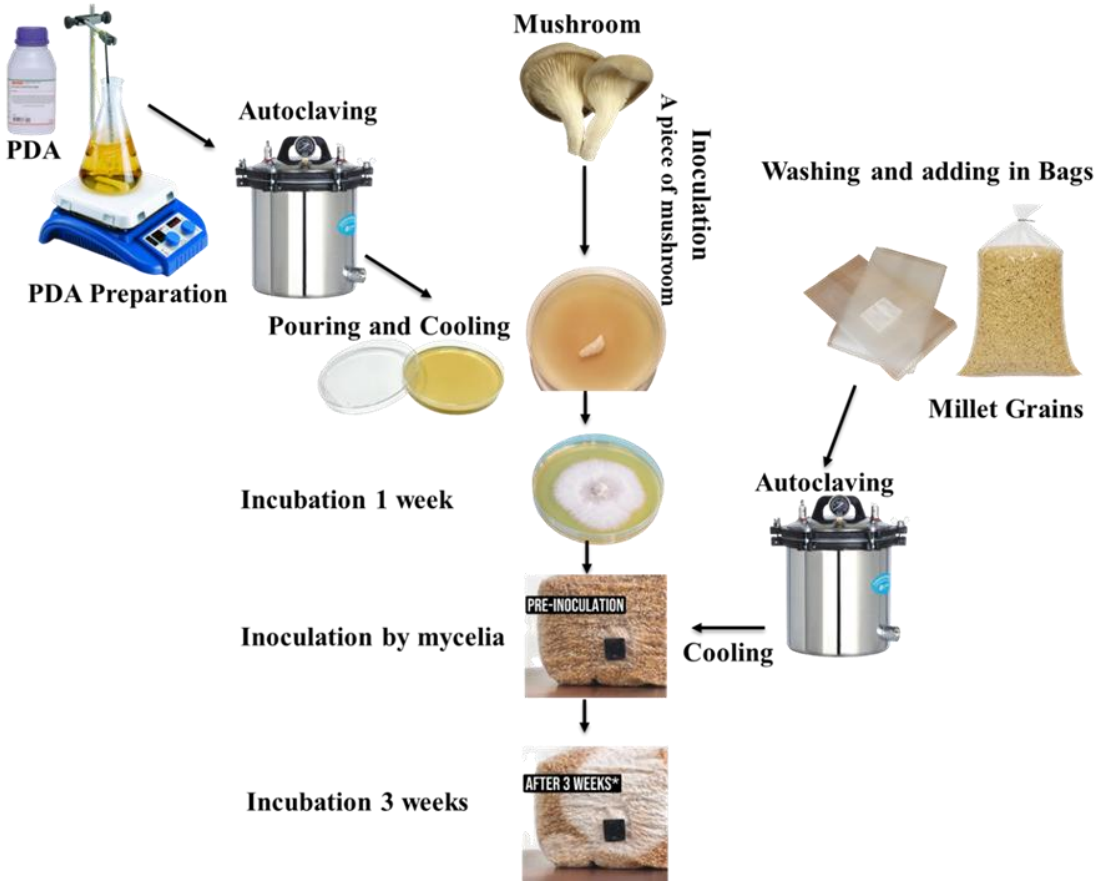

Figure 4. Oyster Mushroom Spawn Production Technique

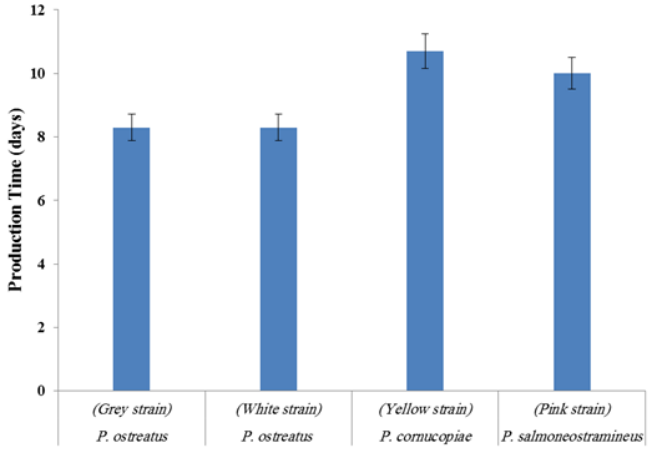

Figure 5. Production time of oyster mushroom spawns on millet grains

The heredity of oyster mushroom strains affected the period of mycelial growth in bags (Shukla & Jaitly, 2011). In addition to the appropriate grain type variance, the development of each mushroom strain is also considered. The type of grain in spawn affects mushroom production (Hakim et al., 2025). That agrees with the results of Tisdale (2004 and Shukla and Jaitly (2011), who reported complete spawn production of several species of *Pleurotus* after 10-14 days when wheat grains were used as the substrate for this process. However, another study assessed the impact of three spawn carriers (wheat grains, date seed powder, and white corn grains) on the production and morphology of *Pleurotus* spp. The results indicated significant differences across treatments, with the fastest complete substrate colonization occurring 23.167 days (Abdullah et al., 2022). But *Agaricus bisporus* (White Mushroom) production in Iraq is conducted on a limited scale. The sector is characterized by a small number of commercial entities (fewer than five nationwide), with the majority of production managed through small, individual projects (Abed et al., 2024). Another study on the last mushroom study demonstrated that higher inoculum rates drastically reduced the cultivation time; the complete mycelial run was achieved in the shortest time (18.3 days) (Shibli & Abed, 2025). Finally, this paper presents a modified method for efficiently producing oyster mushroom spawn on millet grains. Also, this work can be a cost-effective route for mushroom farms locally, as agreed with Singh et al. (2025).

Figure 6. Ready oyster mushroom spawns on millet grains

4. CONCLUSION

This study demonstrated that millet grains are an effective, economical, and readily accessible substrate for producing high-quality oyster mushroom spawn. The improved method described in this work offers a simple and low-cost alternative to traditional spawn production techniques. Local mushroom growers and small-scale farms can readily adopt it. By reducing production time and using inexpensive raw materials, this technique supports sustainable mushroom farming and encourages broader adoption of oyster mushroom cultivation in local communities. The four evaluated *Pleurotus* strains showed apparent differences in their mycelial growth behavior, with the white and grey *P. ostreatus* strains exhibiting the greatest vigor, fastest radial growth, and shortest colonization time. These strains completed spawn production in approximately 8 days, making them ideal candidates for rapid and efficient cultivation. However, the pink and yellow strains required longer colonization periods, reflecting inherent differences in their physiological growth potential.

Ethical Statement

Not Applicable.

Conflicts of Interest

The authors declare no competing interests.

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