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Growth Performance of the Mycelial Culture of Grey Oyster Mushroom *Pleurotus pulmonarius* Prepared on Durum Wheat Waste Glucose Agar on Sawdust Substrate

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ABSTRACT

Grey oyster called *Pleurotus pulmonarius* is an edible mushroom known for its smooth texture, mild flavour, high nutritional value and bioactive compounds. *P. pulmonarius* grows well on agricultural waste and widely grown for food and medicinal purposes. It has attracted attention because of their rapid growth, and potential economic benefits. The primary growth media for culturing the mycelia of oyster mushrooms is potato dextrose agar. This study aimed to evaluate the growth performance of grey oyster mushrooms using whole powdered durum wheat waste glucose agar (WPDWWGA) medium for preparation of mycelia culture and fruiting body production on sawdust substrate. Mycelial culture of the fungus was prepared on WPDWWGA, and the linear growth measured by a scale at every day and the 11 day fully ramified culture was used for the experiment. The spawn was produced with sorghum grains, and the sawdust substrate was supplemented with calcium carbonate and rice bran, inoculated with the spawn and incubated till fruiting body production in a controlled environment. The spawn run period, number and weight of carpophores, pinhead count, number of flushes and biological efficiency (BE) of *Pleurotus pulmonarius* were determined. *P. pulmonarius* produced three flushes of mushroom production which varied significantly in yield but the least yield (86.84±12.31) on the third flush. The number of pin heads; weight and number of carpophores decreased with each subsequent flush. Biological efficiency (BE) ranged from 108.55 - 187.48%. This indicates that the locally sourced and cost-effective WPDWWGA is a viable medium for mycelia growth culture and fruitification of *P. pulmonarius* on sawdust substrate supplemented with rice bran.

Keywords: *Pleurotus pulmonarius*, sawdust, biological efficiency, carpophores.

Introduction

Mushrooms are members of phylum Basidiomycetes and are highly nutritious food consumed as medicine, tonic and dietary food due to their high protein content (de Mattos-Shipley *et al.,* 2016; Thakur, 2020). In the last few decades, scientists have started exploring mushroom's pharmacological potential because of the presence of different biochemical compounds like benzoic acid derivatives, terpenes, vitamins B, C and D, anthraquinones and cholines (Ferraro *et al*., 2022). The vitamins such as niacin, riboflavin and thiamin are found

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in oyster mushrooms, also minerals such as ferrous sulfate, phosphorus, sodium and calcium are present in oyster mushrooms (Swamydhas and Lakshmanan, 2019) Mushrooms, particularly grey oyster mushrooms (*Pleurotus pulmonarius),* are highly valued for their nutritional and medicinal benefits, including their high protein content, essential amino acids, vitamins, and minerals (Roy *et al*., 2021). The consumer demand for mushrooms has increased over the years worldwide and mushroom cultivation has become a highly efficient method for recycling the agricultural residues and producing nutritious food (Thanh *et al*., 2020).

Mushroom cultivation is an indoor activity and can be cultivated in a limited space and land and is an essential part of sustainable agriculture and forestry due to its cost-effective techniques (Abdalla *et al*., 2022; Dey *et al*., 2020). This cultivation has led to generation of revenue, benefiting unemployed young people, farmers, women and self-help groups residing in urban and rural environments. One of the important reasons for the commercial production of mushrooms that should be paid attention to is its ability to utilize agricultural and industrial wastes for growth while playing a dual role: producing food (mushroom) and preventing waste pollution in the environment (Odediran & Ojebiyi, 2017).

The cultivation of mushroom includes selection of adequate mushroom species, preparation of quality fruiting culture, development of active spawn, preparation of substrate, mycelia spawn running, mushroom development and harvest (Ofodile and Yusuf, 2010). However, climatic conditions must be controlled during the stages in mushroom cultivation as any change in temperature, carbon dioxide, humidity and light levels would affect the growth, development and yield of the resulting mushroom (Gunko *et al*., 2021; Balan *et al*., 2022; Stojek *et al*., 2024).

Some of the most cultivated mushrooms include *Agaricus bisporus, Pleurotus spp., Auriculaira auricula-judae, Flammulina velutipes* (Valverde *et al*., 2015; Dimopoulou *et al*., 2022). *Pleurotus* spp. (oyster mushrooms) are the most preferred ones among the edible mushrooms due to their ability to grow quickly and productively in various lignocellulosic media (Frimpong-Manso *et al*., 2011; Gothwal *et al.,* 2012). They have been recognized as mushrooms with dual functions for humans: food and medicine, and they currently rank as the second most vital mushrooms for trade and the third most commercially produced mushrooms globally among edible fungi. Among the various species, *Pleurotus ostreatus* is the most extensively cultivated, followed by *Pleurotus pulmonarius* (Amirullah *et al*., 2023).

Pleurotus pulmonarius, commonly known as grey oyster mushroom, is well-known universally due to its high protein content and medicinal properties and stands out in the food, pharmaceutical and biotechnology industries (Jiang *et al*., 2025; Mokhtar *et al*., 2025). Cultivation of oyster mushrooms has been increased greatly throughout the world during the last few decades (Ilyas & Avin, 2018). However, one of the critical challenges faced by mushroom farmers is the optimization of substrate materials to maximize yield and biological efficiency while maintaining cost-effectiveness (Mane *et al.,* 2007).

The mycelium growth of *Pleurotus* sp. mushrooms is affected strongly by many factors such as culture media, temperature, sources of carbon and nitrogen, grain sources and lignocellulosic substrate sources (Hoa *et al*., 2015; Sofi *et al*., 2014). Potato Dextrose Agar(PDA) is known as the standard media for mycelial culture, isolation, and storage of oyster mushrooms in laboratory (Melanouri *et al*., 2025; Prajapati and Shinde, 2024; Gebru *et al*., 2024; Ofodile *et al*., 2023). PDA being an imported media is expensive and less affordable for many research students, small scale mushroom farmers in Africa. This situation highlights the importance of exploring low-cost, locally available alternatives, particularly agricultural wastes such as durum wheat by-products, which remain largely underutilised despite their rich nutrient content suitable for fungal growth. The high cost of conventional media restricts widespread use in rural and low-resource settings, where affordable and locally sourced options are more practical. This necessity has encouraged the development and use of alternative, cost-effective media such as WPDWWGA. This study, therefore, aimed at determining the growth performance of the

mycelial culture of *P. pulmonarius* prepared with WPDWWGA and grown on sawdust supplemented with and Rice bran.

2.0 Materials and Method

2.1 Study Location

The experiments were carried out at the Mushroom Research and Training Laboratory, Yaba College of Technology, Yaba, Lagos, Nigeria.

2.2 Mycelial Growth Studies

The carpophore (fruiting body) of a freshly harvested *P. pulmonarius* sample was dissected using a sterile surgical blade and the internal tissue was excised, inoculated on whole powdered durum wheat waste glucose agar (WPDWWGA) medium and incubated at 25°C for 11 days. WPDWWGA (Trade name: Fungigro) Patent RP No: NG/P/2024/91 of 21/02/2024 was prepared by dissolving 21 g of the powder in 500 mL of distilled water and sterilized in the autoclave at 121°C and 15psi for 15–20 minutes. After cooling, it was dispensed into Petri dishes in five replicates per medium and allowed to solidify before inoculating with the mushroom tissues.

2.3 Spawn Production

Sorghum bicolor grains were sorted, washed and boiled for 15-20 minutes. After cooling, calcium carbonate was added at a ratio of 20g to 1000g of sorghum grains. The mixture was divided into 250g each and loaded into glass jars, sealed and sterilized for 1hr at 121°C. Each of the sterilized glass jars were inoculated with a 10-day old plate culture of *P. pulmonarius*. The jars were incubated and stored at room temperature to allow full mycelial colonization and daily growth rates were recorded (Ofodile *et al*., 2022).

2.4 Substrate preparation and fruiting experiment

The sawdust (*Triplochiton scleroxylon)* was obtained from Bariga sawmill, Lagos state, Nigeria. The substrate composition used for this study was 78% sawdust, 20% rice bran and 2% calcium carbonate and was done in triplicates. The substrate was properly mixed. Water was added in bits until the mixture was thoroughly mixed after which the substrate was composted for 24-48hrs. After 24hrs, the substrate was bagged in heat-resistant polythene bags, sealed with PVC necks and pasteurized. To prevent microbial contamination, pasteurization was done using steam method for 8hrs. The substrate was then allowed to cool to 10°C and each inoculated with spawn.

The inoculated substrate was incubated in a room with minimal light exposure for a period of 3-4 weeks and maintained at 25-28°C and 70-80% RH. Full colonization of the substrate, which was marked by the presence of white mycelium on the entire surface of the substrate, marked the end of the incubation. Lighting was provided daily using sunlight for 8-12hrs. To allow adequate aeration and allow the pinheads to emerge, holes were made in the substrate bags. Regular misting was done to maintain high humidity; however, care was taken to avoid soaking the pins directly. After fruiting, mushrooms were harvested by gently twisting or cutting them at the base, leaving the substrate intact for subsequent flushes. After the first harvest, the substrate was used to produce additional 2-3 flushes, at intervals of about 7–10 days. To encourage these flushes, the substrate was rehydrated by soaking or heavy misting. Optimal fruiting conditions were maintained during this period which ensured mushroom production continued until the substrate was exhausted. Data collected during the experiment included the mycelial growth across different days, spawn run period, number of days taken until pinhead formation and number of pin head, weight and number of carpophores per flush. The biological efficiency (BE) was calculated as:

$$\frac{\text{Weight of fresh mushroom}}{\text{Dry weight of substrate}} \times 100$$

Total BE was the sum of BE from all flushes and the substrate dry weight was determined by oven-drying a representative sample at 105°C to constant weight prior to the experiment.

2.7 Statistical Analysis

Data collected from all experiments were expressed as mean ± Standard Error of mean (SEM). One-way analysis of Variance (ANOVA)

with Turkey's post-hoc was used for analysis at 5% level of significance.

3.0 Results

3.1 Mycelial Growth of P. pulmonarius

The results of the hyphal extension of *P. pulmonarius* on WPDWWGA are shown in Fig. 1. The mycelial growth was first observed on day 1 after incubation with days 1 to 3 showing a lag phase, which was followed by an exponential phase from day 4-11. This shows a period of adaptation of *P. pulmonarius* on the medium used.

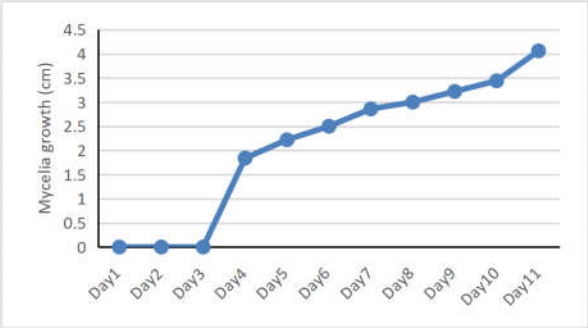

Figure 1: Mean radial mycelial growth of *P. pulmonarius* on WPDWWGA.

3.2 Spawn Preparation for P. pulmonarius Cultivation

The results of the daily growth rate of the spawn used in *P. pulmonarius* cultivation are shown in Fig. 2 below. Mycelial colonization was only observed on the sixth day post-inoculation. Complete colonization was achieved within 13 days, characterized by dense, cotton-white mycelial growth covering the entire grain surface.

This figure showed that sorghum was effective in growing mushroom spawns as shown by the exponential increase from days 5 to 13.

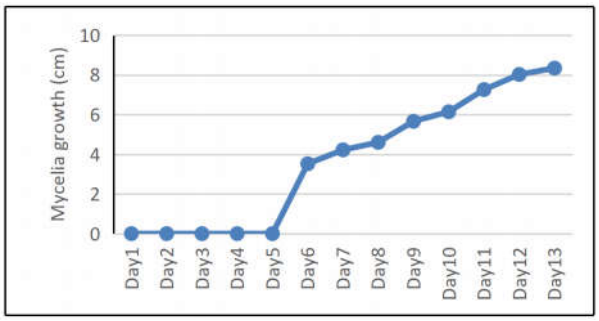

Fig. 2: Daily mycelial growth rate of Spawn.

3.3 Fruiting Body Formation During Cultivation of P. pulmonarius

Table 1 shows the three flushes harvested during the cultivation of *P. pulmonarius.* The production of mushrooms differed significantly among the three flushes during the cultivation of *P. pulmonarius* but was the lowest in the third flush. The values of the pin head count, weight and number of carpophores per bag and the biological efficiency decreased as the flush count increased. The first flush had the highest pin head count (11.2 ± 4.97), followed by flush 2 (8.4 ± 4.22) and flush 3 (6.6 ± 5.86). This suggests that the number of pin heads decreased with each subsequent flush. Also, the first flush produced the highest weight (150.00 ± 12.71 g), while the second flush was lower (114.16 ± 15.99 g), and the third flush had the lowest weight (86.84 ± 12.31 g). This indicates that the yield declined with each flush. The first flush had the highest number of carpophores (26.8 ± 11.61), followed by flush 2 (21.8 ± 8.29) and flush 3 (13.6 ± 4.99). Like the pin head count and weight, the number of carpophores als declined in later flushes

Table 1: Growth Performance of *P. pulmonarius* during different flushes

Flush	Pin head Count	Weight of Carpophores per bag	Number of Carpophores per bag	Biological Efficiency (%)
1	11.2^{a}±4.97	150.00^{b}±12.71	26.8^{v}±11.61	187.48
2	8.4^{a}±4.22	114.16^{c}±15.99	21.8^{v}±8.29	142.71
3	6.6^{a}±3.58	86.84^{d}±12.31	13.6^{v}±4.39	108.55
1,2,&3	8.7±4.4	20.7±9.8	117.0±29.7	438.75

Means within a row followed by different superscript letters are significantly different (p < 0.05, Tukey's HSD tes

Table 1 revealed that Pin head count and number of carpophores (Figure 3a-c) per bag are not significantly different in all the flushes ($p>0.05$). But weight of carpophores per bag in flush 1 was significantly higher compared to flush 2 and flush 3 while that of flush 2 significantly higher than in flush 3.

The mean total weight of the mushroom fruiting bodies produced from the three flushes was 585.00 g. This shows that, on average, each batch of mushrooms harvested from the substrate weighed around 585.00 grams. The number of carpophores (Figure 3c) was approximately 117.0±29.7 while the days to carpophore emergence was 54.2 ± 0.84 days. This suggests that it takes around 54 days for the mushrooms to begin fruiting, with minimal variation in the formation period. The total biological efficiency (BE), which measures effectively the substrate is converted into mushroom biomass was 438.75%.



Figure 3: a-c Pinheads, growing fruiting bodies and the matured fruiting bodies of grey oyster mushroom (*Pleurotus pulmonarius*)

4.0 Discussion

WPDWWGA is formulated from wheat bran base and has relatively different nutrient architecture compared to PDA. It is composed of 30-60% dietary fiber, minerals and micronutrients including phosphorus, magnesium, zinc, iron and manganese, moderate protein content, vitamins and bioactive compound (Onipe *et al*., 2015). The wheat bran base introduces greater variability than PDA, also greater nutrient depth and sustainability most suited for low resource and circular economy laboratory settings such as mushroom laboratories.

The composition of PDA has easily utilisable carbohydrate and nutrient base for fungi because it essentially consists of potato infusion, dextrose 20g/L, agar (15g/L), pH (~5.6) (Ribeiro *et al*., 2025).These may suggest the reasons for more sustained fungal mycelial growth in WPDWWGA than PDA because PDA depletes faster than this innovation during fungal growth (personal observation). The composition of WPDWWGA makes it more suitable for lignocellulolytic and cellulolytic species of fungi such as *Pleurotus s*pecies and other mushroom species. Moreso, WPDWWGA is far cheaper (~50%) than PDA because it is made from locally sourced raw material that is basically waste.

Pleurotus spp. have a high saprophytic ability and can successfully grow on a variety of lignocellulosic substrates (Machado *et al.,* 2016; Iwuagwu *et al*., 2017). In the present study, 78% sawdust and 20% rice bran were mixed and used as substrate for the cultivation of *P. pulmonarius*. The study evaluated the growth performance, spawn preparation, and cultivation success of *P. pulmonarius.*

The growth medium is a key factor in mushroom cultivation because it provides the nutrients needed for healthy mycelial development (Thanh *et al*., 2020). As noted by Shrestha *et al*. (2006), media that are rich in nutrients tend to promote vigorous mycelial growth, likely because they offer all the essential nutrients and create suitable physical conditions for vegetative growth. In this study, the Whole Powdered Durum Wheat Waste Glucose Agar (WPDWWD) agar proved to be the a very supportive medium for the mycelial growth of *Pleurotus pulmonarius*. Earlier studies have shown that PDA is particularly effective for the mycelia growth of *P. eryngii*, *Macrolepiota procera*, and *Cordyceps militaris* (Thanh *et al*., 2020; Shrestha *et al*., 2006; Shim *et al*., 2005), while yeast malt extract medium has been found to favor the growth of *M. procera* and *Ganoderma lucidum* (Shim *et al*., 2005; Jayasinghe *et al*., 2008). These observations highlight that fungal species differ in their nutritional requirements and respond differently to culture media. In this context, the successful spawn run and fruiting body production obtained in this study demonstrate that WPDWWGA can serve as a reliable alternative to PDA, while also confirming that specific media formulations may be better suited to *Pleurotus* species. Mycelial appearance and growth characteristics are also

influenced by factors such as nutrient composition, the presence of bioactive compounds, and substrate concentration within the culture medium (Kim *et al.*, 2002).

The biological efficiency (BE) recorded across the three flushes further indicates efficient substrate utilisation by *Pleurotus pulmonarius*. The highest BE value of 187.48% was obtained during the first flush, followed by 142.71% and 108.55% in the second and third flushes, respectively. The gradual reduction in BE with successive flushes is a common trend in mushroom cultivation and is generally associated with nutrient depletion, reduced availability of easily degradable organic materials, and physiological fatigue of the mycelium after repeated fruiting cycles (Chang & Miles, 2004). Although a decline in biological efficiency was observed across successive flushes, the cumulative biological efficiency of 438.75% indicates a highly efficient conversion of substrate into edible mushroom biomass. Such high BE values suggest that the formulated substrate supplied sufficient nutrients while also providing the structural stability needed to sustain continuous mushroom production. Elevated biological efficiency in oyster mushrooms has been linked to well-optimized lignocellulosic substrates that promote prolonged enzymatic activity and effective nutrient utilisation (Sánchez Carmen, 2010). Similarly, Rivera *et al*. (2023 reported high averages BE of between 93 to 125% for *Pleurotus pulmonarius* grown on different hardwood Sawdust. A biological efficiency of 185% has previously been reported for *Pleurotus ostreatus* cultivated on partially composted sesame stalk supplemented with different proportions of wheat straw (Mesele *et al*., 2024), further confirming the strong capacity of oyster mushrooms to efficiently convert lignocellulosic substrates into edible biomass. In this study, the combination of sawdust and rice bran likely provided a balanced carbon-to-nitrogen ratio together with adequate lignocellulosic components, thereby supporting efficient mycelial colonisation and successful fruit body development. Also, the use of vigorous spawn and proper environmental management, including optimal moisture, temperature, aeration, and humidity, likely enhanced productivity (Sánchez Carmen, 2010).

Despite the nutritional benefits and cost advantage of Wheat Bran Powdered Durum Wheat Waste Glucose Agar (WPDWWGA), some limitations were noted during its use as a fungal culture medium. One major challenge is its lower transparency when compared with Potato Dextrose Agar (PDA). The presence of wheat bran residues and insoluble fibrous materials makes the medium relatively opaque, limiting clear observation of colony morphology and reducing visibility of fungal growth from the underside of Petri dishes. By comparison, PDA provides a clearer background that supports easier morphological examination and routine identification.

Another limitation observed was the development of a noticeable and sometimes unpleasant odor during preparation and use. This odor is likely associated with the natural composition of wheat bran and the breakdown of organic components during heat sterilization. Although this characteristic does not negatively affect fungal growth, it may reduce user comfort and acceptance, particularly in teaching or diagnostic laboratory environments.

Future improvements should therefore aim at enhancing the clarity and sensory acceptability of WPDWWGA while maintaining its nutritional effectiveness. Also, comparative studies assessing fungal growth rate, sporulation, and metabolite production on improved WPDWWGA formulations relative to PDA will be important for refining the medium and broadening its application in research, training, and low-resource laboratory settings.

5.0 Conclusion

The total biological efficiency (BE) of roughly 438% was achieved using WPDWWGA for the successful mycelial preparation and in creation of viable spawns that supported mushroom cultivation on a sawdust substrate. The results of this experiment show that the medium has the potential to be a reliable substitute for traditional culture media (PDA) in the production of mycelia of oyster mushrooms.

In addition, the experiment examined the use of rice bran (*Oryza sativa* L.) and sawdust from *Triplochiton scleroxylon* as growth substrate for the cultivation of *Pleurotus pulmonarius*. Three consecutive flushes from the same substrate

produced mushrooms, and it was observed that there was a progressive decline in pinhead formation, carpophores' quantity and weight as flush number increased and yields as cultivation advanced. This pattern is frequently linked to decreased substrate availability and progressive nutrient depletion after several harvesting cycles. Also, *Pleurotus pulmonarius* maintained very strong growth performance on the substrate, attaining a cumulative biological efficiency of 438.75%, despite the observed decline across subsequent flushes. This result demonstrates that sawdust waste can be successfully used as an appropriate substrate for growing mushrooms.

The capacity of WPDWWGA to produce viable spawns that later contributed to mushroom production on sawdust substrate with a biological efficiency of approximately 438.75% further justifies the relevance of WPDWWGA medium. As an affordable formulation prepared from locally available materials, WPDWWGA has the potential to reduce production costs for small-scale farmers and research students. Therefore, its adoption could enhance access to mushroom cultivation technologies, particularly in resource-limited environments such as Nigeria and other developing countries.

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Conflicts of Interests

The author(s) declares no conflict of interest.

Declaration of AI generative tools

Original images of Figure 3b and c brightened for clarity with ChatGPT.

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