Original Research Article

Mycelia colonization potential of *Pleurotus tuber-regium* (fr) singer and *Pleurotus ostreatus* (Jacq. Ex. Fr) Khum on different culture media and grain types

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ABSTRACT

Aim: To determine the effect of different culture media and grain types on the mycelia growth performance of two *Pleurotus spp* (*P. ostreatus* and *P. tuber-regium*).
Study design: The design this experiment was a completely randomised design (CRD) with three replications.
Place and Duration of Study: This research was carried out in the Department of Plant and Ecological Studies, University of Calabar, Cross River State, Nigeria, between the period of March –May, 2018.
Methodology: The mycelia colonization potential of these mushrooms were assessed on three culture media viz; potato dextrose agar (PDA), palm inflorescence waste agar (PWA), and spent palm fibre agar (SPFA) and different cereal grain types (viz; *Sorghum bisicolor Zea mays*, *Eleusine coracana,* and *Triticum aestivum*) and a combination of the grains were used as spawn substrates. Parameters assessed include linear mycelium extension, mycelia growth, and mycelia density.
Results: Maximum and minimum mycelia colonization rate of both mushroom species was observed in PDA and PWA, respectively. Results showed that *Zea mays* and *Sorghum bisicolor* grains gave the best result in mycelia colonization rate, while *Zea mays* was poor in supporting mycelia density. The combination of grains at ratio of 1:1 did not contribute positively to the mycelia colonization of *P. ostreatus*. However, wheat-millet and sorghum-wheat combination at equal proportions greatly enhanced *P. tuber-regium* mycelia colonization.
Conclusion: This study reveals that PDA was the best medium for culturing both species, while red sorghum was the best grain substrate for *P. ostreatus* and *P. tuber-regium* spawn production.

Key Words: Culture media, Grain types, Mycelia growth, *Pleurotus ostreatus, Pleurotus tuber-reguim*

1. INTRODUCTION

Mushrooms are macro-fungi that grow on soil or wood. They produce microscopic spores, which are easily dispersed by wind. These spores germinate and develop mycelium [2], which ramify available medium to acquire nutrients. Besides the culture medium, fungi also obtain growth nutrients from grains usually prepared as spawn. Thus, mushroom spawn serves as the initial source of culture material for raising mushrooms on the farm. Furthermore, spawn quality is essential for profitable mushroom production since it determines the yield [3,4].

The first stage of mushroom cultivation is the development of actively viable spawn. The main problem facing the mushroom industry in developing countries is the availability of

good quality spawn. The mycelia growth of oyster mushrooms uses soluble carbohydrates such as glucose, molasses, organic nitrogen sources like wheat bran, barley, oat, maize, soybean crust, sunflowers, and mineral sources such as ammonium sulphate [5]. Because of their lignocellulosic enzymes, Oyster mushrooms can utilise cellulose, hemicellulose, and a large or small lignin quantity. Almost all cultivated mushrooms make use of cereals such as maize (corn), millet, wheat, and sorghum (guinea corn) as spawn substrate material [9].

Several grains such as *Zea mays*, *Eleusine coracana, Sorghum bisicolor Oryza sativa*, *Glycine max* and *Triticum aestivum* were reported as substrates for spawn production [3, 7, 8]. In their study, [9] 2014 reported maximum and minimum growth rates of mushrooms spawn observed for corn and millet. Wheat has also been reported as spawn substrate for oyster mushrooms [10].

Reports show that most spawn producing institutes use larger corn and wheat seeds since they contain more nutrients for mycelia growth [11]. Therefore, culture media and grain types are common factors that affect mycelium growth for spawn production.

This study, therefore, evaluated the effect of three different culture media and four-grain types for their mycelia colonization potential of oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus tuber-regium*).

2. MATERIALS AND METHODS

Location of study

This study was carried out in the Research laboratory of the Department of plant and Ecological studies, University of Calabar, Calabar.

2.1 Preparation of potato dextrose agar (PDA) medium

The preparation of PDA medium adopted the method described by [12] with modifications. First, potato dextrose agar (7.8 g) was weighed out and dissolved in 50 ml of water. Then, more water was added to the mixture and stirred to make a mark of 200 ml in a conical flask and sterilised in an autoclave at 121^0 C for 20 minutes. Finally, the medium was allowed to cool to about 40^0 C before dispensing into Petri dishes and allowed to solidify.

2.2 Preparation of palm waste agar (PWA) medium

This medium was prepared as described by [13] with slight modifications. Palm waste was obtained from a dead palm trunk for the preparation of this medium. Seventy-five grams of palm waste was added to water in a 250 ml glass beaker and placed in a steaming water bath for 2 hours. It was allowed to cool to room temperature (28^0 C), filtered with a piece of muslin cloth and the supernatant retrieved. Three and a half grams (3.5 g) of pure agar was added to the palm waste extract and autoclaved at 121^0 C for 15 minutes. The medium was allowed to cool and then dispersed into Petri dishes.

2.3 Preparation of spent oil palm fibre agar (SPFA) medium

This medium was prepared as described by [13] with slight modifications. Oil palm fibre was obtained from a local oil mill in Akpabuyo Local Government Area of Cross River State, Nigeria. Seventy-five grams of palm fibre was added to water in a 250 ml glass beaker and placed in a steaming water bath for one hour. It was allowed to cool to room temperature, filtered with a piece of muslin cloth and the supernatant retrieved. Three and a half grams (3.5 g) of pure agar was added to the palm fibre extract 200 ml and autoclaved at 121^0 C for 15 minutes. The medium was allowed to cool and then dispersed into Petri dishes of 85 mm in diameter.

2.4 Tissue culture

The mushroom fruit body was sterilised with 75 % absolute ethanol to avoid contaminants from the field. A scalpel was flamed to red hot and allowed to cool. Tissue from the point of intersection between pileus and stipe was picked and inoculated on solidified media plates inside a laminar flow cabinet with a spirit lamp. After inoculation, the Petri dishes were labelled and kept in a sterilised culture laboratory for mycelia growth. Sub-cultures were prepared to obtain pure cultures. Radial mycelia growth on Petri dishes were observed until filled with mushroom mycelia for 12-15 days. Mycelial growth and density were measured at three days intervals. Mycelia density was rated as; [14]

i. ++ Scanty (20 % coverage of the Petri dish)

ii. 3+ moderate (30-49 % coverage of the Petri dish)

iii. 4+ abundant (50-69 % coverage of the Petri dish)

iv. 5+ very abundant (70 and above coverage of the Petri dish) methods of [14].

Growth rate = Colony diameter on the last day (cm)/Number of measurement days after inoculation.

2.5 Spawn preparation

The grain types used in the preparation of mother spawn were purchased from open market stalls in Calabar. They include yellow maize, wheat, red sorghum (guinea corn), and millet. Grains were prepared using the modified methods of [15]. A hundred grams each of these grains were weighed out and washed thoroughly up to 3 to 4 times to remove debris. The grains were transferred into a plastic bowl containing 200 ml of water and 2 g of gypsum and soaked for 12 hours. After soaking, water was drained off, and the grains were filled in bottles of 15 cm tall to about ¾ level, plugged with cotton wool, and covered with aluminium foil paper. Grain bottles were sterilised at 121^0 C for 90 minutes. The sterilised grain bottles were allowed to cool and then inoculated with mushroom mycelium from the stock cultures and incubated at room temperature. The experiment was carried out in three replications, and linear mycelia extension was recorded at four days intervals after the first two days for fourteen days.

2.6 Preparation of grain combination

Grains used for this experiment were combined at different ratios to obtain six treatments. The various treatments (grain combination) are shown in Table 1.

TABLE 1: showing Grain combinations

Grain combinations	Ratios	Treatments
Maize + Sorghum	1:1 (50 g maize + 50 g sorghum)	T_1
Maize + Wheat	1:1 (50 g maize + 50 g wheat)	T_2
Maize + Millet	1:1 (50 g maize + 50 g millet)	T_3

Sorghum + Wheat	1:1 (50 g sorghum + 50 g wheat)	T_4
Sorghum + Millet	1:1 (50 g sorghum + 50 g millet)	T_5
Wheat + Millet	1:1 (50 g wheat + 50 g millet)	T_6

2.7 Experimental design and data analysis

The experimental design was a completely randomised design (CRD) with three replications. Data collated were subjected to analysis of variance (ANOVA) using SPSS version 20.0. Mean significant differences at P≤0.05 were compared using one-way analysis of variance and Duncan's multiple range.

3. RESULTS

3.1 Effect of culture media on mycelia growth of *Pleurotus ostreatus*

After fifteen days of inoculation, the three culture media assessments showed significant differences (P≤0.05) between the mycelial colonization rates of the mushrooms cultured on the different media (Figure 1). The three culture media assessment showed that PDA had the fastest mycelial colonization rate within 15 days of incubation. There was no significant difference (P<0.05) in the fungal mycelia growth rate cultured on the different media tested three days after inoculation. However, on the 6th and 9th day, PDA supported the highest colonization diameter of 2.0 cm and 3.0 cm, respectively. This growth level was significantly higher (P<0.05) than the growth obtained on the other two media.

3.2 Effect of culture media on mycelia growth of *Pleurotus tuber-regium*

With *P. tuber-regium*, potato dextrose agar (PDA) supported the highest mycelial colonization rate. This level of mycelia colonization rate was significantly higher (P≤0.05) than those observed on the other media tested throughout the incubation period except on the 12th-day post-inoculation, where those growing on spent oil palm fibre agar (SPFA) produced similar growth values (Figure 2).

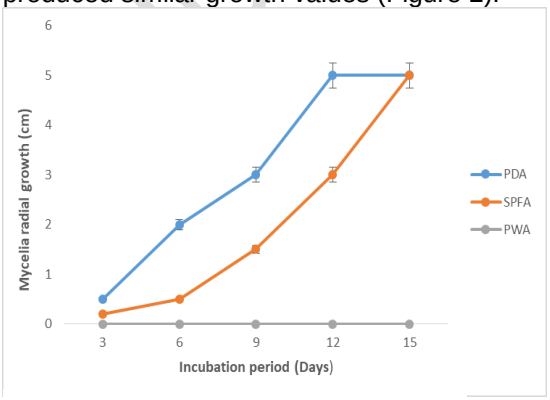

Figure 1: Effect of different culture media on the mycelial radial growth of *Pleurotus ostreatus*

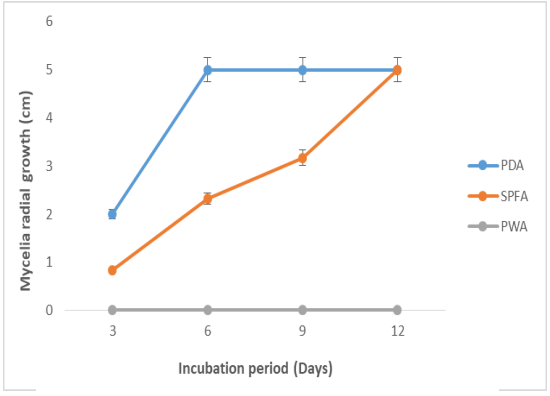

Figure 2: Effect of different culture media on the mycelial radial growth of *Pleurotus tuber-regium*

3.3 Effects of the different grain types on mycelia colonization rate of P. ostreatus

Figure 3 shows the result of the mycelia colonization rate (MCR) of *P. ostreatus* on different grains. Maize had the fastest MCR of 4.47 cm after the second day of inoculation. This value was significantly different (P≤0.05) from other substrates but comparable with sorghum and wheat. The MCR of maize and sorghum grains were significantly higher (P<0.05) than those supported by other grains on the 6th and 14th days after inoculation. In contrast, sorghum and millet were higher on the 10th day.

3.4 Effect of different grain types on the mycelial colonization rate of Pleurotus tuber-regium

The result of the mycelia colonization rate (MCR) of Pleurotus tuber-regium showed that the colonization rates were significantly affected by the different grain substrates (Figure 4). The mycelia colonization rates were recorded for 14 days. On the second day after inoculation (2 DAI), there was no significant difference (P<0.05) in the mycelial colonization level among the four-grain types. However, maize and sorghum supported the mycelial colonization rate and was significantly different (P<0.05) from those produced by other grains on the 6th day. On the 10th day after inoculation, sorghum grain substrates supported mycelia growing significantly faster than others, while maize performed better on the 14th day.

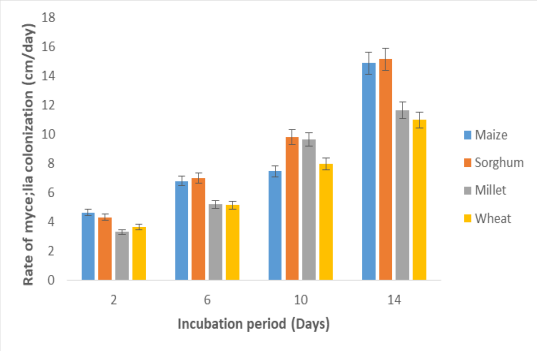

Figure 3: Rate of colonization of different grains by *Pleurotus ostreatus*

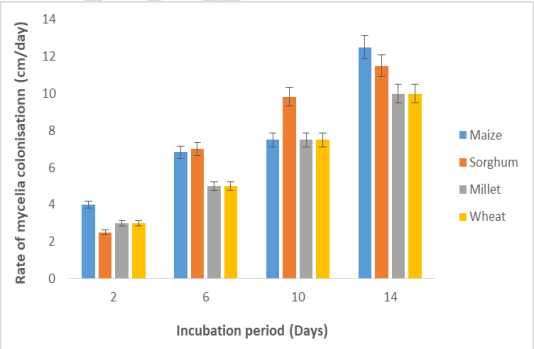

Figure 4: Rate of colonization of different grains by *Pleurotus tuber-regium*

3.5 Effect of the different grain combinations on the mycelial colonization rate (MCR) of *Pleurotus ostreatus*

The result of the grain combination test indicated that the maize/millet (T3) combination had the highest mycelial colonization diameter (8.37 cm) on the 6th and (14.50 cm) on the 10th day after inoculation. On the 2nd and 14th days after inoculation, there were no significant differences (P≤0.05) among the grain combinations. However, on the 6th day, there were significant differences (P≤0.05) in growth support capabilities among the treatments. In contrast, there was no significant (P≤0.05) growth support capability between T2 and T4 on the 10th day (Table 2).

3.6 Effect of the different grain combinations on the mycelial colonization rate (MCR) of *P. tuber-regium*

Two days after inoculation, wheat/millet (T6) combinations recorded the highest MCR, which was significantly different (P<0.05) from other combinations. On the 6th, 10th, and 14th day,

the sorghum/wheat (T4) combination gave a significantly higher (P<0.05) MCR of P. tuber-regium when compared with other combinations. However, on the 14th day, maize/wheat (T2) and sorghum/wheat (T4) were comparable and were significantly (P≤0.05) higher when compared to other grain combinations (Table 3).

TABLE 2: Effect of the different grains combinations on the mycelial colonization rate (MCR) of *Pleurotus ostreatus*

	Mycelial colonization rate (cm)			
Treatment	**2 DAI**	**6 DAI**	**10 DAI**	**14 DAI**
(T_1)	*3.00^{a}±0.00	6.80^{b}±0.00	12.67^{c}±0.29	12.50^{a}±0.00
(T_2)	2.00^{a}±0.00	5.00^{d}±0.00	13.00^{b}±0.00	13.00^{a}±0.00
(T_3)	3.00^{a}±0.00	8.37^{a}±0.37	14.50^{a}±0.00	14.50^{a}±0.00
(T_4)	2.00^{a}±0.00	6.20^{c}±0.00	13.00^{b}±0.00	15.00^{a}±0.00
(T_5)	1.00^{a}±0.00	4.70^{e}±0.00	9.20^{d}±0.00	13.00^{a}±0.00
(T_6)	1.00^{a}±0.00	6.40^{c}±0.00	8.50^{e}a±0.00	13.00^{a}±0.00

*Represents means of three replicates, ± standard error of the mean. Means having dissimilar letters on a column differ significantly at P≤0.05. Duncan's multiple range test. T_1=maize/sorghum, T_2= maize/wheat, T_3=maize/millet, T_4= sorghum/wheat, T_5= sorghum/millet, T_6=wheat/millet.

TABLE 3: Effect of grain combination on the mycelial colonization rate (MCR) of *Pleurotus tuber-regium*

	Mycelia colonization rate (cm)			
Treatment	**2 DAI**	**6 DAI**	**10 DAI**	**14 DAI**
(T_1)	*2.50^{c}±0.29	6.17^{b}±0.17	8.50^{c}±0.00	9.50^{b}±0.00
(T_2)	2.00^{d}±0.00	5.00^{c}±0.00	10.00^{b}±0.00	11.50^{a}±0.00
(T_3)	3.00^{b}±0.00	6.00^{c}±0.00	7.50^{d}±0.00	8.50^{c}±0.00
(T_4)	2.00^{d}±0.00	7.00^{a}±0.00	11.00^{a}±0.00	11.50^{a}±0.00
(T_5)	2.00^{d}±0.00	6.00^{b}±0.00	7.50^{d}±0.00	7.50^{d}±0.00
(T_6)	4.50^{a}±0.00	6.00^{b}±0.00	8.17^{c}±0.33	9.67^{b}±0.00

*Represents means of three replicates, ± standard error of the mean. Means having dissimilar letters on a column differ significantly at P≤0.05. Duncan's multiple range test. T_1=maize/sorghum, T_2= maize/wheat, T_3=maize/millet, T_4= sorghum/wheat, T_5= sorghum/millet, T_6=wheat/millet.

3.7 Effect of the different grain types on the mycelia density and growth rate of the two mushroom species

Table 4 shows the mycelia density and growth rate of the mushrooms on the grains. The fastest growth rate was observed with *P. ostreatus* in sorghum but was not significantly different from *P. ostreatus* in maize. In terms of mycelia density, sorghum performed best with very thick and abundant mycelia for both mushroom species. The fastest growth rate for the grain combinations was with P. ostreatus in T4, which was not significantly different from P. ostreatus in T3. T5 had the best with thick and abundant mycelial density for both mushroom species (Table 5).

Table 4: Mycelia density and growth rate of the different grains

Mushroom species	**Grains**	**Average mycelia density**	**Growth rate cm/day**
PT	Maize	++	0.87^{b}±0.00
PO		++	1.06^{a}±0.00
PT	Sorghum	5+	0.82^{b}±0.00
PO		5+	1.08^{a}±0.00
PT	Millet	4+	0.71^{c}±0.00
PO		4+	0.83^{b}±0.00

PT	Wheat	3+	0.71^{c}±0.00
PO		3+	0.78^{bc}±0.00

++ Scanty (20 %), 3+ Moderate (30-49 %), 4+ Abundant (50-69), 5+ Very abundant (70 % and above). Means having dissimilar letters on a column differ significantly at P≤0.05. Duncan's multiple range test.

PT=*Pleurotus tuber-regium,* PO=*Pleurotus ostreatus*

Table 5: showing Mycelia density and growth rate of the grain combinations

Mushroom species	**Grains**	**Average mycelia density**	**Growth rate cm/day**
PT	T_1	++	0.68^{c}±0.00
PO		++	0.89^{b}±0.00
PT	T_2	++	0.82^{b}±0.00
PO		++	0.93^{b}±0.00
PT	T_3	++	0.61^{c}±0.00
PO		++	1.04^{a}±0.00
PT	T_4	4+	0.82^{b}±0.00
PO		4+	1.07^{a}±0.00
PT	T_5	5+	0.54^{d}±0.00
PO		5+	0.93^{b}±0.00
PT	T_6	4+	0.69^{c}±0.00
PO		4+	0.93^{b}±0.00

++ Scanty (20 %), 3+ Moderate (30-49 %), 4+ Abundant (50-69), 5+ Very abundant (70 % and above). Means having dissimilar letters on a column differ significantly at P≤0.05. Duncan's multiple range test.

PT=*Pleurotus tuber-regium,* PO=*Pleurotus ostreatus*

4. DISCUSSION

The purpose of mushroom culture is to boost its vigorous state such that it will rapidly colonise the selected organic matrix for spawn production [6]. In this study, the two *Pleurotus* species (*P. ostreatus and P. tuber-regium*) were cultured on three different media. The results showed that of the three media used, spent palm fibre agar (SPFA) and potato dextrose agar (PDA) supported the mushroom mycelia's growth though at varying degrees. However, PDA proved to be the best [16, 17]. The best performance observed could be because it contains all the necessary nutrients needed for the mycelium's growth and contains a higher amount of carbohydrates than the other media used [18]. The implication is that the two mushroom species can be cultured on SPFA and PDA, suggesting that the colony characteristics of mycelium on culture media depend on the nutrient composition of the medium and the efficacy of such compounds [19]. [20] reported that amino acids, aspartic acids, and the corresponding keto group accelerated the mycelia growth of *Trichoderma* species. The medium used in this study is rich in some of these compounds.

Grains such as maize, millet, wheat, and sorghum are essential cereals. They are considered a primary staple food in Africa, the Middle East, and Asia due to their high nutrient and dietary fibre content [21]. Aside from being stapled foods for humans, they have been used as substrates to produce mushroom spawn.

This study showed that the mycelium extension, colonization rate, and density varied remarkably among the grain types. Yellow maize and red sorghum were the best in MCR and the growth rate of *P. ostreatus* and *P. tuber-regium.* However, several researchers had earlier reported white maize as the best substrates for spawn production [3, 24, 7, 9].

Although yellow maize had the fastest mycelia colonization and growth rate, the present study observed that it could not produce mushrooms with high mycelial density.

The fast MCR of yellow maize could be attributed to the large surface area and larger food material made available by maize grains for mycelia growth. Also, the porosity provided by maize due to grain size allows for effective mycelia ramification of the substrate [9, 3, 7]. In terms of mycelia density, sorghum and sorghum-wheat combination performed best for both mushrooms with very abundant and thick mycelia. The combination of wheat-millet and Sorghum-Wheat at equal ratios greatly enhanced *P. tuber-regium* mycelia colonization. A combination of sorghum (87.2 %), wheat bran (10%), $CaCO_3$ (1.5 %), $CaSO_4$ (0.5 %), and urea (0.8 %) was reported to give the best medium for *P. ostreatus* spawn production [4].

5. CONCLUSION

This study used different media and grain types for the spawn preparation of two *Pleurotus species - P. tuber-regium* and *P. ostreatus*. PDA was the best medium for culturing both species, while red sorghum was the best grain substrate for oyster mushroom spawn production. However, there is a need for further studies to determine the effect of temperature, pH, and nutritional requirements for these mushrooms' mycelia growth.

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