

Influence of Selected Nigerian Agro-Waste Substrates on the Morphological Characteristics and Yield of *Pleurotus pulmonarius* (Fr.) Quél

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ABSTRACT

Background and Objective: The use of locally available lignocellulosic substrates in mushroom production offers a sustainable approach to waste utilization and food production. However, substrate type and supplementation levels may influence fruit body yield and profitability in oyster mushroom production. The main objective of this study was to determine the effects of selected agro-wastes on the fruiting performance of *Pleurotus pulmonarius*. **Materials and Methods:** Coconut fiber (CF), *Raphia* palm inflorescence (RPI), and oil palm husk (OPH), supplemented with rice husk (RH) at 25% and 50% levels, were made in five replications of 150 g each and were pasteurized at 80°C for 2 hours. Each substrate was inoculated with 50 g of grain-based spawn and incubated in the dark during the spawn run. Data were analyzed using ANOVA, and mean differences were separated using Duncan's Multiple Range Test (DMRT) at the 5% significance level. **Results:** The shortest primordial formation time (18 days) was observed in 100% CF, while 75% CF recorded the longest (32 days). All OPH substrates (pH 10.3) failed to produce fruit bodies. The highest yield (438.0 g/kg) and biological efficiency (58.40%) were obtained from 100% RH, followed by 100% RPI (346.00 and 46.13%), whereas 75% CF recorded the lowest yield (108.0 g/kg) and biological efficiency (14.40%), with significant differences at $p < 0.05$. Morphologically, 100% RPI produced the longest stipe (3.39 cm), while 75% RPI (6.65 cm), 50% RPI (6.39 cm), and 100% RH (6.78 cm) produced a larger pileus diameter and higher fresh weight. Results further showed weak to moderate correlations, indicating that an increase in SL is associated with an increase in CS, FBW, and FBN of the cultivated *P. pulmonarius* fruit bodies. **Conclusion:** The RH, RPI, and CF are suitable substrates for the cultivation of *P. pulmonarius* and are therefore recommended for commercial or large-scale mushroom production.

KEYWORDS

Pleurotus pulmonarius, agro-waste substrates, rice husk, biological efficiency, mushroom cultivation, fruit body yield

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INTRODUCTION

Mushrooms are the fleshy spore-bearing fruit bodies of a fungus belonging to classes Ascomycota and Basidiomycota, typically produced above ground, in the soil or on the other food sources^{1,2}. Most macro-fungi have fruiting body that are large enough and visible to the naked eye, which can be picked up by



hand³. Valério *et al.*⁴ maintained that the word “mushroom”, also means the fruiting body of macro-fungi with a heterotrophic mode of nutrition, which grows above ground with structures including a cap, stem, and hymenium. Spores are their main reproductive structure, but not the same as that of seed plants^{5,6}.

Mushrooms are also saprotrophs and cannot manufacture nutrients by photosynthesis, but derive their nutrients from organic sources^{4,7}. They naturally grow on, decaying materials and have no chlorophyll. They grow in cool and moist places, pastures, dung, meadows, and wood-lands. Some are commercially cultivated in caves, indoors on shelves; filled with partly decomposing plant and animal materials and in green houses, where the average temperature is relatively cool^{7,8}.

Oyster mushroom has been widely cultivated in many countries, such as India, China, US, Singapore etc. It can thrive at a wide range of temperatures and humidity, utilizing various lignocelluloses in its substrate^{9,10}.

Traditionally, morphological features are used as a basic requirement for the identification of mushrooms, while modern identification is quickly becoming molecular. The standard method of identification still combines both morphological and molecular examinations³. The most important microscopic feature for the identification of a mushroom is the spore. Their colour, shape, size, attachment, orientation, and reaction to chemical tests can be the crux of identification. Mushrooms reproduce by spores under proper conditions, and spores germinate into hyphae, which finally differentiate into the cap and stipe, called the fruit body^{2,4,7}.

Mushrooms obtain nutrients from substrates, through the network of an interwoven mass of hyphae called mycelia, at a specific level of pH¹¹. Lime is usually used to optimize the pH of the substrate during mushroom cultivation. Rapid mycelia growth of oyster mushroom takes place at pH 6.4-7.8^{2,12}, *Pleurotus* spp. can grow on a wider range of substrate materials than any other mushrooms¹³.

This study will help identify the most suitable agro-waste substrates for large-scale production of *P. pulmonarius* fruit bodies. The findings of this research will contribute to sustainable agricultural waste management, improve oyster mushroom production, enhance food security, and provide an additional source of income to Nigerian mushroom farmers.

MATERIALS AND METHODS

Study area: The study was conducted in the research laboratory of the Department of Biology, Alvan Ikoku Federal University of Education, Owerri, Nigeria (between January and April, 2026), located between Longitude 7.0 and 7.0240E and Latitude 5.0 and 5.49910N, with a humid tropical climate. Rainfall is bi-modally distributed with peaks between July and September of each year. Annual rainfall is approximately 170mm, spread between April and November each year. The air temperature ranges between 21.20°C and 33.60°C, with relative humidity of 60-83%¹⁴.

Source of culture: A pure mycelium (mother spawn) culture of *P. pulmonarius* was obtained from the Mushroom Research laboratory of the Department of Biology, Federal University of Technology, Owerri, Nigeria. Mother spawn culture of the oyster mushroom was subsequently multiplied using sterilized sorghum grains^{15,16}.

Experimental procedure: Substrates such as Coconut fibre (CF), *Raphia* palm Inflorescence (RPI), and oil palm husk (OPH), supplemented with Rice Husk (RH) were used for the study in a Completely Randomized Design (CRD) experiment². Each substrate (100%) was also supplemented with 25% and 50% rice husk at 150 g of 5 replicates, making 10 experimental units of 750 g dry weight each and 50 observations. All substrates were steeped in tap water for 2 hrs, drained, and pasteurized for 2hrs at 80°C^{15,17}, before it was inoculated with 50 g spawn of *P. pulmonarius* per replicate^{2,18}.

Measurement of morphological characters

Stipe length of fruit bodies: The effect of substrate on the stipe length of fruit bodies was determined by placing a meter rule along the length of the stipe, and readings were taken, according to Nwoko *et al.*¹⁶ and Okwulehie and Okwujiako¹⁹.

Cap diameter: This was done by placing a transparent meter rule at the margin and across the centre of the pileus and reading off the diameter¹⁹.

Fruit body number: The effect of substrate on the number of fruit bodies produced by the mushroom was determined by harvesting the mushrooms, counting and recording the number of fruit bodies for each substrate, and later comparing these values^{2,18}.

Determination of yield and biological efficiency: The total fresh weight (g) of all the fruit bodies harvested from each of the five replications was measured as the total yield of the mushroom³. The biological efficiency (BE), i.e., the percentage yield of fresh mushroom fruit bodies per dry weight (g/kg) substrate, was calculated as:

$$\text{Biological efficiency} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

Statistical analysis: Data collected was analyzed by Analysis of Variance (ANOVA) as described by Ewens and Brumberg²⁰. The Duncan Multiple Range Test (DMRT) at a probability level of 5% was used to separate the means and standard errors (S.E.).

RESULTS AND DISCUSSION

Results comparing duration of primordial initiation on various substrates showed spawn run duration of *P. pulmonarius* across various substrates. Time taken for Pinhead initiation of the mushroom varies according to substrate and substrate supplementations. Pinheads (primordia), as the name implies, are the tiny fruit bodies of mushrooms with a size greater than 0.01 mm¹⁶. In this investigation, they appeared after the completion of mycelia growth on the substrates. A pH and environmental conditions of the substrate affect the growth of the primordia directly. The comparison shows that the number of days taken for primordial initiation differs significantly. A 100% CF took 18 days to initiate primordia, while RVF 75% and RH 75% took 31 days each to initiate primordia shown in Table 1. The longest was recorded at 75% CF, which initiated fruit body primordia 32 days after inoculation (DAI). Low mycelial colonization was observed in CF, compared to that of RPI. This result is evidently supported by Taskirawati *et al.*²¹, who investigated the feasibility of using coconut husk as a substrate for the cultivation of oyster mushrooms. They reported that the percentage of mycelium colonization in the substrate significantly reduced as the quantity of coconut residue in the composite substrate was increased.

The result obtained from this study showed that as the percentage supplementation increased, the number of days for primordial initiation increased, hence the 32 days taken for the formation of primordia in 75% CF and 20 days in 50% CF. The result also showed that primordial formation started at the 18th day in 100% CF. Taskirawati *et al.*²¹ also reported a similar observation, where they recorded primordia initiation in coconut residues 15 DAI. Studies²² cultured oyster mushrooms on waste material from industry, and reported that *P. pulmonarius* took 17-20 days to complete spawn running, while pinhead formation started after 23-27 days of spawning. The result presented above showed that rice husk took 100% of 31 days to produce pinhead. The result is also in agreement with Pettipher *et al.*²³, who cultivated oyster mushrooms (*P. pulmonarius*) on cellulosic residues from rice straw. They noted that it took 35 days for complete colonization of the substrate before the initiation of primordia. Hoa *et al.*²⁴ stated that due to the fact that rice husk dries up very fast, it is advisable to use it as an additive rather than using it as a whole substrate, hence the long duration taken for it to initiate primordia.

Table 1: Comparison of the number of days taken for primordial initiation across various substrates and substrate supplementations

Substrates (g/kg)	Time taken for primordial initiation (days)
100% CF	18
75% CF	32
50% CF	20
100% RPI	24
75% RPI	31
50% RPI	30
RH 100	31
OPH 100%	No growth
OPH 75%	No growth
OPH 50%	No growth

100% CF, 75%, 50% -Coconut fibre, 100% RPI, 75%, 50%: *Raphia* palm inflorescence, RH 100: Rice husk, OPH 100%, 75%, 50%: Oil palm Husk

Table 2: Effect of different substrates on the stipe length of individual fruit bodies of *P. pulmonarius*

Substrates (g/kg)	Mean stipe length (cm)
100% CF	2.19 ^a
75% CF	2.44 ^e
50% CF	2.59 ^c
100% RPI	3.39 ^a
75% RPI	2.38 ^f
50% RPI	2.47 ^d
100% RH	2.79 ^b

100% CF, 75%, 50% -Coconut fibre, 100% RPI, 75%, 50%: *Raphia* palm inflorescence, RH 100: Rice husk. Means followed by different superscript letters (a-g) within the column are significantly different at $p \leq 0.05$

Table 3: Effect of substrates on mean pileus diameter of individual fruit bodies of *P. pulmonarius*

Substrates (g/kg)	Mean pileus diameter (cm)
100% CF	3.80 ^g
75% CF	4.54 ^f
50% CF	5.03 ^e
100% RPI	5.16 ^d
75% RPI	6.65 ^b
50% RPI	6.39 ^c
100% RH	6.78 ^a

100% CF, 75%, 50% -Coconut fibre, 100% RPI, 75%, 50%: *Raphia* palm inflorescence, RH 100: Rice husk. Means followed by different superscript letters (a-g) within the column are significantly different at $p \leq 0.05$

In the present study, 100% RPI took 24 days, while 75% RPI and 50% took 31 and 30 days to initiate primordia. The longer days taken for 75% RPI and 50% to initiate Pinheads may be a result of the effect of an increase in the concentration of the rice husk. All OPH substrates with supplementations did not support the growth of any fruit bodies.

Results in Table 2 show the mean stipe length values of *P. pulmonarius* as affected by different substrates. From the result, 100% RPI gave the highest stipe length (3.39 cm), followed by 100% RH at 2.79 cm, while the least was recorded in 100% CF (2.19 cm). This may be because rice husk provides enough aeration and complete colonization and fructification by the mushroom²³. Environmental conditions, substrate supplementation, and carbon to nitrogen ratio have been reported to affect growth yield and quality of mushrooms²⁴⁻²⁶.

Result in Table 3 represents the mean pileus diameter (cm) of *P. pulmonarius* cultivated on various substrates. Results showed that the highest pileus size was obtained from 100% RH, with a mean pileus size of 6.78, followed by 75% RPI (6.65) and 50% RPI (6.39), while the least cap/pileus size was obtained from 100% CF (3.80). The result obtained is consistent with that obtained from Stipe length, with 100% RH having the highest, while 100% CF has the lowest in both categories. Megersa *et al.*²⁵ noted that rice

Table 4: Effect of substrates on mean fresh weight of individual fruit bodies *P. pulmonarius*

Substrates (g/kg)	Mean fresh weight (g/kg)
100% CF	4.48 ^a
75% CF	3.72 ^a
50% CF	4.91 ^d
100% RPI	3.43 ^f
75% RPI	5.50 ^c
50% RPI	5.63 ^b
100% RH	5.73 ^a

100% CF, 75%, 50% -Coconut fibre; 100% RPI, 75%, 50% = *Raphia* palm inflorescence, RH 100: Rice husk

Table 5: Effect of percentage substrate inclusion on number of fruit bodies of *P. pulmonarius*

Substrates (g/kg)	Fruit body number
100% CF	34
75% CF	32
50% CF	29
100% RPI	103
75% RPI	38
50% RPI	67
100% RH	80

100% CF, 75%, 50% -Coconut fibre; 100% RPI, 75%, 50%: *Raphia* palm inflorescence, RH 100: Rice husk

bran and the seed coat of corn waste are rich in nutrients required for fungal mycelial colonization and faster sclerotial formation. High nutrient composition in RH may be responsible for the extensive mycelia produced as confirmed by Chiejina and Olufokumbi²⁶; hence, the long stipe and large pileus sizes recorded. Relatively, a smaller mushroom cap diameter is an undesirable market quality²⁷. The small pileus size, cap diameter, and B.E. recorded in CF suggest that fiber is not very effective for growing *Pleurotus* spp., as noted by Galappaththi *et al.*²⁸ with *P. flabellatus*.

Result of Table 4 the effect of substrates on the mean fresh weight of individual fruit bodies of *P. pulmonarius*. 100% RH gave the highest fresh mushroom weight of 5.73 kg, followed by 50% RPI (5.73 g/kg) and 75% RPI (5.63 g/kg). The lowest fresh fruit body weight was recorded in 75% CF (4.48 g/kg). The findings show that the mycelial growth, pileus size, stipe size, and fresh fruit body weight did not follow a regular trend. This finding is supported by Yang *et al.*²⁹ in their study of yield and size of oyster mushrooms grown on a rice/wheat straw basal substrate supplemented with cottonseed hull. Increasing the amount of rice husk in the substrates increased the uniformity of the mushroom fresh weight and enlarged the cap diameter of the oyster mushroom as observed in 100% RPI, 75% RPI, and 50% RPI. But this was also observed in CF, 75% and 50%, respectively. This may be as a result of increasing the amount of substrate, which increased the fresh weight of the mushroom²¹. The present result showed a slight deviation from the result of the influence of the agro-waste on the fruit body yield and fruit body fresh weight¹⁸.

Results in Table 5 show the effect of percentage substrate inclusions on the number of fruit bodies of the mushroom produced. Results showed that 100% RPI had the highest number of fruit bodies (103), followed by 100% RH (80). The lowest fruit body number (29) was produced by 50% CF. Although 100% RPI produced the highest number of fruit bodies, it did not give the highest yield and biological efficiency (Table 4), showing that the number of fruit bodies produced does not guarantee yield and B.E. This result is in line with the work of Chiejina and Olufokumbi²⁶; in their study on the effects of different substrates on the yield and protein content of *P. tuber-regium*, Khan *et al.*³⁰ believed that the production of a healthy sporophore depends on favourable environmental conditions, especially relative humidity, light and temperature. Most species of oyster mushroom grow and give maximum production at more than 85% humidity, 400-500 lux and 15-25°C temperature³¹. The result above shows that *P. pulmonarius* performed better on RPI compared to CF and OPH, which failed to produce any fruit body.

Table 6: Relationship effect between stipe length (SL (cm)), cap size (CS)

	SL	CS	FBWT	FBN
SL	1.000	0.1995**	0.2447**	0.35007**
CS	-	1.000	0.5669**	-0.0261 ^{ns}
FBWT	-	-	1.0000	-0.0897 ^{ns}
FBN	-	-	-	1.0000

SL. (cm): Sstipe length, CS (cm): Cap size, FBWT (g/kg): Fruit body weight, FBN: Fruit body number. pearson correlation co-efficient, N= 355. $P > /r/$ under H_0 : $R_h O$: 0, r : 1.000. *: Significant correlation, **: Highly significant correlation, ***: Very highly significant correlation, ns: No significant correlation. Values are means of 5 replicates

Table 7: Effect of different substrates on the yield (g/kg) and biological efficiency (%) of *P. pulmonarius*

Substrates (g/kg)	Yield (g/kg)	BE (%)
100% CF	139.00	18.53 ^e
75% CF	108.00	14.40 ^g
50% CF	114.00	15.20 ^f
100% RPI	346.00	46.13 ^c
75% RPI	186.00	24.80 ^d
50% RPI	349.00	46.53 ^b
100% RH	438.0	58.40 ^a
OPH 100%	No growth	
OPH 75%	No growth	
OPH 50%	No growth	

100% CF, 75%, 50% -Coconut fibre, 100% RPI, 75%, 50%: *Raphia* palm inflorescence, RH 100: Rice husk, OPH 100%, 75%, 50%: Oil palm Husk and BE: Biological efficiency

Table 6 presents a Pearson correlations coefficients which shows the levels of relationship between SL, CS, FBWT, and FBN of the individual fresh fruit bodies of *P. pulmonarius* on different substrates. Stipe length (SL) exhibited significant positive correlations with CS ($r = 0.1995$, $p < 0.001$), FBW ($r = 0.2447$, $p < 0.001$) and FBN ($r = 0.3501$, $p < 0.001$). The results shows a weak to moderate correlations and also indicate that increase in SL is associated with increase in CS, FBW and FBN of the cultivated *P. pulmonarius* fruit bodies. Therefore, the results suggests that increase in the overall fruitbody size is associated with increase in FBW¹⁹. However, these size traits do not translate into more FBN, indicating that both fruitbody production and size may be as a result of different or environmental factors^{15,18}.

The results of yield and BE of *P. pulmonarius* as affected by different substrates and substrate supplementations showed that 100% RH gave a significantly higher yield and BE (438.0 g/kg and 58.40%) shown in Table 7, respectively. This was followed by 50% RPI (349.0 g/kg) and 46.53%, respectively. 75% CF gave the lowest yield and BE (108.0 g/kg) and 14.40%, respectively. This result supports the fact that *P. pulmonarius* is in the class of edible mushrooms that has a high saprophytic ability and are capable of growing on a variety of lignocellulosic wastes³². This causes the bioconversion of cellulosic wastes into edible biomass¹⁸. Cellulose is singled out as the main characteristic feature of substrates in order to be deemed fit for the cultivation of oyster mushrooms. This is because they belong to the kingdom Fungi and as such require cellulose, which is finally produced in decomposition, for their survival and growth³¹. A 100% RH gave the highest yield and B.E.%. This is probably because not only is it rich in cellulose, it also contains and retains a high volume of moisture. However, Liu *et al.*³³ reported that RH sawdust gave maximum yield in their own study of *P. pulmonarius*, and the least was its leaves, while Kuforiji and Fasidi¹² maintained that substrates like corn cobs, rice straws, and cotton waste supported the growth of *P. tuber-regium*.

Studies¹⁴ investigated paper wastes supplemented with rice husk, chicken manure, and peat for *P. pulmonarius* cultivation and noted that the highest yield for fresh weight was recorded in the substrate containing 20% rice husk. Stanley and Umolo⁷ agreed that unsupplemented corn cob gave the highest yield from his own study. This may probably be why 100% RH gave the highest yield and biological efficiency. On the supplementation of coconut and *Raphia* wastes with 25% and 50% rice husk, 50% RPI gave the highest yield and BE of 349.0 g/kg and 46.53%, respectively. Studies²⁴ stated in their study that

among the different concentrations of rice husk used as an additive for the cultivation of *P. pulmonarius*, rice husk at 2% supplementation supported the best yield and growth of the fungus. This may be why 50% RPI gave a better yield than 75% RPI. Pettipher²³ also agree that the lower the supplementation, the better the yield as well as the biological efficiency. However, yield and BE on CF were lower than those of RPI. Mycelial colonization in coconut husk was considerably low, compared to RPI with very dense mycelial colonization. This finding was supported by Taskirawati *et al.*²¹ from their experiment with RPI and other agro-waste substrates. Thus, Amerikanou *et al.*³⁴ stated that the yield of the mushrooms is directly dependent on the spread of mycelium into the substrate.

CONCLUSION

In this study, CF substrates initiated fruiting body primordia earlier compared to RPI and RH substrates, while oil palm husk (OPH) failed to support any observable growth throughout the experiment.

Regarding yield performance and biological efficiency, RH 100% demonstrated the highest yield (438.0 g/kg) and biological efficiency (54.84%), followed by RPI 50% (349.00 g/kg and 46.53), indicating its suitability in the production of the oyster mushroom.

It is therefore recommended that rice husk (RH) be utilized as an effective substrate for the commercial cultivation of oyster mushrooms. Conversely, OPH is not suitable for *P. pulmonarius* production and should not be considered for use in commercial mushroom production, due to its inability to support fungal growth.

SIGNIFICANCE STATEMENT

This study demonstrates the potential of locally available agro-waste substrates for sustainable cultivation of *Pleurotus pulmonarius*. Rice husk, Raphia palm inflorescence, and coconut fiber supported improved yield and desirable morphological characteristics, whereas oil palm husk was unsuitable. These findings promote cost-effective waste valorization, environmentally sustainable mushroom production, and provide practical substrate recommendations for commercial cultivation.

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