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Synergistic Substrate Formulation for Enhanced Oyster Mushroom Production Using *Tectona grandis* Leaves, Waste Paper, and Straw

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ABSTRACT

A biotechnologically viable way to convert lignocellulosic waste into high-protein, nutritional food is through mushroom cultivation. The purpose of this study is to examine the efficacy of *Tectona grandis* leaves combined with wheat straw as a growing medium for *Pleurotus sajor-caju* oyster mushrooms. The productivity and biological efficiency (BE) of various substrate compositions were assessed in a number of experiments. The highest BE of 43.1 % was achieved with a substrate mix that included 50% waste paper and 50% rice straw; the lowest BE of 11.52% was attained with waste paper alone. The study also looked into the potential use of sodium nitrate and *Tectona grandis* leaves as dietary supplements in the substrate mixture. The findings demonstrated that compared to standard substrates, a combination of *T. grandis* leaves, discarded paper, and straw produced a significantly higher number of mushrooms. Strong mycelial growth and the development of fruiting bodies were the results of further increasing the nutritional content with the addition of sodium nitrate.

Keywords Oyster mushroom cultivation, Substrate formulation, *Tectona grandis leaves,* Waste paper, Sodium nitrate supplementation, Biological efficiency, Sustainable agriculture.

INTRODUCTION

Due to its potential as a sustainable and profitable agricultural practice, oyster mushroom (*Pleurotus* spp.) production has attracted a lot of interest lately (Wan Mahari *et al.* 2020). The medical benefits, high nutritional value, and very quick growth in comparison to other mushroom species make oyster mushrooms highly prized. Furthermore, they are especially well-suited for cultivation in a variety of environmental circumstances due to their capacity to grow on a broad range of substrates (Ivarsson *et al.* 2021). The type of substrate that mushrooms grow on is essential to their effective cultivation. The substrate facilitates the growth of the mushroom mycelium by giving it the necessary nutrition and support to develop into a fruiting body. The choice and make-up of suitable substrate materials have a significant impact on the production, quality, and overall performance of mushroom cultivation projects (Silva *et al*. 2024).

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Under these circumstances, investigating substitute substrate formulations has become an important field of study. In addition to providing an affordable substrate for mushroom cultivation, the use of waste materials from forestry and agriculture offers a sustainable approach to waste management (Koul *et al.* 2022). Waste paper, straw, and leaves from *Tectona grandis* (Teak) are examples of plentiful and easily accessible biomass sources that could be used again for mushroom farming (Balan *et al.* 2022). A viable and sustainable method of turning lignocellulosic waste into nutrient-dense, protein-rich food is through mushroom farming. Due to their high concentration of vital nutrients like folic acid, vitamins B1, B3, B5, B12, C, and D, as well as protein, fiber, and minerals like calcium, iron, zinc, phosphorus, potassium, selenium, and zinc, oyster mushrooms, in particular (Effiong *et al.* 2024), are considered functional foods. Optimizing mushroom production is of great interest due to its health advantages and the increasing demand for sustainable agriculture practices.

The purpose of this study is to assess the oyster mushroom culture efficacy of a substrate mix that includes straw, scrap paper, and *T. grandis* leaves. Additionally, the study investigates how adding sodium nitrate to the substrate affects the nutritional content of the substrate and how that impacts mushroom growth and yield. This study aims to optimize oyster mushroom production and help sustainable agriculture and waste valorization initiatives by offering insights into sustainable substrate formulations and cultivating practices (Antunes *et al.* 2020).

MATERIALS AND METHODS

Teak (*Tectona grandis*) leaves, a vital component of the lignocellulosic substrate, are a rich source of organic matter and essential nutrients, making them an ideal environment for mushroom proliferation. Waste paper, a cellulose-rich substance, acts as the primary carbon source in the substrate mix, facilitating the colonization of fungus and metabolic processes essential for mycelium development. Straw, derived from agricultural byproducts like wheat, rice, or barley, is purified and chopped to enhance its surface area and stimulate microbial activity. Straw functions as a foundational component, facilitating the establishment of mushroom mycelium and fruiting bodies. It also improves the aeration of the substrate and helps retain moisture, creating an optimal microclimate for mushroom cultivation.

Table 1. Combination of substrates used for oyster mushroom cultivation.

Treatment	Rice straw (%)	Waste paper (%)	Teak Leaves (%)
T1	100	0	0
T2	0	100	0
T3	0	0	100
T4	50	50	0
T5	50	0	50
T6	0	50	50
T7	30	30	40

Nitrogen fertilizer, sodium nitrate, is a water-soluble chemical used as a nutrient supplement to enhance the nutritional content of substrates. It provides easily accessible nitrogen, crucial for fungi growth and protein production (Asadu *et al.* 2024). The addition of sodium nitrate increases the nitrogen available in the growing medium, stimulating mycelium growth and accelerating oyster mushroom fruiting bodies. The substrate mix used in culture trials was carefully combined in specific amounts to ensure optimal nutritional content. Table 1 illustrates different substrate combinations for different treatments.

Spawn preparation

The initial preparation of substrates involved submerging them in hot water at 60°C for four hours to eliminate pathogens and microorganisms. This process maintained the substrate's structural integrity and nutritional characteristics. After draining excess water, the substrates were cooled to ambient temperature. A pH buffering solution of 1% calcium carbonate was added to boiled grains and filled in polypropylene bags (250g/bag) to improve the substrate's conditions for mushroom mycelium growth and metabolic processes. Additionally, sodium nitrate ($NaNO_3$) was added as a nutritional supplement, with a concentration of 5 g per kilogram of substrate. The bags were cooled and inoculated with pure oyster mushroom culture in a laminar flow (Chukwu *et al.* 2022). The inoculated bags were incubated for 15 days at 25 °C or till complete mycelia colonization

(Chan *et al.* 2021).

Substrate preparation, sterilization, bag filling and spawning

The substrate components were carefully and systematically mixed based on the experimental design, with three replicates created for each formulation. The materials used in the experiment were pure straw, pure paper waste, and pure *Tectona grandis* leaves. Additionally, different combinations were tested, including a 50:50 mixture of paper and straw, a 50:50 mixture of paper and leaves, a 50:50 mixture of leaves and straw, and a mixture of straw, paper, and leaves in proportions of 30:30:40, respectively. The substrates were evenly divided into polypropylene bags, with dimensions of 35 × 55 cm, with each bag containing up to 3000 g. Placing layers of substrate involved spreading a pure culture of oyster mushroom in a clean environment. This was done with a laminar flow hood at a spawning rate of 2%. In order to enhance the circulation of air and encourage the growth of mycelium, a wooden stick was used to cut 10-15 holes in each bag.

Incubation and spawn run

The bags were placed in a controlled environment at a temperature of 25 ± 2 °C. The humidity in the environment was maintained at 70–85% by spraying water twice daily. The incubation phase lasted 15 to 20 days, during which the bags were regularly observed to ensure full mycelial colonization. Opening the bags allowed the fungal mycelium to fully colonize them and begin the process of producing fruiting bodies. Data was collected about the occurrence of pinhead formation, the date of the initial harvest, and the yield throughout a maximum of three harvests. The duration of the first, second, and third harvests was also recorded to evaluate the growth performance and productivity of the various substrate compositions.

Mushroom pins emerge from the substrate and undergo a gradual process of maturation, transforming into fully developed fruiting bodies over a period of many days to weeks. When they reach maturity, they are collected by delicately twisting or severing them from the surface they are attached to. Post-harvest management includes additional processes such as pasteurization or composting to prepare the substrate for future use in subsequent crop cycles. Utilizing recycled or composted substrates can further enhance sustainability in mushroom production methods. By adhering to these protocols, cultivators can optimize the success of oyster mushroom growth and attain superior mushroom yields.

Parameters measured and methods of data collection

Oyster mushroom cultivation tests entail assessing multiple parameters to determine growth performance, yield, and quality. The rate at which mycelium grows is an important indicator of how well a substrate is suited for fungal development and how strong the fungus is. Typically, the amount of substrate that the fungus colonizes over time is what determines this. The emergence of fruiting bodies is crucial for successful cultivation, and it involves monitoring the quantity, size, and quality of these structures. Mushroom yield refers to the overall weight of mushrooms that are collected from a specific amount of substrate. It is calculated by weighing the mushrooms obtained from each replicate or treatment group. Biological efficiency (BE) quantifies the effectiveness of mushroom production in relation to the substrate utilized, where higher BE values indicate a superior ability to transform substrate into mushrooms (Liang *et al.* 2019).

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

RESULTS AND DISCUSSION

Spawn run duration

The duration of the spawn run differed among several treatments, spanning from 16.0 to 21.4 days (Table 2). The treatment with the shortest incubation period was T4 (R1), which involved a mixture of 50% waste paper and 50% rice straw and was completed in 16.0 days. On the other hand, treatment T1 (R3) achieved the longest duration of 21.4 days by exclusively using 100% rice straw. Oyster mushrooms exhibited vigorous growth and produced large yields when planted

Table 2. Effect of different substrates and supplements on days required for spawn run.

Treatments	Replication treatment	Spawn run days	Days to pin head formation	Days to first harvest	Days to second harvest	Days to third harvest
T1	R1	20.0	18.00	39.00	47.20	66.50
	R2	19.6	18.80	37.60	48.00	63.80
	R3	20.4	18.50	36.20	51.60	61.00
T2	R1	-	-	-	-	-
	R2	-	-	-	-	-
	R3	-	-	-	-	-
T3	R1	17.8	15.00	30.00	53.20	65.80
	R2	20.6	15.60	29.00	44.50	56.20
	R3	21.4	15.20	29.60	43.80	65.00
T4	R1	16.0	18.00	38.80	46.50	67.20
	R2	16.8	17.40	38.80	47.20	61.00
	R3	18.0	16.20	30.50	48.00	61.50
T5	R1	20.2	15.40	37.20	47.00	65.50
	R2	21.2	15.20	34.80	44.50	60.60
	R3	19.6	15.20	28.00	45.00	64.00
T6	R1	17.0	14.00	28.00	35.60	55.10
	R2	18.6	15.20	29.00	45.40	63.90
	R3	20.0	15.20	39.20	44.60	55.50
T7	R1	16.8	17.00	27.00	39.80	55.60
	R2	16.6	16.80	34.80	46.00	64.00
	R3	17.0	15.40	32.00	44.80	66.80

on groundnut processing waste. Similarly, excellent outcomes were found when grown on lignocellulosic substrates such as paddy straw, wheat straw, and soybean straw.

Initiation of pinhead formation and fruiting

Once the mushroom mycelium completely colonized the substrate, indicating the completion of the vegetative development phase, the process of fruiting began. The transition from vegetative to reproductive growth was achieved through the utilization of several methods, such as reducing temperature or improving fresh air circulation. The manipulation of fruiting stimuli, including light exposure and environmental fluctuations, was executed with great attention to detail to ensure the successful initiation of fruiting. For each treatment, observations about the number of days required for pinhead formation were made (Table 2). According to the results, treatment T6 (R1), which used a 50% waste paper and 50% leaf mixture, took the least amount of time of just 14.0 days to develop pinheads. In contrast, treatment T1 (R1), which employed 100% rice straw, had the longest duration (18.8 days).

On the substrate's surface, mushroom primordia, or pins, start to form in the right environmental circumstances. Depending on the type of mushroom and the particular climatic conditions, these pins progressively mature into fruiting bodies over the course of many days to weeks (Herman & Bleichrodt 2022). When the mushrooms are ready to be harvested, they are sliced or twisted carefully out of the substrate (Fig. 1).

Days required for harvest of mushrooms

The study tracked the number of days required for the first, second, and third harvests of oyster mushrooms, with results detailed in Table 2. The data revealed that the shortest time to reach the first, second, and third harvests was observed in treatment T7 (R1), consisting of 30% rice straw, 30% waste paper, and 40% leaves. The recorded times for this treatment were 27 days for the first harvest, 39.80 days for the second, and 55.60 days for the third. In contrast, the longest durations were associated with treatment T6 (R3), where the times to harvest were 39.2 days for the first, 44.60 days for the second, and 55.60 days for the third harvest.

Fig. 1. Different stages of mushroom development on polybags.

Table 3. Effect of different substrates and supplements on harvest and biological efficiency.

Treatments	Replications treatments	Mushroom substrate (g)	Harvest 1 (g)	Harvest Harvest 2 (g)	Harvest 3 (g)	Total yield (g)	Biological efficiency (%)
T1	R1	1398	267	26	86	379	37.9
	R2	1263	88	30	78	196	19.6
	R3	1105	205	125	20	350	35.0
T2	R1	2400	-	-	-	0	0
	R2	2900	-	-	-	0	0
	R3	3100	-	-	-	0	0
T3	R1	1360	78	40	23	141	14.1
	R2	1206	58	30	27	115.2	11.52
	R3	1464	86	120	55	261	26.1
T4	R1	2200	253	27 g	30	310	31.0
	R2	2100	105	50 g	127	282	28.2
	R3	2200	290	43 g	97	430	43.0
T5	R1	1535	206	39 g	79	324	32.4
	R2	1348	183	105	101	389	38.9
	R3	1373	200	150	20	370	37.0
T6	R1	2200	94	73	80	247	24.7
	R2	2100	91	98	45	234	23.4
	R3	2200	97	120	70	287	28.7
T7	R1	1663	200	20	10	230	23.0
	R2	2200	178	64	30	272	27.2
	R3	1982	96	165	25	286	28.6

Yield and biological efficiency

The flush-wise yield and biological efficiency (BE) findings are summarized in Table 3. With a BE of 43% and a fresh mushroom yield of 430 g/kg of dry substrate, treatment T4 (R3) had the highest yield. On the other hand, the treatment consisting solely of leaves (T3) produced the least amount of dry substrate of only 115.2 g/kg with a BE of 11.52%. Notably, the waste paper alone treatment (T6) produced no mushroom yield. These results highlight the significance of supplementing substrates, especially those with low protein content, in order to encourage the best possible growth and yield of mushrooms.

CONCLUSION

According to the study, *Pleurotus sajor-caju* oyster mushrooms can be grown on *Tectona grandis* leaves and wheat straw as a sustainable alternative. According to the study, waste paper by itself had a low biological efficiency of 11.52%, while waste paper combined with rice straw had a high biological efficiency of 43.1%. By adding *T. grandis* leaves, mushroom production increased by 35.8%. Stronger mycelial growth and quicker fruiting bodies were encouraged by the addition of sodium nitrate as a nutrient supplement to the substrates. This strategy aids in efficient waste management in addition to encouraging sustainable farming methods.

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