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Comparative study of growing edible oyster mushrooms (*Pleurotus ostreatus*) using different cultivation techniques and organic substrates in Guyana

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ABSTRACT

Mushrooms are vital decomposers in ecosystems, breaking down complex organic matter and lignocellulosic substrates. Growing substrates like wheat straw, vermicompost, wood shavings, and coconut coir vary in their nutrients and moisture retention capacity, which are crucial for mushroom growth and are key for optimizing yield and quality. The bag method supports large-scale production and ease of management, while the bottle method allows for precise control and is ideal for smaller batches or experiments. The study investigated the growth and nutritional content of oyster mushrooms (*Pleurotus ostreatus*) using different types of substrates and cultivation techniques. Four treatments were tested: T1 (wheat straw and vermicompost), T2 (coconut coir and vermicompost), T3 (wood shavings and vermicompost), and a control substrate (C - wheat straw). These treatments were applied using two cultivation methods, bags and bottles. *Pleurotus ostreatus* on wheat straw are lighter in colour, while those on coconut coir are darker due to substrate composition. Ordinal rank analysis indicated that both cultivation methods (bag and bottle) produced similar results across six physical parameters, with the control substrate ranking first in bags and T1 ranking first in bottles. Statistical analysis using the Kruskal-Wallis test indicated no statistically significant differences in *Pleurotus ostreatus* cap thickness ($p = 0.09509$), stipe length ($p = 1$), cap diameter ($p = 0.6514$), stipe thickness ($p = 0.4621$), or fresh and dry weights ($p = 0.1173$) across treatments and cultivation methods. Vermicompost in addition to other substrates boosts mushroom nutrients. Our data showed that wheat straw with vermicompost in bottles is the most effective and sustainable substrate combination for cultivating *Pleurotus ostreatus.*

Keywords: Oyster mushrooms, cultivation techniques, growth parameters, organic substrates, elemental composition, yield performance

Mushrooms such as *Pleurotus ostreatus* (oyster mushrooms) have gained significant attention due to their adaptability, rapid growth, and nutritional value (Sánchez, 2010). Cultivation of oyster and button mushrooms has evolved significantly driven by technological advancements, commercial demands, and environmental considerations. Oyster mushrooms, which are saprophytic organisms, obtain nutrients by breaking down complex lignocellulosic materials, making them essential decomposers in their ecosystems (Van der Westhuizen and Eicker, 1994; Seecharran *et al*., 2018).

Substrate choice is a critical factor in mushroom cultivation, influencing both yield and nutritional content. Organic substrates such as vermicompost,

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wheat straw, coconut coir, and wood shavings have been widely studied for their effectiveness in mushroom growth. Research conducted in Guyana demonstrated that vermicompost and rice straw are particularly effective, with vermicompost leading to higher nutrient content and better growth parameters in oyster mushrooms (Seecharran *et al*., 2018; Singh *et al*., 2021). Additionally, the use of autoclavable bags in these studies helped maintain sterile conditions, crucial for preventing contamination during the cultivation process (Smith *et al*., 2022).

Mushrooms like oyster and button varieties are highly valued for their nutritional benefits. Oyster mushrooms, for instance, are rich in essential minerals such as potassium, phosphorus, and iron, as well as B vitamins like riboflavin and niacin (Majesty *et al*., 2018). They also contain bioactive compounds like beta-glucans, which have been shown to possess immunomodulatory and cholesterol-lowering properties (Chugh *et al*., 2022). Similarly, button mushrooms are a good source of antioxidants, which help protect against oxidative stress and may reduce the risk of chronic diseases (Gallego *et al*., 2021).

From an economic perspective, mushroom cultivation presents significant opportunities, particularly in rural communities. It offers a sustainable means of livelihood, providing both food security and income generation. The collaboration between Guyana and China in enhancing mushroom cultivation capabilities underscores the growing importance of this agricultural sector in the region.

This research aimed to identify the most suitable and effective cultivation technique and organic substrate for oyster mushroom cultivation. The study intended to investigate the impact of these techniques and substrates on key growth parameters and yield in Guyana and composition of nutritional qualities such as nitrogen, phosphorus and potassium (NPK), etc. in oyster mushrooms (*P. ostreatus*) grown in three different organic substrates (wheat straw, coconut coir, wood shavings).

MATERIALS AND METHODS

Wheat straw, coconut coir, and wood shavings were used to cultivate *P. ostreatus* as per the bottle and bag method earlier described (Kaur *et al*., 2021; Johnnie *et al*., 2023; Seecharran *et al*., 2018). Vermicompost was made following the method described by Ansari (2010). The cultivation trials were conducted during four months of February 2023 to May 2024 at the mushroom house at the University of Guyana, Turkeyen Campus, Guyana.

According to Seecharran *et al*. (2018), different growth parameters of mushrooms were measured i.e. number of pins, fruit bodies, size, weight of the fruiting bodies (fresh and dry) and elemental composition (substrates and fruiting bodies). The University of Guyana's Department of Chemistry and the Government Analyst, Food and Drug Department tested samples of mushrooms for N, P, and K.

Preparation of the mushroom spawn

Pure cultures of *Pleurotus ostreatus* were maintained on Potato Dextrose Agar plates. Mushroom spawn was prepared using white millet bird seeds. Millet seeds were soaked for 12 hours, boiled for 10-15 minutes, and dried for 12 hours. Seeds were filled in glass bottles, sealed with cotton gauze, and autoclaved at 121°C for 2 hours. After cooling, three mycelial discs from the actively growing mother culture were inoculated into each bottle using a sterile spatula. The bottles were stored in a dark place until fully colonized by mycelia (Seecharran *et al*., 2018).

Cultivation of oyster mushroom

Oyster mushrooms (*Pleurotus ostreatus*) were grown on four substrate treatments: the control (C) with wheat straw, T1 with wheat straw and vermicompost, T2 with coconut coir and vermicompost, and T3 with wood shavings and vermicompost. The growth of mushrooms was observed for each treatment.

Treatments used in the study

Sr No	Treatments	Composition
1	T1-Bt	100g wheat straw and 100g vermicompost + 2 g Calcium carbonate in bottles
2	T1-Bg	100g wheat straw and 100g vermicompost + 2 g Calcium carbonate in bags
3	T2-Bt	100g coconut coir and 100g vermicompost + 2 g Calcium carbonate in bottles
4	T3-Bg	100g wheat straw and 100g vermicompost + 2 g Calcium carbonate in bags
5	T3-Bt	100g wood shavings and 100g vermicompost +2 g Calcium carbonate in bottles
6	T3-Bg	100g wheat straw and 100g vermicompost + 2 g Calcium carbonate in bags
7	C-Bt	Control (200 g Wheat Straw + 2 g Calcium carbonate) in bottles
8	C-Bg	100g wheat straw and 100g vermicompost + 2 g Calcium carbonate in bags

Preparation of vermicompost

Three 4-gallon compost units were set up with a base to top layer with broken pebbles (4.5 cm), coarse sand (5 cm), loamy soil (6 cm), damp rice straw (200 g), and 100 g of cattle dung. Each unit contained 50 earthworms (*Eisenia foetida*). Finally, an additional 200 g of damp rice straw was added, and the units were watered. Organic materials were added twice a week. After about 30 days, watering was stopped for 2-3 days to allow the earthworms to settle. The vermicompost was then harvested and prepared for bagging once it reached the correct moisture content (Ansari, 2010).

Preparation of one time used plastic bottles

Single-use plastic bottles were cleaned with soap and water, then disinfected overnight in a 6% sodium hypochlorite solution. The following day, the bottles were rinsed twice with distilled water and dried. Finally, the bottles were cut in half with a box cutter.

Mushroom cultivation and harvesting

Wheat straw was dried, cut, soaked, and pasteurized. A mix of 200 g straw and 2 g calcium carbonate with 60 ml water was autoclaved. After cooling, it was inoculated with colonized bird seed, sealed, and incubated for the bag technique. Sterilized substrate mixtures were cooled and placed into reassembled plastic bottles for the second technique type with cotton wool plugs to ensure aeration. After mycelium colonized, slits were made for fruiting bodies, which were then watered daily and exposed to light after 14 days. Similar to the control, but with 100 g vermicompost added to the substrate. Coconut coir was processed and mixed with 100 ml vermicompost. The mixture was autoclaved, inoculated, and incubated similarly to the control. Wood shavings were processed and combined with 100 g vermicompost. The mixture followed the same steps as the other phases. Mature fruiting bodies were harvested by gently twisting the stipe. The substrate was continuously watered and aerated following the initial harvest. Measurements were taken for size of fruiting bodies, texture, number of pins and clustered fruiting bodies, color, and fresh & dry weights of fruit bodies. The dried fruit bodies were ground and stored for analysis, with each sample needing about 5 g. Both original and final substrates were prepared for elemental analysis.

Elemental Analysis

A 5 g mushroom sample was digested with sulfuric acid and a catalyst, heated gradually to 350°C with hydrogen peroxide added, until the solution was clear. After cooling, the solution was transferred to a

flask, diluted, and filtered. It was then analyzed for nitrogen (Kjeldahl automated analyzers), phosphorus (spectrophotometry), and potassium (Atomic absorption spectrophotometer).

Data Analysis

An ordinal rank analysis was conducted in Excel to compare data across different cultivation methods (bag and bottle). Bar graphs and summary tables were created using the 'ggpubr' package in R. The Kruskal-Wallis test, a non-parametric one-way ANOVA based on ranks, was used to identify significant differences ($p<0.05$) in physical parameters.

RESULTS AND DISCUSSION

The study assessed the growth of oyster mushrooms using various treatments and cultivation methods. Treatment T1-Bt emerged as the most effective, yielding the highest number of fruiting bodies and superior growth parameters, including cap diameter and thickness (Tables 1 and 2). Treatments such as T2-Bg and T2-Bt also demonstrated significant growth but were less effective than T1-Bt Conversely, T3 treatments were ineffective, showing no growth.

The analysis of growth metrics revealed that the Bottle method, particularly with Treatment 1, resulted

Table 1. Total growth parameters of fruiting bodies at harvest

Treatments	No. of cluster fruiting bodies	No. of pins fruiting bodies
C-bg	9.00	59.00
C-bt	8.00	50.00
T1-bg	12.00	56.00
T1-bt	13.00	88.00
T2-bg	12.00	62.00
T2-bt	7.00	83.00
T3-bg	No growth	No growth
T3-bt	No growth	No growth

Table 2. Growth parameters of fruiting bodies at harvest (Mean ±SD)

Treatments	Growth Parameters				
	Trials No.	Stipe length (mm)	Stipe thickness (mm)	Cap diameter (mm)	Cap thickness (mm)
C-bg	1st trial	23.8±7.3	3.3±2.2	27.7±8.0	3.5±2.0
C-bt	1st trial	20.9±5.9	5.8±1.8	40.3±10.0	3.1±0.3
C-bg	2nd trial	20.3±3.6	5.1±1.3	32.0±7.5	0.9±0.3
C-bt	2nd trial	30.2±6.1	5.0±1.3	40.0±10.5	1.3±0.4
T1-bg	1st trial	19.8±6.3	3.2±1.3	22.4±8.8	0.8±0.2
T1-bt	1st trial	20.2±7.8	5.7±1.7	40.2±11.1	2.6±1.3
T1-bg	2nd trial	31.8±10	5.0±1.5	42.8±10.5	2.0±0.7
T1-bt	2nd trial	20.6±4.5	1.8±0.7	10.7±2.3	0.7±0.2
T2-bg	1st trial	17.0±8.7	5.3±2.9	35.4±11.0	2.9±1.1
T2-bt	1st trial	19.77±5.5	1.5±0.7	31.6±10.0	1.0±1.0
T2-bg	2nd trial	23.6±4.8	5.2±1.2	17.5±4.8	1.0±1.1
T2-bt	2nd trial	36.7±1.2	14±0.8	49.0±7.3	2.7±1.2
T3-bg	-	-	-	-	-
T3-bt	-	-	-	-	-

in the highest production of fruiting bodies and pins. Despite this, there were no statistically significant differences in cap thickness, stipe thickness, stipe length, or cap diameter across treatments and methods. These findings suggest that while T1-Bt is the best treatment for increasing mushroom yield and growth parameters, the cultivation method (Bag vs. Bottle) does not significantly influence the physical characteristics of the mushrooms.

Ordinal rank analysis revealed that both bag and bottle cultivation methods performed similarly across six physical parameters, meaning neither method significantly affects mushroom quality. For treatments, 'Control' and 'Treatment 1' showed the best results, with 'Treatment 2' consistently ranking lower. T1-Bt in the 1st trial was the top performer, but variability between trials highlights the need for multiple trials to confirm results.

Mushrooms grown in bags (Bg) had higher moisture content and yields compared to those in bottles (Bt). For example, the C-Bg treatment had a fresh weight of 93.89 g with 73.59% moisture, while C-Bt had a fresh weight of 50.75 g and 73.87% moisture. Treatment 1 (T1) consistently showed higher moisture content and weight, especially in bags, with T1-Bg at 77.17% moisture and a fresh weight of 133.64 g (Table 3). A Kruskal-Wallis test showed no significant differences in weight across treatments and cultivation methods (chi-squared = 8.8, df = 5, p-value = 0.1173), suggesting that weight variability is likely due to random factors rather than systematic effects.

Mushroom cultivation, particularly of *Pleurotus ostreatus*, relies heavily on substrate choice, which affects moisture content, fresh weight, and dry weight of the mushrooms, influencing their freshness and overall yield. High moisture content, indicative of fresher mushrooms, was observed in Treatments T1-Bg and T2-Bg, with moisture contents of 82.06% and 79.28%, respectively, in the second trial. This aligns with the findings of Gogavekar *et al*. (2014), Morais *et al*. (2000), Sánchez (2010), and Ahmed *et al*. (2013), who reported that optimal mushrooms contain about 90% water. The ability of vermicompost to retain moisture, as demonstrated by Singh *et al*. (2021), likely contributed to the higher moisture content observed in these treatments.

T1 and T2 treatments, particularly in plastic bottles, outperformed autoclavable bags in fruiting body formation and structural characteristics, with vermicompost enhancing clustering and growth (Yamanaka, 2017; Jo *et al*., 2008). Plastic bottles also better controlled contamination due to reduced

Table 3. Weight and moisture content for fruiting bodies at harvest (Mean ±SD)

Treatments	Weight of fruiting bodies		
	Fresh weight (g)	Dry weight (g)	Moisture % ± SD
C-Bg	46.95±7.69	12.40±3.17	71.84±5.21
C-Bt	25.38±8.06	6.63±2.08	73.85±2.86
T1-Bg	133.64±71.78	30.51±3.58	74.15±11.22
T1-Bt	63.45±5.79	20.90±3.46	67.19±2.48
T2-Bg	88.89±49.74	22.46±7.87	72.99±6.93
T2-Bt	27.64±25.28	7.18±7.30	76.09±4.52
T3-Bg	0.00±0.00	0.00±0.00	0.00±0.00
T3-Bt	0.00±0.00	0.00±0.00	0.00±0.00

exposure during inoculation, unlike autoclavable bags (Seecharran *et al*., 2018). Vermicompost combined with substrates like wheat straw or coconut coir significantly improved yield and quality, including stipe length, thickness, and cap diameter, while wood shavings (T3) proved ineffective, producing no fruiting bodies (Girmay *et al*., 2016; Singh *et al*., 2021). These findings suggest that substrate selection is crucial for optimizing mushroom cultivation.

Both bag and bottle methods yielded similar mushroom characteristics, though bottles might offer better environmental control. This supports the flexibility in choosing cultivation methods without compromising quality, with specific tailoring based on substrate and environmental conditions (Singh *et al*., 2021; Johnnie *et al*., 2023). If button mushrooms *Agaricus bisporus* had been inoculated alongside *Pleurotus ostreatus*, different outcomes would have been anticipated due to their distinct substrate preferences, as noted by Seecharran *et al*. (2018).

The fresh and dry weights also varied across treatments, with T1-Bg showing the highest fresh weight of 184.40 g in the second trial and a corresponding dry weight of 33.04 g. This supports Ahmed *et al*. (2013) and Samuel and Eugene (2012), who found that higher moisture content correlates with greater fresh weights, indicative of higher yields. The Kruskal-Wallis test indicated no significant differences in mushroom weights across treatments, suggesting that substrate variations and cultivation methods did not markedly impact the final weights.

Vermicompost, known for its rich microbiota and nutrients (Tomati *et al*., 1987; Edwards and Burrows, 1988), improved mushroom growth in Treatments T1 and T2. This finding is consistent with Seecharran *et al*. (2018) but contrasts with El-Sayed *et al*. (2019), who found significant differences in growth parameters across various substrates. Calcium carbonate was consistently used in all treatments to buffer pH and ensure structural integrity, ensuring observed differences were due to substrate and vermicompost composition.

Wheat straw, imported and used due to logistical reasons, outperformed other substrates like wood shavings, aligning with findings by Sharma *et al*. (2013) and Yang (2013). Coconut coir, noted for its moisture retention, also proved effective, consistent with Purnomo *et al*. (2022). Using organic substrates like vermicompost aligns with sustainable practices (Ansari, 2010).

The Nutritional analysis of the fruiting bodies grown in different treatments suggested that the substrate added with vermicompost is the most effective substrate for enhancing nutrient uptake in oyster mushrooms, while other substrates may need additional nutrient supplementation. Earlier reports also showed the similar results that the mushroom grown on substrate supplemented with vermicompost has the highest nutrient content, with 3.96% nitrogen, 1.87% phosphorus, and 4.17% potassium (Seecharran *et al*., 2018). Rice straw, with 3.28% nitrogen, 0.90% phosphorus, and 3.43% potassium (El-Sayed *et al*., 2019), is also a good substrate but not as nutrient-rich as vermicompost. Coconut coir and wood shavings have significantly lower nutrient levels, particularly in nitrogen and potassium, at 0.68% and 0.33% respectively (Johnnie *et al*., 2023).

Atomic Absorption Spectroscopy (AAS) was used to measure the concentrations of Magnesium (Mg), Iron (Fe), Zinc (Zn), and Sodium (Na) by standard curves in the oyster mushrooms (*Pleurotus ostreatus*) grown on different substrates and using different cultivation techniques, Magnesium analysis showed that bottles generally had higher Mg concentrations than bags, except for Treatment 1 (Wheat Straw + Vermicompost), where bag samples (95.19 ppm) exceeded bottle samples (74.01 ppm). The highest Mg concentration in bottles was observed in Treatment 2 (90.02 ppm), suggesting that coconut coir enhances Mg retention in liquid environments. Iron

concentrations were highest in Treatment 1 bag samples (8.879 ppm), indicating vermicompost significantly increased Fe availability, while the lowest was in control bottles (4.743 ppm). Zinc analysis showed the highest concentration in control bottles (18.08 ppm), suggesting wheat straw alone retained more Zn in bottles, while the lowest was in Treatment 1 bottles (11.68 ppm). Treatment 2 had relatively stable Zn levels in both bottles and bags. Sodium concentrations showed highly variable trends; the highest was in Treatment 1 bags (73.51 ppm), while the lowest was in control bags (3.34 ppm). This suggest that vermicompost increased Mg and Fe levels, coconut coir contributed to stable Zn retention, and Na accumulation varied based on substrate and storage method

The study showed that substrate type significantly affects the growth, color, and productivity of oyster mushrooms. Mushrooms from T2 exhibited a darker grey color due to the substrate's nutrient profile and moisture retention, aligning with Demiray's findings on drying impacts (Demiray, 2013). T1-Bg showed the highest fresh and dry weights, indicating optimal growth and moisture retention, while T3 showed no growth, emphasizing the importance of substrate choices (Kotwaliwale *et al*., 2007). Wheat straw produces lighter mushrooms due to its cellulose and moisture retention (Zhang *et al*., 2002; Kumla *et al*., 2020). Coconut coir leads to darker mushrooms due to its lignin and hemicellulose content affecting pigment production (Bellettini *et al.*, 2019; Xu *et al*., 2021). Cultivation method (bags vs. bottles) has minimal impact on color compared to substrate factors (Mohapatra *et al*., 2010).

CONCLUSION

During this study, we tried to compare how effectively edible oyster mushrooms can be cultivated on various agricultural waste substrates with added calcium carbonate and vermicompost. The best results were achieved with wheat straw and vermicompost (T1), especially when grown in bottles, which provided the highest fresh and dry weights, along with excellent growth parameters such as a high number of fruiting bodies, long and thick stipes, and large cap diameters. Coconut coir, calcium carbonate and vermicompost (T2) also performed well but less consistently than T1. Wood shavings, calcium carbonate and vermicompost (T3) failed to produce any growth. The bottle cultivation method outperformed the bag method, offering better control and higher yields. Oyster mushrooms on wheat straw are lighter (pale white to light grey) due to lignin and cellulose, while those on coconut coir are darker (grey to darker grey) because of hemicellulose and nutrients enhancing melanin. Previous research indicates that integrating vermicompost with various substrates enhances the nutrient profile and yield of mushrooms by increasing NPK levels. Although differences in cultivation techniques may exist, they are not guaranteed to affect the outcome. Results suggest the effectiveness of wheat straw, coconut coir and vermicompost in enhancing mushroom growth and quality, suggesting that wheat straw with vermicompost in bottles is a highly efficient and sustainable approach for *Pleurotus ostreatus* cultivation.

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