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Growth and production of oyster mushroom (*Pleurotus ostreatus*) using peanut as substrate supplement

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Abstract Oyster mushroom cultivation is a sustainable practice that converts agricultural waste into valuable resources, enhancing food security and economic stability. This research examined oyster mushroom growth and production using peanut hulls and nuts as substrate supplements. The study assessed growth parameters (stipe length and diameter, pileus diameter and thickness) and production parameters (number of mushrooms per cluster, number of clusters harvested, and weight per cluster) on different substrate formulations: sawdust-molasses-limestone (S_1), sawdust-molasses-limestone with 2% peanut hull (S_2), sawdust-molasses-limestone with 2% nut (S_3), and sawdust-molasses-limestone with 2% molasses (S_4). Substrates were prepared using a completely randomized design through drying, mixing, and fermentation. The sawdust and molasses substrate produced the longest stipe and largest pileus diameter, while the sawdust and nuts substrate resulted in the largest pileus diameter, highest number of mushrooms per cluster, and greatest cluster weight. ANOVA analysis indicated no statistically significant differences between substrates, suggesting similar effects among the combinations. The study recommends using sawdust and nuts for optimal growth and production, avoiding molasses due to its inhibitory effects on cluster formation. Further research should investigate the nutrient contributions and pH levels of each substrate component to refine recommendations. Mushroom cultivators should monitor substrate pH and incorporate nuts to enhance production, with agricultural extension services providing training on effective substrate optimization.

Keywords: oyster mushroom, substrate supplement, growth and production parameters

1. Introduction

Mushroom cultivation is a technology of growing mushrooms utilizing mostly plant waste. The culture of mushrooms is gaining popularity in the Philippines and worldwide because of its dietary fiber and protein value. Medenilla (2020) noted mushroom production has existed in the Philippines since the 19th century. Despite this long history, the nation's current contribution to the local mushroom market is just 10%. While mushroom cultivation has the potential to be both profitable and a source of sustainable income, many Filipinos remain hesitant to engage in this industry due to limited awareness.

Growing mushrooms requires minimal space and time (Stamets, 2011). Rice straws, dried banana leaves, rice hulls, or other plant waste materials can be utilized as substrates (Adenipekun, 2011). Provided there is a good supply of plant materials, mushrooms can be cultivated year-round. Mushroom cultivation is an environmentally friendly practice that converts agricultural waste into valuable resources (Mahari et al., 2020). Instead of burning agricultural waste such as rice straw and rice hulls (Rabena et al., 2008), these materials can be used to produce mushrooms, thereby reducing waste. Additionally, mushroom cultivation can provide individuals with an opportunity to earn more income cost-effectively.

In the Philippines, ten varieties of edible and medicinal mushrooms are cultivated, including Paddy straw, Oyster, Shiitake, Button, Ear fungi, Milky, Yellowish oyster, Reishi, Lion`s mane, and King tuber oyster mushrooms. According to Chang et al. (2014), fostering the mushroom industry is crucial for rural economic development, as it boosts employment and income opportunities in rural areas and provides financial benefits to small-scale farmers.

One of the most common types of mushrooms cultivated worldwide is the oyster mushroom, the common name for the species *Pleurotus ostreatus* (Seethapathy et al., 2023). It has broad, white, fan-shaped caps with gills on the underside. It ranks as the third most widely cultivated mushroom. Oyster mushrooms are beneficial in treating anemia due to their folic acid content (Regula et al., 2016). They are also ideal for individuals with hypertension, obesity, and diabetes, thanks to their low sodium-to-potassium ratio and minimal starch, fat, and caloric content (Lai et al., 2024). The high fiber and alkaline ash content in oyster mushrooms makes them suitable for those with hyperacidity and constipation (Sar et al., 2024). Additionally, a polycyclic aromatic compound called pleurotin, which has antibiotic properties, has been isolated from *P. griseus* (Daanne,

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2001). This dual economic and health potential underscores the importance of oyster mushroom cultivation in both local and global contexts.

Recent global studies underscore the importance of substrate optimization in enhancing the yield and quality of *Pleurotus ostreatus*. While traditional substrates like rice straw, wheat straw, and sawdust are widely used, rising costs have prompted the exploration of alternatives. Sawdust combined with rice husk (SR) improves biological efficiency, polyphenol content, and reduces harvest time (Akter et al., 2022). A 3:2 mix of sugarcane bagasse and rice straw enhances yield, protein content, and accelerates fruiting (De et al., 2023), while sugarcane trash alone also supports high yield and efficient decomposition (Karpagavalli et al., 2024). Cotton seed hulls, when added to rice or wheat straw, increase mushroom size and cap diameter, though they may delay early growth (Yang, 2013). Waste tea leaves (WTL), though ineffective alone, boost yield and nutritional value when mixed with sawdust (Ahmed et al., 2024). Additionally, combining sawdust with materials like teff straw, waste paper, or banana pseudostems consistently yields better results than single substrates (Besufekad et al., 2020).

Though mushroom culture is gaining popularity, its present cultivation, especially in the province, is limited, perhaps due to the insufficiency of planting materials, the unavailability of grain spawn, and the limited local knowledge about how it is being cultured. Although there are mushroom growers in the province, the demand still surpasses the supply. To meet the demand, mushroom supplies come from nearby regions.

One of the projects implemented by the University of Northern Philippines in cooperation with other schools and agencies was oyster mushroom production as an alternative livelihood source among Ilocanos devastated by calamities (Rosal & Tabunan, 2005). The university also conducted training and seminars on mushroom cultivation to encourage more growers and intensify the technology.

Successful cultivation of oyster mushrooms is a way of utilizing different agricultural waste. This research study investigated the suitability of combining sawdust peanut and nuts as supplements in oyster mushroom cultivation. The implication of this study is to strengthen the promotion of oyster mushroom cultivation, facilitate technology adoption using the aforementioned substrate combination, improve the sustainability of small mushroom growers, and improve the life of the local community.

2. Materials and Methods

This study employed an experimental research design and utilized a complete randomized design with five treatments. The procedures were adopted from Raboy (2021) with modifications. The experimental part of the research involves testing different substrates to evaluate their impact on the growth and production parameters of oyster mushroom cultivation. This includes assessing growth parameters such as stipe length, stipe diameter, pileus diameter, and pileus thickness, as well as production parameters such as the number of mushrooms per cluster, the number of clusters harvested, and the weight of mushrooms per cluster.

2.1. Preparation of the substrate

The sawdust was dried for two weeks. After drying, the substrate was prepared using a shortened composting method. Initially, the dried sawdust, limestone, and molasses were thoroughly mixed in the following proportions: 98% sawdust, 1% limestone, and 1% molasses. Water was added gradually until the correct moisture content was achieved, as this step is crucial for successful mushroom substrate preparation. The substrate was placed into a sack once the correct moisture level was attained. Following the sacking, the substates were fermented for seven days. After this fermentation period, the sawdust substrates were ready for bagging.

2.2. Preparation of the supplement

Hulls and nuts were used as supplements in the study. They were dried for two weeks and subsequently crushed. Three formulations were prepared as supplements: (1) 100% or 20g peanut hull, (2) 100% or 20g nut, and (3) 100% or 20g molasses. These formulations were selected based on the findings of Zeids et al. (2019), as outlined in the related literature. As a reference, one substrate (S_1) served as the control without any supplement, enabling comparison of the formulations' viability and substrate efficiency. The supplements containing peanut hull, nuts, and molasses were added to the substrate at a dose of 2% of the substrate's wet weight.

2.3. Preparation of the fruiting bag

After fermenting the substrate, it was unpacked and divided into three portions to represent the three substrates. For the second substrate (S2), 2% or 20 grams of peanut hulls were incorporated into the mixture before bagging. For S3, 2% or 20 grams of nuts were similarly added, and for S4, 2% or 20 grams of molasses were included in the mixture before bagging. Each treatment had three replicates, and for each replicate, ten fruiting bags were prepared. Each mixture was then packed and compressed into polyethylene bags, which were covered using PVC pipes and cotton. The fruiting bags were sterilized for 6-8 hours and cooled overnight before inoculation.

2.4. Inoculation

After the substrate cooled, one teaspoon of spawn, obtained from the Mushroom Research and Development Center of the University, was aseptically inoculated into it. Following inoculation, the substrate was incubated for a month at relatively higher temperatures and humidity levels. Following inoculation, the substrate was incubated for a month at a temperature of 28–30°C, under very low light exposure, and at a relative humidity of 70–80%. These environmental conditions are crucial for mushroom development, as the optimal temperature, humidity, and limited light exposure promote vigorous mycelial growth and successful colonization. Once the spawn fully colonized the substrates, they were transferred to a cooler area (growing house) to induce fruiting. After 5 days, an opening was cut at the back of the polyethylene bag to ensure that the substrate containing mushrooms remained adequately moist. This was achieved by daily sprinkling of water over the compost to maintain high humidity levels.

2.5. Statistical analysis

The data that were collected include the following: for the growth parameters, stipe length, stipe diameter, pileus diameter, and pileus thickness were measured and expressed in centimeters. For the production parameters, (i) the number of mushrooms per cluster, (ii) the number of clusters harvested, and (iii) the weight of mushrooms per cluster. The number of mushrooms produced in each substrate was counted, and the weight of mushrooms harvested from each fruiting bag was recorded. The following statistical tools were used in analyzing the data gathered in the study. The mean was used to represent the average growth and production parameters of oyster mushrooms. One-way repeated-measures ANOVA was used to compare the increase in oyster mushrooms' growth and production parameters, and Scheffé's Test was used to determine which substrates differ significantly from each other. The use of repeated-measures ANOVA is justified as measurements were taken repeatedly from the same fruiting bags (experimental units) across the different parameters, rather than from independent groups. This allowed the researchers to observe trends and variations within the same experimental units under different substrate treatments.

3. Results and Discussion

3.1. Growth of the oyster mushroom

The growth parameters of oyster mushrooms in terms of stipe length, stipe diameter, pileus diameter, and pileus thickness are presented in Figure 1. The result demonstrates that the Sawdust and Molasses (S_4) combination produced the longest stipe, measuring 79.38 mm long and 13.04 mm in diameter. In contrast, using sawdust (S_1) resulted in a stipe length of 70.25 mm and a diameter of 13.19 mm. Sawdust + peanut hull (S_2) exhibit a nearly similar stipe length (66.97 mm) to sawdust (S_1) but slightly increased diameter (14.06 mm). Sawdust + nuts (S_3) showed a decreased stipe length (63.38 mm) but significantly increased diameter (15.69 mm), which might indicate enhanced nutrient uptake. Oyster mushrooms' stipe length and diameter can differ based on the cultivation substrates. While sawdust generally supplies vital nutrients, it might be deficient in specific micronutrients. Incorporating peanut hulls, nuts, and molasses can add extra nutrients such as sugars, proteins, and fats, impacting stipe growth. Peanut hulls and nuts affect the stipe's diameter, whereas molasses might promote stipe elongation when adequate hydration is maintained.

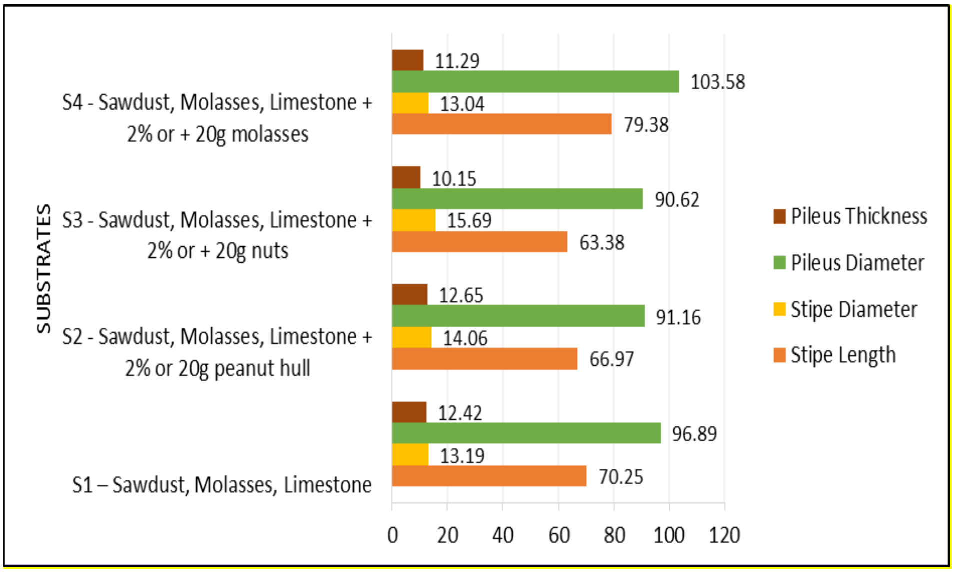

Figure 1 Growth Parameters of Oyster Mushroom.

The table further indicates that the combination of sawdust and molasses (S_4) led to the largest pileus diameter, measuring 103.58 mm, with a thickness of 11.29 mm. Sawdust (S_1) is second to the highest pileus diameter (96.89 mm) and thickness (12.42 mm). Sawdust + peanut hull (S_2) has a decreased pileus diameter (91.16 mm) compared to Sawdust (S_1) but with the thickest pileus (12.65 mm). Sawdust + nuts (S_3) has the lowest pileus diameter (90.62 mm) and thickness (10.15 mm).

The pileus diameter and thickness in oyster mushroom cultivation also depend on the substrate type. Molasses and peanut hulls provide extra sugars and nutrients that can boost metabolic processes, potentially leading to increased pileus diameter and thickness, respectively. The results imply that the different substrates used in oyster mushroom cultivation can affect stipe length, stipe diameter, pileus diameter, and pileus thickness through various mechanisms related to nutrient availability, water retention, and biochemical interactions. Results also imply that although sawdust is a primary substrate, it may require supplementation for optimal growth parameters. Peanut hulls and nuts improve water retention and supplement nutrients, potentially resulting in thicker and more extensive stipes and piles. Molasses also serve as a nutrient reservoir and help regulate moisture, fostering robust growth in stipe length and pileus diameter.

The study's results were supported by different research. Hasan et al. (2015) found that supplementing sugarcane bagasse with 30% wheat bran and molasses led to increased stipe length and thicker pileus diameter, demonstrating beneficial effects on mushroom growth. Similarly, Afify et al. (2012) reported that molasses, serving as a primary energy source, can enhance mushroom growth and yield when 5% is added to substrates, significantly outperforming control substrates. Erkel (2009) was cited by Afify, noting that molasses supplies sugar, nitrogen, and other nutrients essential for better cell growth. Additionally, Zhiyuan (2014) suggested that incorporating peanut hulls into the oyster mushroom cultivation medium is a cost-effective strategy that can improve both yield and quality.

3.2. ANOVA Results on the Significant Difference Between and among the Different Substrates on the Growth of Oyster Mushroom

Table 1 presents the significant differences between and among the different substrates used in mushroom growth in terms of stipe length, stipe diameter, pileus diameter, and pileus length. The table presents the f-values and corresponding p-values for the following growth parameters of oyster mushrooms: stipe length, stipe diameter, pileus diameter, and pileus thickness. For all growth parameters listed, the p-values are greater than 0.01. This indicates that no statistically significant difference is observed among the different substrates for these growth parameters. This suggests that the different substrates have more or less the same effect on the stipe length and diameter as well as the pileus diameter and thickness of oyster mushrooms.

Table 1 One-factor ANOVA of the Growth Parameters Between and Among Substrates.

Growth Parameters	F-value	p-value
Stipe Length	2.707	p>.01
Stipe Diameter	1.143	p>.01
Pileus Diameter	1.186	p>.01
Pileus Thickness	.293	p>.01

3.3. Production of oyster mushroom

The production parameters of oyster mushrooms in terms of the number of mushrooms per cluster, number of clusters harvested, and weight of mushrooms per cluster are presented in Figure 2. The production parameters of oyster mushrooms in terms of the number of clusters harvested, number of mushrooms per cluster, and weight per cluster reveal notable differences based on the substrate used. Sawdust (S_1) resulted in the highest mean number of clusters harvested (1.33 grams), whereas Sawdust + molasses (S_4) had the lowest (1.00 grams), suggesting that molasses may negatively affect cluster formation. When considering the mean number of mushrooms per cluster, Sawdust + nuts (S_3) had the highest number (3.69 grams), indicating a potential positive influence of nuts on mushroom proliferation within clusters. Conversely, Sawdust + molasses (S_4) again performed the lowest (2.67 grams), showing that molasses might inhibit mushroom growth. Regarding mean weight per cluster, Sawdust + nuts (S_3) also led with 50.00 grams, suggesting that adding nuts enhances overall cluster weight, likely due to larger or denser mushrooms. Meanwhile, Sawdust (S_1) had a lower mean weight (44.86 grams) than Sawdust + nuts (S_3) but higher than Sawdust + peanut hull (S_2) and Sawdust + molasses (S_4) with 46.48 grams and 46.00 grams, respectively.

The results imply that adding nuts showed the most promise for improving the number of oyster mushrooms per cluster, weight per cluster, and number of clusters harvested. Molasses did not appear beneficial and might even impede growth. Molasses can alter the pH of the substrate, which could create unfavorable conditions for mycelium growth. According to United Molasses, the pH of cane molasses is typically 4.8-5.5 pH. According to Mardiana et al. (2023), pH affects oyster mushroom growth by influencing nutrient absorption, and the suitable pH for oyster mushroom growth is 6.5-8.0. Hassan and

Hisamudin (2022) also found that pH 7.7 showed the most favored mycelium growth condition in oyster mushrooms. This highlights the importance of substrate optimization in mushroom cultivation to enhance its yield or production.

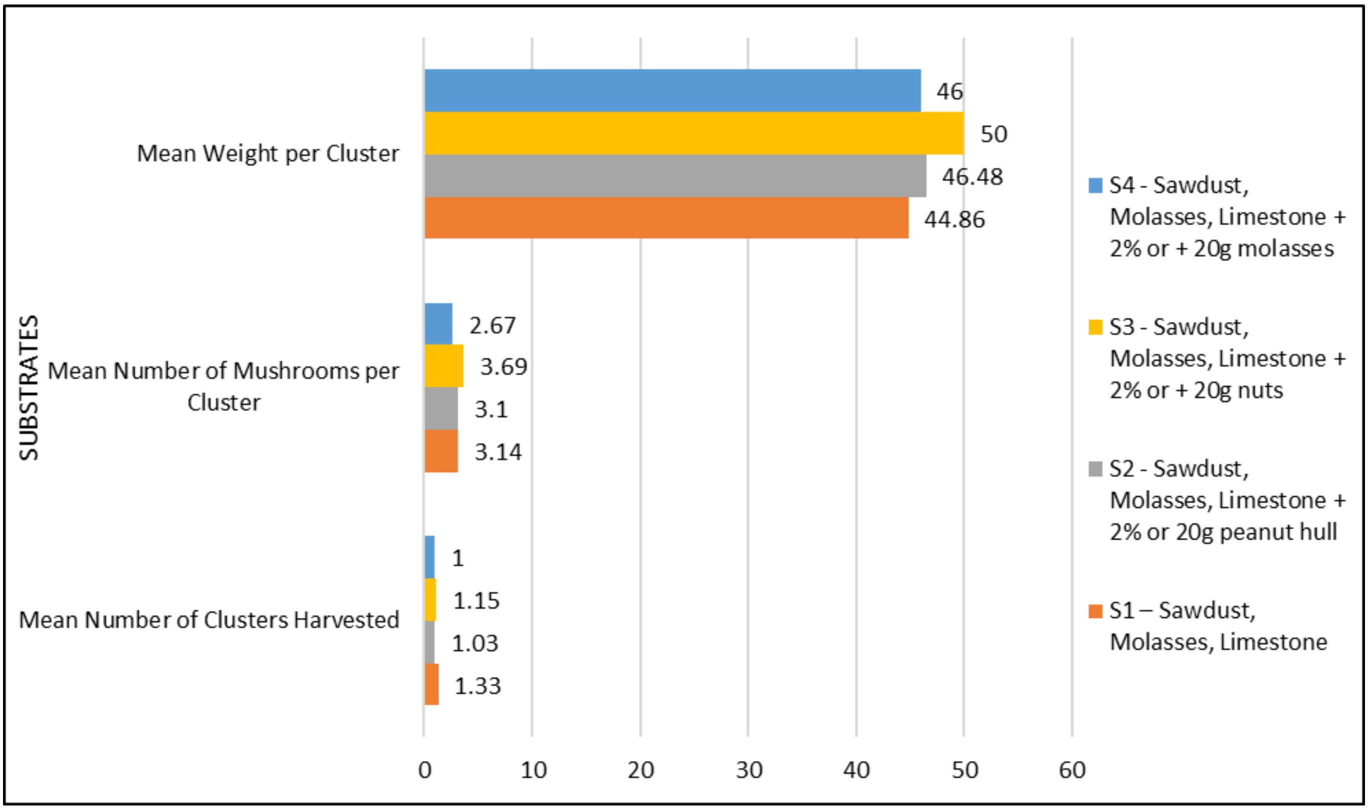

Figure 2 Production Parameters of Oyster Mushroom.

3.4. Results of ANOVA on the Significant Difference Between and among the Different Substrates on the Production of Oyster Mushroom

Table 2 presents the significant differences between and among the different substrates used in mushroom production parameters regarding the number of clusters harvested, the number of mushrooms per cluster, and the weight per cluster. Based on the results presented in Table 2, none of the production parameters (number of clusters harvested, number of mushrooms per cluster, and weight per cluster) showed statistically significant differences between the different substrates. This suggests that the different substrates applied did not have a significant effect on the production of mushrooms in terms of these parameters. This implies that the different substrates have more or less the same effect on the production of oyster mushrooms in terms of the number of clusters harvested, the number of mushrooms per cluster, and the weight per cluster.

Table 2 One-factor ANOVA of the Production Parameters between Treatments.

Production Parameters	F-value	p-value
Mean Number of Clusters Harvested	1.057	p>.01
Mean Number of Mushrooms per Cluster	.535	p>.01
Mean Weight per Cluster	.223	p>.01

4. Conclusions

The substrate composed of sawdust and molasses produced the longest stipe and largest pileus diameter in oyster mushrooms, while the substrate with sawdust and nuts resulted in the greatest pileus diameter and highest number of mushrooms per cluster and cluster weight. Although the ANOVA analysis revealed no statistically significant differences between the different substrates for all measured growth and production parameters, observable trends in the mean values suggest practical advantages favoring certain treatments. Specifically, the substrate supplemented with nuts consistently demonstrated higher production parameters. These trends, while not statistically confirmed, offer valuable insights for guiding practical recommendations, particularly for small-scale mushroom growers aiming to enhance yield and production efficiency. Based on these findings, it is recommended to use sawdust and nuts as a substrate for oyster mushroom cultivation to achieve improved growth and production performance, while avoiding the use of molasses due to its potential inhibitory effects on cluster formation and mushroom proliferation.

Further research should investigate each substrate component's specific nutrient contributions and pH levels to refine these recommendations. In addition, it is recommended that future studies conduct detailed biochemical and nutrient analyses of the substrates, including assessments of moisture content, nitrogen levels, sugar concentrations, and pH stability. Such analyses would substantiate the observed trends and enhance the reliability of the findings, providing a stronger scientific basis for optimizing substrate formulations in oyster mushroom cultivation. Moreover, mushroom cultivators are encouraged to monitor substrate pH levels and consider incorporating nutrient-rich supplements, such as nuts, to optimize production outcomes. Agricultural extension services should also provide training on effective substrate preparation and optimization techniques to improve mushroom farming practices.

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Ethical considerations

Not applicable.

Conflict of Interest

The authors declare no conflicts of interest.

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