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EXPLORING AGRICULTURAL WASTE SUBSTRATE FOR ENHANCEMENT OF *PLEUROTUS OSTREATUS* CULTIVATION.

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ABSTRACT

The proximate composition of the organic waste substrates used in cultivating Pleurotus ostreatus was analyzed, and the results revealed significant variations in various nutritional components among the substrates. Palm Chaff had the highest ash content (7.14%), followed by Oil Palm Empty Bunch (5.08%), Yam Peel (4.29%), and Saw Dust (1.36%). This indicated that Palm Chaff was the richest in minerals, while sawdust had the lowest mineral content. In addition, Yam Peel had the highest moisture content (8.46%), followed by Palm Chaff (5.87%), Oil Palm Empty Bunch (4.62%), and Saw Dust (2.83%). This suggested that Saw Dust was the driest substrate. Furthermore, Oil Palm Empty Bunch had the highest crude lipid content (1.94%), followed by Palm Chaff (1.38%), Yam Peel (1.13%), and Saw Dust (0.04%). Moreover, Oil Palm Empty Bunch had the highest crude protein content (2.56%), followed by Yam Peel (2.1%) and Palm Chaff (2.02%). Saw Dust had the lowest crude protein content (1.74%). Besides, Oil Palm Empty Bunch had the highest crude fiber content (48.27%), followed by Palm Chaff (36.94%), Saw Dust (26.17%), and Yam Peel (14.36%). In addition, Saw Dust had the highest carbohydrate content (67.86%), followed by Yam Peel (69.64%) and Palm Chaff (46.65%). Oil Palm Empty Bunch had the lowest carbohydrate content (37.58%). These findings offered valuable insights into the suitability of each substrate for cultivating Pleurotus ostreatus Additionally, it enabled growers to make informed decisions about substrate selection based on the specific nutritional needs and growth requirements of the mushrooms. The impact of various organic waste substrates on the growth dynamics of Pleurotus ostreatus (oyster mushroom), revealed significant variations in several key parameters. In primordial Formation, the number of early-stage fruiting bodies formed varied between 12 and 36 across different substrate formulations. The highest primordial formation was observed in treatment A + B + C + D (36), while treatments involving individual substrates or their combinations had the lowest primordial formation (12). Furthermore, in Fruiting Body Production, the number of mature mushrooms produced varied across treatments. Treatment A + C resulted in the highest number of fruiting bodies (26), while treatments involving combinations of substrates (B + C + D, A + B + C, A + B + C + D, C + D) had the lowest number of fruiting bodies (3). Overall, the findings highlight the influence of specific substrate combinations on various aspects of mushroom growth dynamics. However, the choice of growth substrates did not significantly impact the overall weight and biological efficiency of Pleurotus ostreatus in this study. These results provide valuable insights for optimizing mushroom cultivation practices.

Keywords: *Pleurotus ostreatus*, primordial formation, substrate formulations.

INTRODUCTION

Organic waste refers to any waste material that is derived from natural sources and can decompose over time (O'Connor et al., 2021; Ayilara et al., 2020). This includes food waste, agricultural waste, yard waste, and sewage sludge, among others (Battista et al., 2022; Mu et al., 2020; Gonawala & Jardosh, 2018). Organic waste is a major environmental concern, as it is one of the largest sources of greenhouse gas emissions and contributes to global warming (Maria et al., 2020; Al-Rumaihi et al., 2020; Kristanto & Koven, 2019). However, organic waste also has the potential to be used as a valuable resource through a process called valorization (Rao & Rathod, 2019; Abdel-Shafy & Mansour, 2018). Valorization is the process of converting waste materials into products of value (Duan et al., 2021; Cecilia et al., 2019). In the case of organic waste, valorization can involve the conversion of the waste material into products such as biofuels, fertilizers, and animal feed (O'Connor et al., 2021; Galanakis, 2020). For instance, a recent study by Koutrotsios et al. (2019) explored the potential of using olive mill wastewater and olive pomace for the production of mushroom-based poultry feed. Similarly, another study by Hassan et al. (2020) investigated the use of mushroom waste for broiler feed production. Other studies by Mahfuz et al. (2019) and Adetunji and Adejumo (2019), found that using spent mushroom substrate as a feed ingredient for broilers reduced the amount of waste going to landfills, thus decreasing greenhouse gas emissions. The process of valorization can help to reduce waste and mitigate environmental pollution, while also creating new economic opportunities (Kapoor et al., 2020; Fetene et al., 2018). Additionally, this method also contributes to the circular economy by converting waste into a valuable resource (Duan et al., 2022; Leder et al., 2020).

The increasing volume of organic waste generated from agricultural and food processing activities poses a significant challenge for waste management systems worldwide (Sharma et al., 2019; Abdel-Shafy & Mansour, 2018).By exploring this alternative feed source, this paper provide insights into the feasibility of utilizing agro-wastes such as sawdust, yam peel, oil palm empty bunch, and palm chaff as substrates for cultivating mushrooms.

MATERIAL AND METHOD

Development of mushroom tissue culture

A solution of 70% ethanol and 5% sodium hypochlorite in distilled water for sterilizing the equipment and workspace was prepared as described by Petrova et al. (2019). After sterilizing the workspace and equipment, the potato dextrose agar (PDA) medium used for cultivating the *Pleurotus ostreatus* was prepared and sterilized using a pressure cooker as described by Xu et al. (2021) and Hou et al. (2019). The potato dextrose agar (PDA) was prepared using 200 g of fresh, locally available potato and industrially produced agar and glucose. The potato was washed, sliced, and added to 1 liter of boiling water in a brass flask. The mixture was boiled for 15 minutes and then filtered using a piece of cheesecloth to obtain potato broth. To this broth, 20 g of glucose and 20 g of agar were added, and the volume was made up to 1 liter with water. The mixture was then sterilized in a pressure cooker for 30 minutes, and 25 ml of the resulting PDA was poured aseptically into petri dishes. The PDA was then allowed to cool before inoculation and incubation. A small piece of fresh tissue from a healthy fruiting body of *Pleurotus ostreatus* obtained from the University of Port Harcourt Demonstration Farm was collected using sterile forceps and inoculated on the PDA medium. Afterward, the inoculated culture medium was incubated in a sterile environment at a temperature range of between 20 and 25°C for a period of 10 to 14 days or until the mycelium had grown over the surface of the medium.

Production of mushroom spawn

The grain-based medium used for sub-culturing the *Pleurotus ostreatus* in larger

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amounts was prepared and sterilized as described by Xu et al. (2021) and Hou et al. (2019). Sorghum, maize, and rice grains were manually cleaned to remove debris. The cleaned grains were soaked in tap water overnight and then boiled in a stainless-steel pot. After soaking and boiling the grains were drained to remove the excess water. Next, wheat bran was added at a rate of 10% and chalk ($CaCO_3$) at a rate of 2% on a dry weight basis of the grains. The additives were mixed evenly and thoroughly with the grains. The grain-based medium was then filled into heat-resistant glass bottles, which were plugged with cotton wool and sterilized using a pressure cooker for 30 minutes. After sterilization, the bottles were allowed to cool for approximately six hours and inoculated with the mycelial culture of *Pleurotus ostreatus* that was maintained on potato dextrose agar (PDA). The inoculated bottles were then incubated at room temperature (25-28°C) and humidity levels between 70-80% for about 14-21 days, or until the mycelium fully colonized the grains inside the bottles. The bottles containing the spawns were stored in a cool, dry place until they were ready to be used for cultivating the mushroom on the organic waste materials. When the spawns were ready, they were transferred onto the organic wastes so that mushrooms could shoot out.

Experimental design for cultivating the mushroom on waste substrates

To determine the effect of organic waste substrates including yam peel, palm chaff, sawdust and oil palm empty bunch on the growth of *Pleurotus ostreatus*, the mushroom was cultured on different amounts and combinations of the substrates using the completely randomized block design (CRBD) shown in Table 3.1 (Wachira et al., 2022; Prasad et al., 2021). The independent variables included the type and amount of the organic waste, while the dependent variables included growth parameters such as the number of primordial formations, number of fruiting, cap diameter, height, girth, and biological efficiency of the mushroom. The cap diameter, girth, and height of the mushroom were monitored and determined using a caliper with a meter rule (Rakib et al., 2020).

Table 1: Experimental design for cultivation of *Pleurotus ostreatus* on waste substrates

Treatment	Label	Replications		
Treatment 1	A	A1	A2	A3
Treatment 2	B	B1	B2	B3
Treatment 3	C	C1	C2	C3
Treatment 4	D	D1	D2	D3
Treatment 5	A+B	A+B1	A+B2	A+B3
Treatment 6	A+C	A+C1	A+C2	A+C3
Treatment 7	A+D	A+D1	A+D2	A+D3
Treatment 8	C+B	C+B1	C+B2	C+B3
Treatment 9	C+D	C+D1	C+D2	C+D3
Treatment 10	A+B+C	A+B+C1	A+B+C2	A+B+C3
Treatment 11	B+C+D	B+C+D1	B+C+D2	B+C+D3
Treatment 12	A+B+C+D	A+B+C+D1	A+B+C+D2	A+B+C+D3

A = Saw Dust; B = Yam Peel; C = Oil palm empty bunch; D = Palm chaff

Collection and proximate analysis of the organic wastes and *Pleurotus ostreatus*

Fresh organic wastes such as sawdust, palm chaff, oil palm empty bunch, and yam peel were collected at the source of their generation within and around the University of Port Harcourt main campus in Choba. After collecting the wastes, samples were sun-dried, cleaned to remove debris, powered using a grinding machine, and then taken to the laboratory to determine their proximate composition according to the procedures described in AOAC (2016).

RESULTS AND DISCUSSION

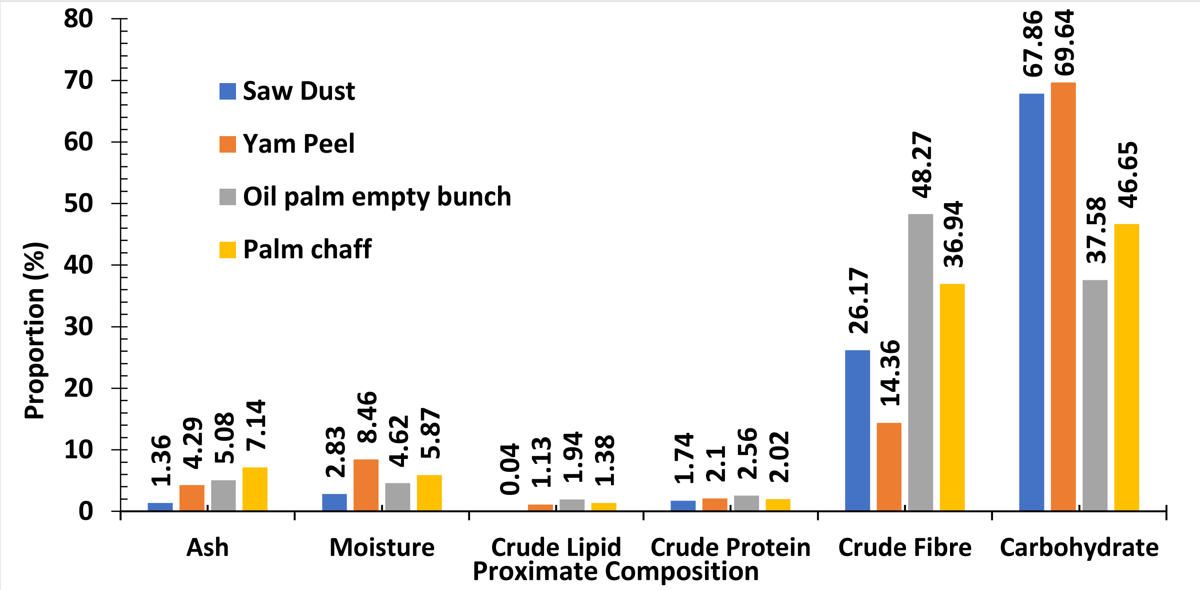

Figure 1: Comparison of proximate composition of the mushroom growth substrates

The proximate compositions of organic waste substrates used in cultivating the mushroom (*Pleurotus ostreatus*) are shown in Figure 1 above. Among the growth substrates, Palm Chaff (D) exhibited the highest ash content at 7.14%, followed by Oil Palm Empty Bunch (C) at 5.08%, and Yam Peel (B) at 4.29%. Saw Dust (A) recorded the lowest ash content at 1.36%. This indicates that Saw Dust (A) possessed the lowest mineral content, while Palm Chaff (D) had the highest.

Yam Peel (B) displayed the highest moisture content at 8.46%, followed by Palm Chaff (D) with a moisture content of 5.87%, and Oil Palm Empty Bunch (C) with a moisture content of 4.62%. Saw Dust (A) had the lowest moisture content at 2.83%. This implies that Saw Dust was the driest among the substrates. Oil Palm Empty Bunch (C) exhibited the highest crude lipid content at 1.94%, followed by Palm Chaff (D) at 1.38%, and Yam Peel (B) at 1.13%. Saw Dust (A) had the lowest crude lipid content at 0.04%. This indicates that Oil Palm Empty Bunch (C) possessed the highest fat content, while Saw Dust (A) had the lowest.

Oil Palm Empty Bunch recorded the highest crude protein content at 2.56%. Yam Peel (B) and Palm Chaff (D) had similar crude protein contents at 2.1% and 2.02% respectively. Saw Dust (A) recorded the lowest crude protein content at 1.74%. Oil Palm Empty Bunch (C) exhibited the highest crude *fiber* content at 48.27%, indicating a significant amount of indigestible plant material. Palm Chaff (D) also contained a substantial amount of crude *fiber* at 36.94%, followed by Saw Dust (A) at 26.17%. Yam Peel (B) had the lowest crude *fiber* content at 14.36%, indicating the lowest amount of indigestible plant material compared to the other substrates. Saw Dust (A) displayed the highest carbohydrate content at 67.86%, followed by Yam Peel (B) at 69.64% and Palm Chaff (D) at 46.65%. Oil Palm Empty Bunch (C) had the lowest carbohydrate content at 37.58%.

Proximate analysis of the growth substrates provided valuable insights into their suitability for cultivating *Pleurotus ostreatus*. The ash content indicates the mineral content of the substrates (Onyegeme-Okerenta et al., 2021; Patil et al., 2020). Therefore, substrates with higher ash content, such as Palm Chaff and Oil Palm Empty Bunch, offered a richer source of inorganic nutrients for mushroom growth. Conversely, a substrate like Saw Dust, with the lowest ash content, was relatively mineral-poor. Growers could use this information to select substrates based on the desired mineral profile for their mushroom cultivation.

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The moisture content of a mushroom growth substrate is crucial, as it affects the water availability for the mushrooms. Substrates with higher moisture content, like Yam Peel, could potentially provide more water to support mushroom growth. On the other hand, Saw Dust, with the lowest moisture content, may require additional hydration before use. Our findings align with studies conducted by Mahari et al. (2020) and Karavani et al. (2018), which emphasize the importance of moisture content for mushroom growth, suggesting that higher moisture content can lead to increased water availability for mushrooms. However, Akter et al. (2022) and Iwuagwu et al. (2020) propose alternative methods for maintaining moisture levels, which may offer additional insights for growers. Crude Lipid represents the fat or oil content in the substrate (Ritota & Manzi, 2019). Substrates with higher lipid content, such as Oil Palm Empty Bunch, might have served as a source of energy for the mushrooms during their growth (Sadh et al., 2018; Salami & Bankole, 2018). This information is relevant for understanding the potential energy supply available to the mushrooms during their growth cycle.

Moreover, protein is an essential component for the growth and development of mushrooms (Akter et al., 2022; Peter et al., 2019). Substrates with higher protein content, like Oil Palm Empty Bunch, provided a more substantial source of this essential nutrient. This knowledge could be used to select substrates that offered optimal protein levels for mushroom cultivation.

Furthermore, crude *fiber* represents the indigestible portion of the substrate (Ewekeye et al., 2023; Kumla et al., 2020). Substrates with higher *fiber* content, like Oil Palm Empty Bunch and Palm Chaff, contained more structural plant material that may not have been directly utilized by the mushrooms. This information is crucial for understanding the digestibility and potential nutritional value of the substrate.

Carbohydrates served as the primary energy source for mushroom growth in this study. Substrates with higher carbohydrate content, such as Saw Dust and Yam Peel, offered more readily available energy for the mushrooms (Yamauchi et al., 2019; Peter et al., 2019). This information is vital for selecting substrates that can sustain vigorous mushroom growth. Our findings on the importance of carbohydrates as an energy source for mushroom growth are supported by studies conducted by Grimm et al. (2021) and Xiong et al. (2019). Besides, studies conducted by Raman et al. (2021) and Gong et al. (2020) also provide additional insights into the types of carbohydrates that are most readily utilized by mushrooms, which could be beneficial for substrate selection.

Overall, the results indicated that each organic waste substrate had its unique composition, influencing its suitability for mushroom cultivation. For example, substrates with higher carbohydrate content (like Saw Dust and Yam Peel) could potentially serve as good sources of energy for mushroom growth, while those with higher protein content (like Oil Palm Empty Bunch) could provide essential nutrients like amino acids that are crucial for the development and structure of mushrooms. Growers could leverage this information to make informed decisions about substrate selection based on the specific nutritional needs and growth requirements of *Pleurotus ostreatus*.

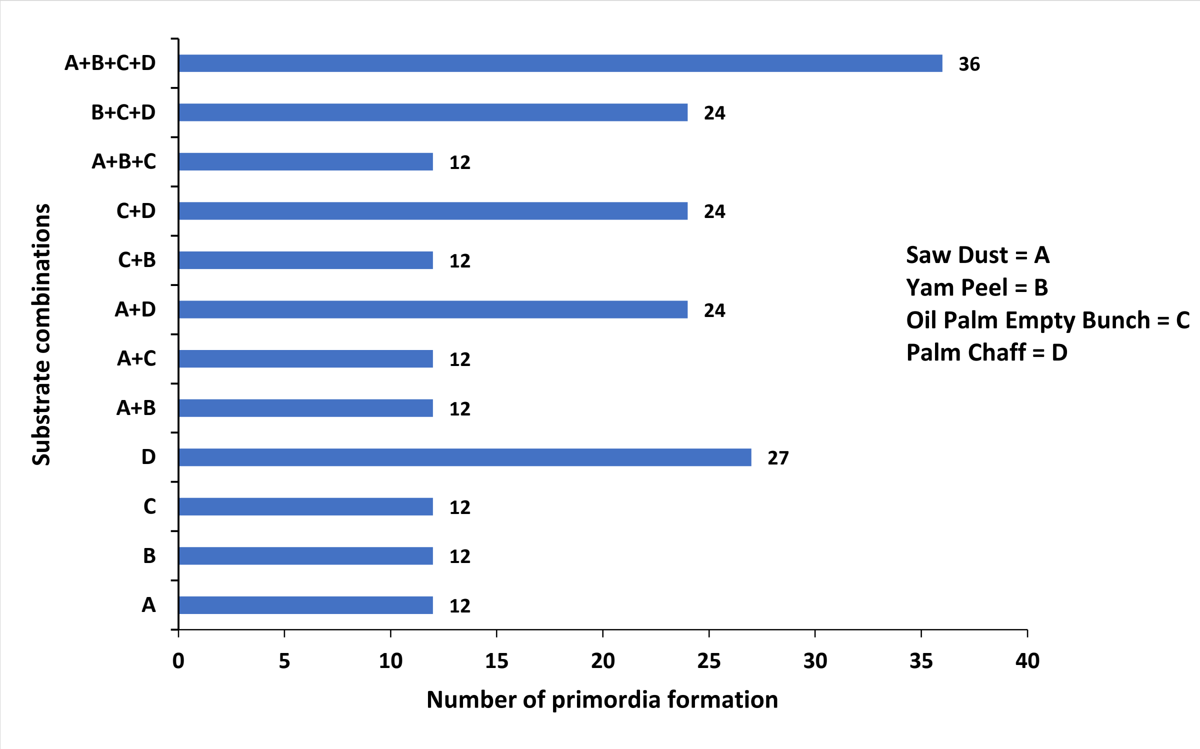

Figure 2: Primordia formation of *P. ostreatus* cultivated on the substrates

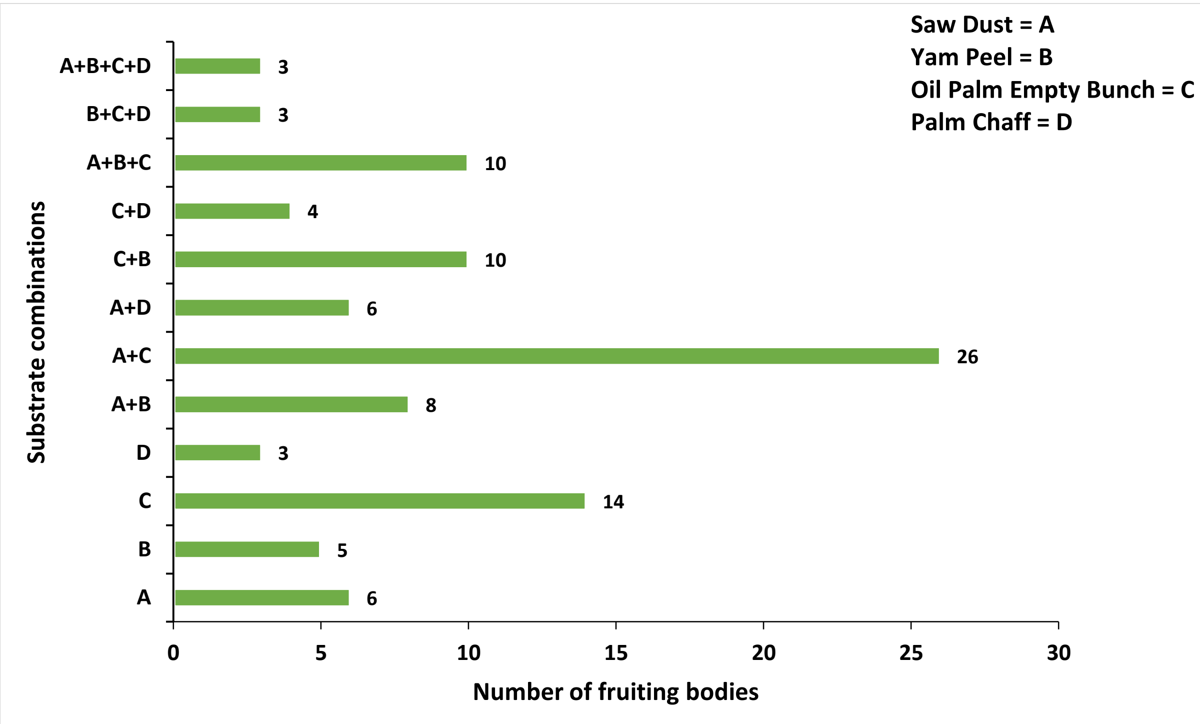

Figure 3: Fruiting bodies of *P. ostreatus* cultivated on the substrates

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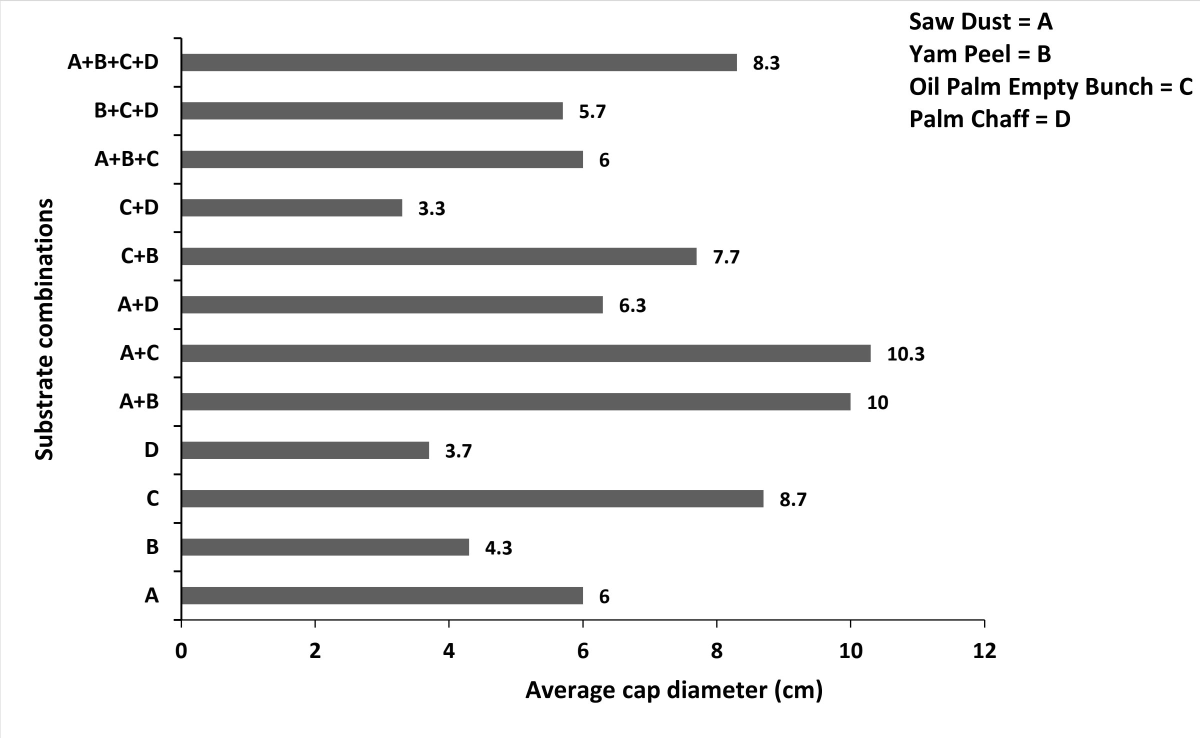

Figure 4: Cap diameter of *P. ostreatus* cultivated on the substrates

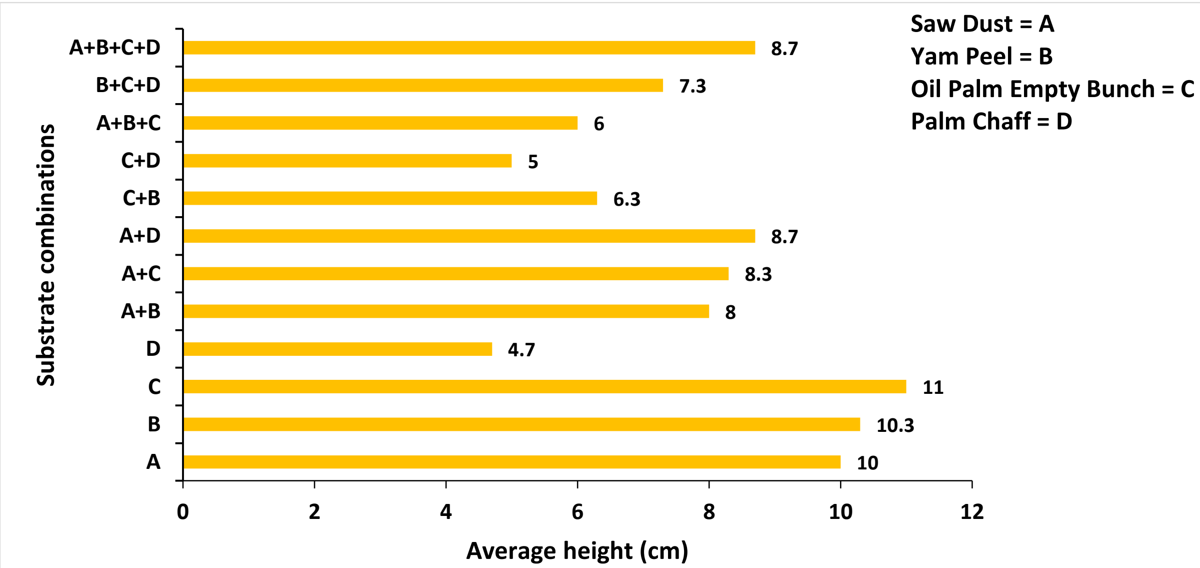

Figure 5: Height of *P. ostreatus* cultivated on the substrates

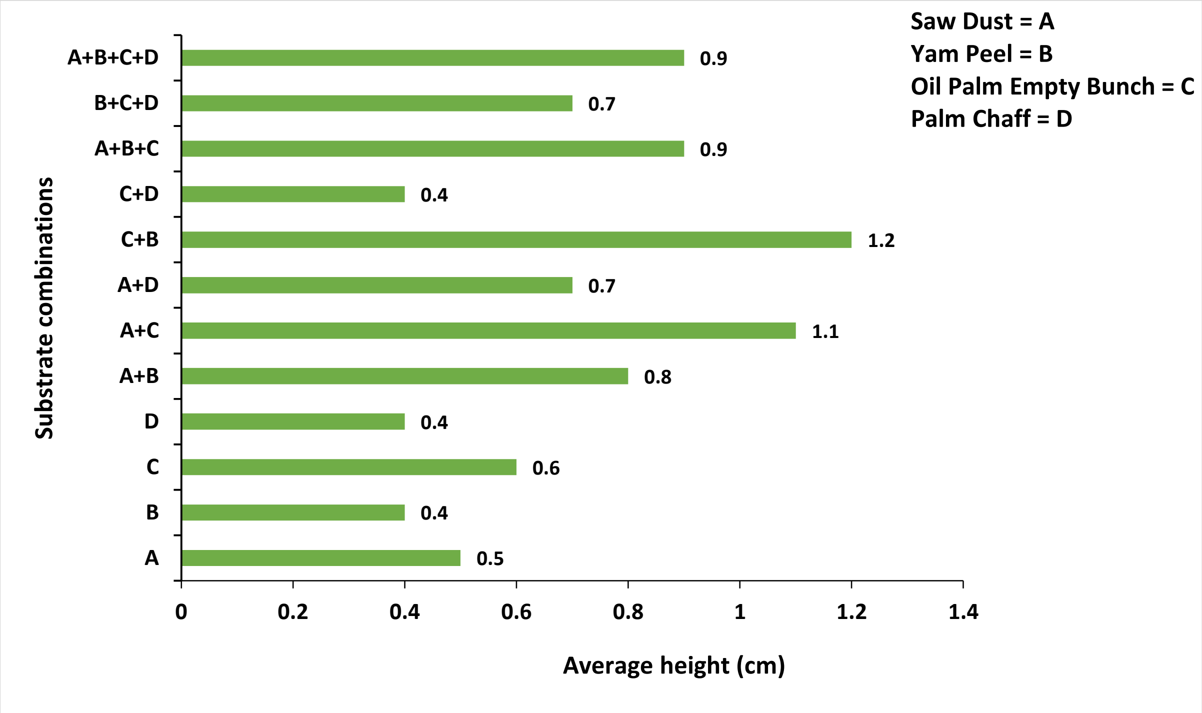

Figure 6: Girth of *P. ostreatus*cultivated on various substrates combinations

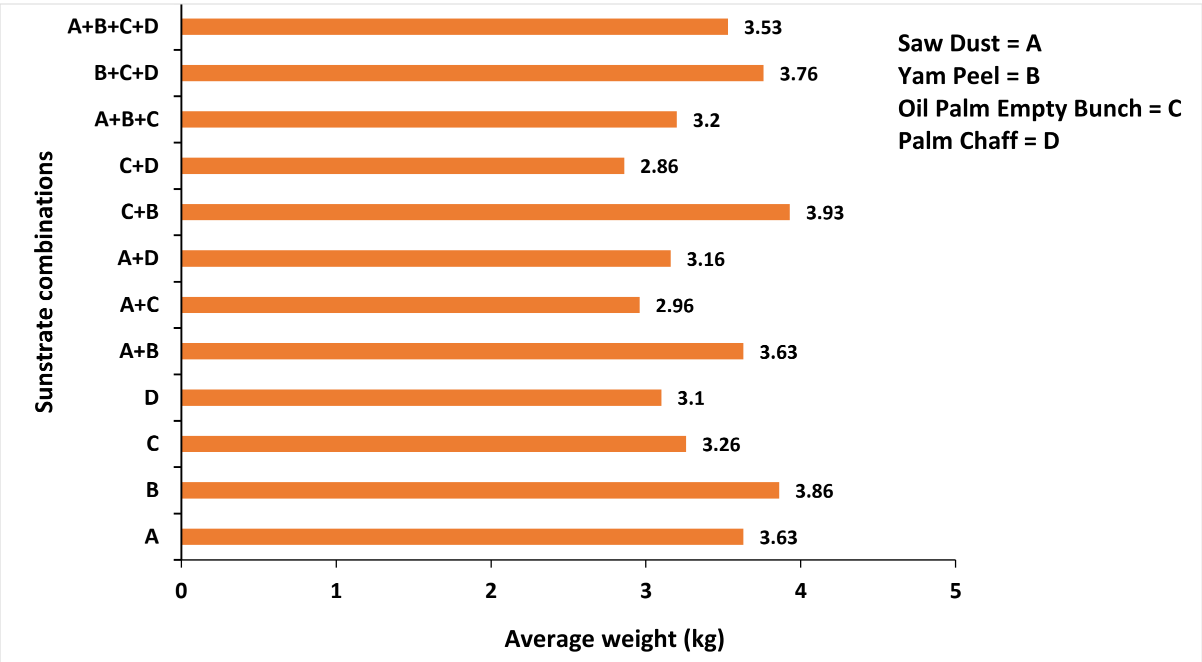

Figure 7: Weight of *P. ostreatus* cultivated on the substrates

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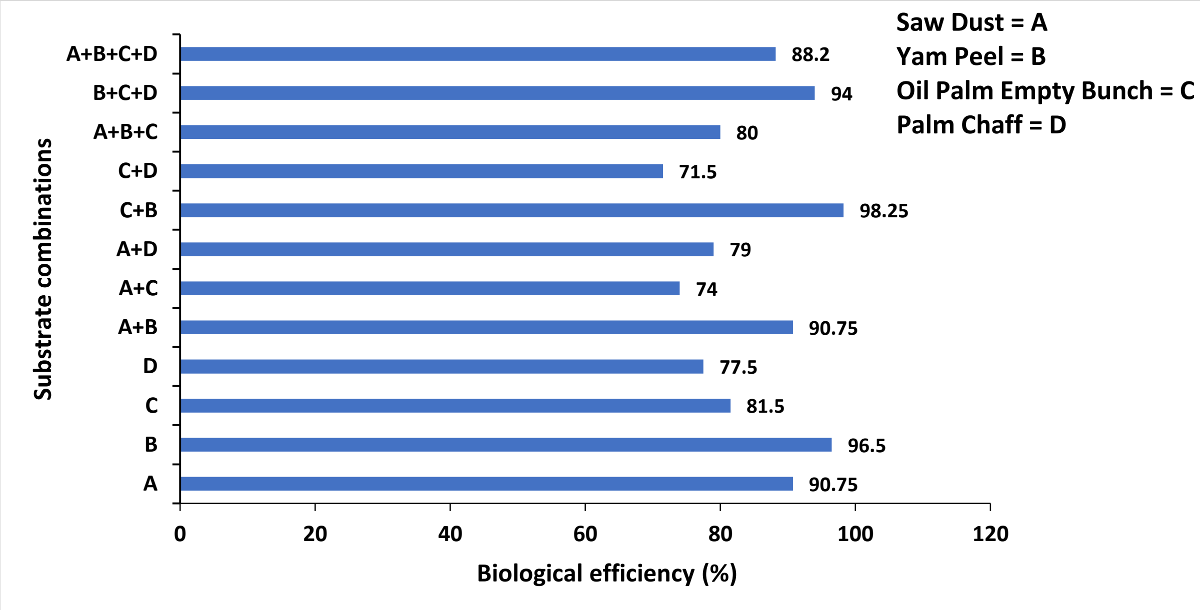

Figure 8: Biological efficiency of *P. ostreatus* cultivated on the substrates

The results obtained for the effect of the substrates on growth dynamics of *Pleurotus ostreatus* (oyster mushroom) about various combinations of the growth substrates were presented in figure 2 to 8 above. The primordial formation of the mushroom ranged from 12 to 36 across the various substrate formulation treatments (Figure.2). The highest primordial formation of the mushroom was recorded in treatment A + B + C + D at 36, followed by treatment D at 27, treatment C + D at 24, and treatment B + C + D at 24. Treatments A, B, C, A + C, A + B, A + C, and A + B + C had the lowest primordial formation, each recording 12. This parameter referred to the number of primordia (early-stage fruiting bodies) formed. It is an important indicator of successful mushroom growth.

Furthermore, the number of fruiting bodies produced varied across the treatments (Figure 3). Treatment A + C had the highest number of fruiting bodies at 26, followed by treatment C at 14, and treatments B + C and A + B at 10 each. Treatments B + C + D, A + B + C, A + B + C + D, and C + D had the lowest number of fruiting bodies, each recording 3. This parameter indicated the number of mature mushrooms produced by the various treatments.

The average cap diameter of the mushrooms ranged from 3.3 cm to 10.3 cm across the different substrate formulations (Figure 4). The highest average cap diameter was observed in treatment A + C at 10.3 cm, followed by treatment A + B at 10.0 cm. The smallest average cap diameter was recorded in treatment C + D at 3.3 cm.

In addition, the average height of the mushrooms ranged from 4.7 cm to 11.0 cm (Figure 5). Treatment C had the tallest mushrooms with an average height of 11.0 cm, followed by treatment B at 10.3 cm, treatment A at 10.0 cm, and treatment A + C at 8.3 cm. Treatment D had the shortest mushrooms with an average height of 4.7 cm.

Moreover, the average girth (circumference) of the mushrooms varied between 0.4 cm and 1.2 cm (Figure 6). Treatment B + C had the highest average girth at 1.2 cm, followed closely by treatment A + C at 1.1 cm. The smallest average girth was recorded in treatments A, B, and D, each at 0.4 cm.

The average weight of the freshly harvested mushrooms ranged from 2.86 kg to 3.93 kg

(Figure 7). Treatment C + B had the highest average weight at 3.93 kg, followed by treatment B at 3.86 kg. The lowest average weight was recorded in treatment C + D at 2.86 kg. The ANOVA in Table 7b (See Appendix) suggested that there was no significant difference in the weight of the fresh mushrooms between the different treatments. This was based on the very high p-values of 0.9839 and 0.4151 for the "Rows" and "Columns" sources of variation. In other words, the variation in weight observed between the treatments was more likely due to random chance rather than a true difference in treatment effects. Since the p-value was much higher than the conventional alpha level (0.05), we failed to reject the null hypothesis. This meant that there was insufficient evidence to conclude that there were significant differences in the weight of the fresh mushrooms between the treatment groups.

Furthermore, the biological efficiency, which measured the efficiency of converting substrate into mushrooms, varied from 71.50% to 98.25% (Figure 8). Treatment C + B had the highest biological efficiency at 98.25%, followed by treatment B + C + D at 94.00%. The lowest biological efficiency was recorded in treatment C + D at 71.50%. The ANOVA in Table 8b (See Appendix) indicated that there was no significant ($P > 0.05$) difference in the biological efficiency between the different treatments. This was based on the very high p-values of 0.9839 and 0.4151 for the "Rows" and "Columns" sources of variation. In other words, the variation in biological efficiency observed between the treatments was more likely due to random chance rather than a true difference in treatment effects. Since the p-value was much higher than the conventional alpha level (0.05), we failed to reject the null hypothesis. This meant that there was insufficient evidence to conclude that there were significant differences in the biological efficiency between the treatment groups.

The results presented in figure 2 to 8 demonstrated the significant influence of different substrate formulations on the growth dynamics of *Pleurotus ostreatus* (oyster mushroom). Several key parameters were assessed, including primordial formation, fruiting body production, cap diameter, mushroom height, girth, weight, and biological efficiency.

The primordial formation, which indicates the early stage fruiting body development, exhibited a wide range across the various substrate combination treatments as shown in Figure 2. This highlights the substantial impact of specific substrate combinations on the initial stages of mushroom growth, indicating that certain formulations were more conducive to primordial development. Studies by Nguyen and Ranamukhaarachchi (2019) and Tarko and Sirna (2018) align with our findings, emphasizing the significant impact of specific substrate combinations on primordial formation. However, studies by Chukwu et al. (2022), Meng et al. (2021), and Attaran et al. (2019) suggest that alternative factors like the environment and genetics may also affect primordial development, indicating the need for further investigation.

In addition, the number of fruiting bodies produced also varied significantly across treatments as shown in Figure 3, further emphasizing the importance of substrate selection. This parameter provided insight into the ultimate yield of the mature mushroom, underscoring the significance of substrate choice in maximizing production. Our results regarding the influence of substrate selection on fruiting body production are supported by the findings of Muswati et al. (2021) and Besufekad et al. (2020).

In terms of size and dimension, the average cap diameter of the mushrooms exhibited a considerable range as shown in Figure 4. Mushroom height and girth also varied significantly with different substrate formulations as shown in Figure.5 and Figure 6 respectively. These findings indicated that certain substrate combinations not only affected the number of mushrooms produced but also impacted their size and dimension, potentially influencing their market value and

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culinary applications (Zięba et al., 2020; Ritota & Manzi, 2019). Studies by Wachira et al. (2022) Agba et al. (2021), and Khalaphallah et al. (2020) corroborate our observations regarding the impact of substrate combinations on mushroom size and dimensions.

While there was substantial variation in cap diameter, height, and girth, the average weight of the freshly harvested mushrooms showed less pronounced differences (P > 0.05) across the treatments as shown in Figure 7. This implies that substrate choice may have had a more modest impact on mushroom weight compared to other growth parameters. The studies conducted by Kurd-Anjaraki et al. (2022) and Raschad et al. (2019) align with our findings that substrate choice may have a more modest but insignificant impact on mushroom fresh weight compared to other growth parameters. In contrast, the findings of Wachira et al. (2022) and Akter et al. (2022) demonstrated the significant impact of substrate choice on the average weight of mushrooms (*P. ostreatus*). Moreover, the investigations of Pardo-Giménez et al. (2020),

Finally, the biological efficiency, which measures the effectiveness of converting substrate into mushrooms, varied from 71.50% to 98.25% as presented in Figure 8. However, similar to the fresh weight parameter, statistical analysis indicated that the observed variation in biological efficiency was not statistically significant (P > 0.05). This suggested that differences in biological efficiency between the treatments were more likely due to random chance rather than a true difference in treatment effects. Investigations by Akter et al. (2022) and Elsisura & Figueroa, (2022) bring into with our findings that substrate choice may have a more modest but insignificant impact on the biological efficiency of mushrooms compared to other growth parameters.

In this study there was no statistically significant differences in mushroom weight and biological efficiency across treatments, it's important to consider external factors like environmental conditions and genetic variability that may have influenced these outcomes (Pattanayak & Das, 2022; El-Nour et al., 2021).

Overall, the results obtained in this study underscored the pivotal role of substrate formulation in shaping various facets of mushroom growth dynamics. While parameters like primordial formation, fruiting body count, cap diameter, height, and girth exhibited notable variations across different substrate formulations, the weight of fresh mushrooms and their biological efficiency emerged as reliable benchmarks for overall growth assessment. These findings held substantial practical implications for refining mushroom cultivation practices. Specific substrate combinations showed promise in yielding higher quantities, larger and sturdier mushrooms, and enhanced biological efficiency.

CONCLUSION

The study addressed the environmental challenges posed by organic waste. The objectives of the study included investigating the suitability of various agro-wastes as substrates for mushroom cultivation, analyzing the nutritional content of the waste-grown mushroom-based poultry feed, proximate composition of the organic waste substrates used in cultivating *Pleurotus ostreatus* was analyzed, and the results revealed significant variations in various nutritional components among the substrates. These findings offered valuable insights into the suitability of each substrate for cultivating *Pleurotus ostreatus* Additionally, it enabled growers to make informed decisions about substrate selection based on the specific nutritional needs and growth requirements of the mushrooms.

This study investigated the impact of various organic waste substrates on the growth dynamics of *Pleurotus ostreatus* (oyster mushroom), and the results revealed significant variations in several key parameters. In primordial Formation, the number of early-stage fruiting bodies formed varied between 12 and 36 across different substrate formulations. The highest primordial formation was observed in treatment A + B + C + D (36), while treatments involving individual substrates or their combinations had the lowest primordial formation. The study also investigated the influence of different primary waste substrates (sawdust, yam peel, oil palm empty bunch, and palm chaff) on the proximate composition of freshly harvested *Pleurotus ostreatus* mushrooms, and the results revealed significant variations in several key nutritional parameters.

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