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Growth and Yield Performance of Oyster Mushroom Cultivated in Combined Cassava Peels, Coconut Residue and Coffee Waste Substrates

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ABSTRACT

The oyster mushroom (*Pleurotus ostreatus*) is an edible mushroom that belongs to the class of Basidiomycetes. It has reached sufficient market maturity because of its flavor, shelf-life durability, and protein and fiber content. Besides their nutritional, medicinal, and economic value, they may help the country's agricultural waste management, bridge environmental issues, and contribute to climate change resolution advancements. A study on different varieties of agricultural substrates derived from waste materials such as cassava peels, coconut residue, and coffee waste was investigated and compared to sawdust, the common substrate for oyster mushrooms. The effects of different substrates on the morphological characteristics of *P. ostreatus*, percent contamination, and yield parameters were recorded and analyzed using the Analysis of Variance in Completely Randomized Design, and their significant results were compared using Tukey's HSD. Results showed that different substrate mixtures did not significantly influence the morphological characteristics of *P. ostreatus*. Moreover, sawdust, the common substrate for oyster mushrooms, showed the lowest percent contamination as compared to other substrate mixtures. Contaminants found in cassava substrates include *Trichoderma spp., Aspergillus spp., Fusarium spp., Neurospora spp.,* and *Penicillium spp.* 80% of cassava peels combined with 10% coconut residue and 10% coffee waste significantly increased the number of fruiting bodies and produced the heaviest fresh weights of oyster mushrooms. Stipe length and pileus diameter were also significantly influenced by this substrate mixture, which is comparable to the common substrate. However, further research on the varying proportions of these substrate mixtures on the performance of oyster mushrooms is recommended.

INTRODUCTION

Oyster mushrooms have reached sufficient market maturity because of their delightful flavor, durability in shelf life, extraordinary protein and fiber content, and nutritional and medicinal features. These kinds of mushrooms can establish and degrade a variety of lignocellulosic substrates and other wastes, which are produced primarily through the activities of the agricultural, forest, and food-processing industries.

Because of the more favorable climate conditions in Southeast Asian countries such as the Philippines, growing one's food poses few to no challenges. The country's main crops are rice, corn, coconut, sugarcane, bananas, pineapple, coffee, mangoes, tobacco, and abaca. Secondary crops include peanuts, cassava, sweet potatoes, garlic, onion, cabbage, eggplant, lime, lemon, rubber, and cotton. These are all export-quality products that the country is most proud of. But with the assortment of waste materials it produces, it piles it up to the extent of harming the environment itself, which supplies all of it, ironically. But the ballooning effect of our population reflects the agricultural and waste management of our country.

Today, only a part of this waste is used, mainly as provisions for goats and other pastured animals. In many regions where cassava is cultivated and processed, volumes of cassava peels are dumped along the roads, especially in wet seasons, and can generate offensive odors and unsanitary conditions. A valorization of cassava waste would not only resolve this environmental predicament but also contribute to the local economy.

The coconut residue generated after milk extraction is a food waste by-product, but only a fraction of it is used as fertilizer or cow feed. As a result of these activities, the by-products can contribute to the additional trash in the community.

By-products from coffee beans are useful substrates for the cultivation of *P. ostreatus* in coffee-producing countries like the Philippines because this species is fast-growing, substrates are easily accessible, and the mushrooms (or fruiting bodies) are a beneficial source of nutrition and revenue.

The researcher recognized the fact that there was not enough identified data that involved the previously indicated substrate combinations in developing *P. ostreatus*, although individual studies have shown that these substrates are effective in growing oyster mushrooms as previously mentioned. *P. ostreatus* is uncommon in our area, and the researcher was strongly convinced that the need to discover and innovate ways to cultivate it was significant. It was anticipated in this research to compare and contrast the yield performance of oyster mushroom (*P. ostreatus*) cultivation in a rural setting using different agricultural waste materials such as cassava wastes, coconut residue, and used coffee grounds, along with a constant percentage of supplementations from rice bran, limestone, and molasses and sawdust as common substrate. The researcher compared five

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substrate mixtures based on the yield performance in the production of oyster mushrooms. Subsequently, the study determined which of these substrates produced the most viable yield.

Through this study, Philippine households and farmers, particularly those in Mainit, Surigao del Norte, can have the opportunity and means to learn more about cultivating edible mushrooms such as oyster mushroom. Making these concepts a reality on a micro-to-commercial scale can suit the people's nutritional demands, not only as a supplement but also with profitable future engagements. It can also provide an opportunity for everyone with the resources to address and contribute to the country's current understanding of agricultural waste management not only ecologically but also economically.

LITERATURE REVIEW

For numbers of years, oyster mushroom (*P. ostreatus*) has displayed as one of the most cultivated mushrooms in the world, largely in Brazil (Lee et al., 2002; Sánchez, 2010; Royse, 2013). Pleurotus species' nutritional value, comprises high protein of 25-50 %, nine essential amino acids, and very low 2-5 % fat content, which makes it fitting as a diet food for health-conscious individuals. The sugar content is reasonable and ranges 17-47%, including minerals such as calcium, potassium, sodium with vitamins such as niacin, riboflavin, vitamin B1, B5, B6, C, and D (Caglarirmak, 2007; Syed et al., 2009). In addition, oyster mushrooms are good source of extraordinary stamina and vigor, and they are utilized in the manufacturing of many continental recipes. They also have medical properties such as anti-cancer, anti-cholesterol, and anti-tumorous. Mushrooms can help with diabetes, ulcers, and lung illness (Quimio, 1976). Mushroom protein is a combination of animal and vegetable protein (Kurtzman, 1976). Mushrooms are also potent in Niacin, Pantothenic acid, and Biotin (Subramanian, 1986). The good thing is, we can easily grow it on agricultural and industrial waste. More than half of the overall yield from the land is wasted as straws, leaves, stalks, roots, and so on (Zadrazil, 1978). These pollutants can be repurposed into food, and the environment may be less polluted as a result (Hayes, 1978). The population of the Philippines is climbing to 110,623,413 (as of Sunday, March 21, 2021; based on web). With this increase in population is the increase in the demand for food and agricultural products. As a result, it had been estimated that only about 50% of the city household's solid wastes are pulled by garbage collectors, while 38% of the households incinerate their garbage, and a further 12% of it is dumped in vacant lots (Holmer et al., 1999). The bigger portions of the solid waste that are being dumped at the city landfill site are from private households (54%) and commerce and institutions (28%). About 40 to 50% of the city wastes are biodegradable. However, if organic waste is properly processed to form compost, it can be put to good use in urban agriculture and horticulture as a fertilizer and soil improver. Farmers and gardeners can benefit from

it diligently. The use of organic waste provides a lasting increase in the waste control situation in cities. However, this assumes that urban waste management is integrated consistently with urban horticulture and agriculture (Guanzon & Holmer, 2015).

In Thailand, they can easily grow oyster mushrooms (*P. ostreatus*) in local conditions if the appropriate specifications of food and moisture for growth are available (Shah et al., 2004). *Pleurotus ostreatus* demands few environmental controls, and their fruiting bodies are not often attacked by diseases and pests, and they can be cultivated simply and cheaply. All this makes *P. ostreatus* cultivation an excellent alternative for the production of mushrooms when compared to other mushrooms (Sánchez, 2009).

Cassava (*Manihot esculenta*) belongs to the higher classification *Manihot*; and in order *Malpighialesis*, which is a dominant food crop for approximately 700 million people, especially in African countries. A substantial quantity of waste is usually produced throughout the processing, mainly consisting of tuber peels (Sonenberg et al., 2015). Previous reports on the utilization of cassava waste for the cultivation of *P. ostreatus* and *P. pulmonarius* have shown that yields were reasonable (Adebayo et al., 2009; Onuoha et al., 2009; Obodai et al., 2014). Cassava by-products as substrates can give yields of up to 100 percent BE of oyster mushrooms which compare well to traditional substrates such as sawdust (Frimpong-Manso et al., 2011). Cassava-based composites thus have the potential to be economically profitable for the production of oyster mushrooms (Sonnenberg et al., 2015).

Coconut palm (*Cocos nucifera* L.) is a tropical plant that belongs to the Kingdom Plantae, division Magnoliophyta, class Liliopsida, order Arecales, and family Arecaceae. The adult de-husked coconut generates 50% wet meat or core, 33% shell, and 17% water. Raw coconut meat has a chemical composition that contains about 3.33 percent protein, 33.49 % total fat, 15.23 % starch, 3.23 % total fiber, and a range of minerals and vitamins. The meat of the coconut kernel is used to make coconut milk in mostly Asian counties like the Philippines. Fresh coconut kernel is finely shredded and hand-squeezed or expeller pressed to produce coconut milk. As a result, a significant volume of coconut waste by-product after squeezing out milk is razed to the ground as waste (Sopit, 2007).

Coffee grounds have been investigated as a potential remnant for mushroom growth. Coffee grounds' chemical and structural properties enable for the reuse of lignin (23.90 g/100g dry product), nitrogen (12.79 g/100g dry product), and dietary fiber (60.40 g/100g dry product). The storage capacity of water is influenced by the particulate matter produced by coffee pressing (Ropciuc et al.,2016). Oyster mushroom cultivation on SCG for human consumption is totally possible, and there is significant potential for this extensive form of waste to be utilized and the caffeine content reduced. (Cabrera, 2018). Lime (CaCO3) is a key component in mushroom development; commercial mushroom production is dependent on correct substrate pH adjustment. (Khan

et al., 2013). Furthermore, molasses has been shown to promote the growth of several bacteria. Molasses contains sugar, nitrogen, and other nutrients that aid in cell development. As a result, it produced good results in the production of oyster mushrooms. (Erkel, 2009). Rice bran is clearly the most widely used and widely available organic component in substrates for the production of a wide range of edible mushrooms (Peng et al., 2000)

MATERIALS AND METHODS

Table 1. Research material, equipment, and raw materials used.

Materials	Equipment	Raw materials
Metal strainer	Improvised mushroom bag sterilizer	Cassava peels
Cheesecloth	Shovel	Coconut residue
6x12 inches Polypropylene cellophane	Shredder	Coffee waste
Empty bottle	Pressurized sprayer	Sawdust
PVP pipe (2.0 cm thick)	Triple burner gas tank	Limestone
Cotton wool	Water drum	Molasses
Rubber bands		Rice bran
Soap		Water
Bleach		
Small-eyed net		
Basin		
Tarpaulin		
Black cloth		
Pail		
Spoon		
70% Alcohol		
Denatured alcohol		
Denatured alcohol lamp		
Sacks		
LED light bulb		

The following materials, equipment, and raw materials were used in the study (Table 1).

Acquisition of *Pleurotus ostreatus* Grain Spawn and Substrate's Raw Materials
The oyster mushroom spawn was procured from a commercial laboratory located in Taguibo, Butuan City. Different raw materials needed for preparing substrates for the study were gathered from the local area in Mainit, Surigao del Norte. Specifically, cassava wastes and coconut residue were from the farmers and neighborhood within Mainit, Surigao del Norte. The coffee-pressed grounds were collected from the nearest coffee shop within Surigao City, Surigao del Norte.

Preparation of Mushroom Substrates
Cassava wastes. Fresh cassava wastes were carefully cleaned with water, packed into nylon sacks and were submerged in water for three days to mimic natural fermentation. After three days, the fermented cassava wastes were drained for a day, and sun-dried for two days using a net or a table. The sun-dried cassava wastes were shredded using a shredder. When the desired texture and sizes were attained, it was then packed in a clean sack and stored in a dry place.
Coconut residue. Finely shredded fresh coconut meats were soaked in warm water. The soaked shredded coconut meats were hand-squeezed and pressed through a metal strainer, and cheesecloth. In order to get lighter coconut milk, the process was repeated once or twice. After the milk extraction, the coconut leftovers were sun-dried for at least two days. The dried coconut residues were stored in a cool and dry place.
Coffee grounds. Coffee-spent grounds that were gathered from a coffeehouse were dumped in hot water to eliminate chemicals that might limit fungal development and trigger contamination. To eliminate the extra moisture, cheesecloth was used to softly pressed it to allow any leftover moisture to travel through. The coffee waste was left to cool off in a room temperature for about a day or two. It was placed in a dry container and stored in a cool and airy corner.

Building and Maintenance of Mushroom House
The mushroom house was four meters from floor to top part, and with an area of four-square meters. It only has one door, no window, and was made of bamboo matting with a nipa roof.
The mushroom house was cleaned thoroughly in its entirety by using pressurized water hose with soap, and bleach to ensure the removal of almost every unwanted bacterium or foreign biological substance that could alter the growing process and was regularly sanitized.

Preparation and Bagging of Mushroom Substrates
Recently prepared substrates were packaged in a 6x12

inch polypropylene cellophane bags containing 500 grams each based on the different substrate mixtures (Table 2). The bottom ends of the bags were folded to allow them to stand on their own. As additional compost was added to the ultimate weight required, an empty bottle was used to compress it. To act as a bottle neck, a 2.0 cm thick and 2.5 cm long PVC pipe was put at the neck of each bag. A piece of cotton wool was inserted into the bags' necks then covered with paper, and was secured with a rubber band. Then, it was labelled carefully and accordingly before proceeding to the next step.

Sterilization of Bagged Substrates

Bagged substrates were sterilized for six hours using the improvised mushroom bag sterilizer, fired with a triple burner stove. The sterilized bags were then removed from the improvised mushroom bag sterilizer to allow the second batch to be accommodated.

After sterilization, the substrates were allowed to totally cool-off for six to ten hours before spawn inoculation.

Table 2. Experimental treatments.

Treatment Numbers	Oyster Mushroom Substrate Mixtures							Total
	Major Substrate Ingredients (78%)				Supplemental Ingredients			
	Sawdust (Control)	Cassava peels	Coconut residue	Coffee waste	Rice bran	Molasses	Lime-stone	
1	100%	-	-	-	20%	1%	1%	100%
2	-	100%	-	-	20%	1%	1%	100%
3	-	80%	10%	10%	20%	1%	1%	100%
4	-	70%	20%	10%	20%	1%	1%	100%
5	-	50%	40%	10%	20%	1%	1%	100%

Grain Spawn Inoculation to Sterile Bagged Substrates

The oyster mushroom grain spawn was inoculated to the sterile bagged substrates in a biosafety cabinet. The grain spawn was loosened first before inoculation by shaking it. Bags were opened carefully, grain spawn were aseptically inoculated to the substrates at one tablespoon per bag, and then was covered again for incubation.

Incubation of Inoculated Fruiting Bags

The inoculated fruiting bags were arranged properly by treatments and replication, then they were placed in a well-ventilated dark area, covered in black cloth, and layered by tarpaulin or any plastic material to prohibit the direct light to our fruiting bags, until fully colonized. Fruiting bags were regularly monitored for colonization period.

Hanging of Fully-colonized Fruiting Bags

After colonization, the bags were sorted and hung using a rope and a wire as a lock to the thread. To depict each replication, fifteen mushroom fruiting bags were arranged in a row of every treatment piled at one foot apart. These were then regularly monitored until the appearance of pinheads' formation.

Management of Fruiting Bags

Spraying water at the back portion of the fruiting bags were done twice a day, at nine o'clock in the morning and three o'clock in the afternoon. The fruiting bags were enclosed in a net to avoid insect and pests' infestations. The floor was completely covered with a damp cloth to keep the mushroom house's humidity level stable. The time pinheads were observed, the cotton and paper cap of the fruiting bags were removed to allow the fruiting body to develop. During the stage of fruiting body formation, the room was illuminated with diffused light. Bags were checked for contamination on a daily basis. To prevent the spread of contaminants, defective bags were disposed or eradicated as soon as they were observed. Contaminations were identified based on their morphological characteristics and were compared to published and identified common contaminants of oyster mushrooms.

Harvesting of Oyster Mushroom Fruits

Harvesting was done when the cap of the oyster mushroom reached its maturity with its maximum diameter and in convex shape, by hand picking. Fruiting bodies were twisted and pulled carefully from the base of the stem to avoid contamination.

Data Gathered

During the course of the study, the following parameters were recorded.

1. Morphological characteristics. This parameter includes period of colonization or spawn run, pinhead formation, fruiting body formation and full maturity of oyster mushroom.

a) Days from Spawning to Complete Spawn Run (S-CSR). This refers to the number of days the substrates were fully colonized by the fungus from the day of inoculation.

b) Days from Spawning to Pinhead Formation (S-PF). This pertains to the number of days the fungus produces pinheads' formation from the day of inoculation

c) Days from Pinhead Formation to fruiting body formation (PF- FBF). This refers to the number of days the fruiting body was observed after the pinhead formation, and

d) Days from Fruiting Body Formation to Full Maturity (FBF- FM). This represents the ideal number of days to harvest after fruiting body formation.

2. Percentage Contamination. It refers to the percentage of discarded fruiting bags due to contamination in every treatment.

This was computed using the formula:

$$\% \text{ Contamination} = \frac{\text{No. of contaminated bags}}{\text{No. of sample bags}} \times 100$$

3. Harvest Data. This parameter includes number of fruiting bodies per treatment, average mycelia height, average mycelia diameter, and weight of fresh fruiting bodies of oyster mushroom on different substrate mixtures.

a) Number of fruiting bodies. Immediately every after harvest, fruiting body was counted by determining the caps of the fruits.

b) Average stipe height. It was gathered by measuring the tip of the stem or the stipe of the oyster mushroom to the base of the cap. A ruler was used and was expressed in centimeters.

c) Average pileus diameter. The pileus or the cap of the oyster mushroom was measured across using a ruler and was expressed in centimeters.

d) Weight of fresh fruiting bodies of oyster mushroom. It was gathered every after harvest. Electronic balance was used to gather the data and was expressed in grams.

Statistical Analysis

The study was analyzed using Analysis of Variance (ANOVA) in Completely Randomized Design and the differences among treatment means were compared using Tukey's Honest Significant Difference (THSD) as Post-Hoc analysis.

RESULTS AND DISCUSSION
Morphological Characteristics of P. ostreatus Grown in Different Substrate Mixtures

Days from Spawning to Complete Spawn Run (S-CSR). The colonization period of oyster mushroom using different substrate mixture is presented in Table 3. Results of the Analysis of Variance (ANOVA) showed no significant differences. Results of the study showed that 100% cassava peel has the shortest colonization period (33.33 days from inoculation), which was followed by 80% cassava peels + 10% coconut residue + 10% coffee waste with 34.67 days of colonization period, and 70% cassava peels + 20% coconut residue + 10% coffee waste with 35 days of inoculation. The longest period of colonization was recorded in substrate mixtures of 100% sawdust (Control) and 50% cassava peels + 40% coconut residue + 10% coffee waste with both 35.33 days. These results suggest that different substrate mixtures did not influence the period of colonization of *P. ostreatus*.

Days from Spawning to Pinhead Formation (S-PF). The pinhead's formation period of oyster mushroom using different substrate mixtures is also presented in Table 3. Same results of the ANOVA were recorded showing no significant differences among treatment means. Shortest pinhead formation was recorded in substrate mixtures with 100% cassava peels with 37.33 days. Eighty percent cassava peels + 10% coconut residue + 10% coffee waste and 70% cassava peels + 20% coconut residue +10% coffee waste have shorter period of pinhead formation with both took 38.00 days from inoculation. These were followed by the control (100% sawdust) with 38.67 days of pinhead formation. Substrate mixture with 50% cassava peels + 40% coconut residue + 10% coffee waste registered the longest number of days of pinhead formation with 40.00 days from inoculation. However, general results of this parameter suggest that these substrate mixtures did not significantly influence the pinhead formation of *P. ostreatus*.

Days from Pinhead Formation to Fruiting Body Formation (PF- FBF). The period of fruiting body formation of oyster mushroom using different substrate mixtures is presented in Table 3 as well. ANOVA results showed no significant differences among treatment means. Data showed that whatever substrate mixtures used, there is no differences in period of fruiting body formation which only took three days from pinhead formation in all substrate mixtures.

Days from Fruiting Body Formation to Full Maturity (FBF- FM). The time taken from fruiting body formation to pin maturation is also presented in Table 3. Results of the ANOVA revealed no significant differences among treatment means. Results of the study showed that all substrate mixtures used had no significant influenced on the period of full maturation of *P. ostreatus* which only took three days from fruiting body formation in all substrate mixtures.

Generally, results imply that different substrate mixtures used in the experiment do not influence the colonization period, pinhead formation, fruiting body formation, and full maturation period of *P. ostreatus*. These findings could be attributed to the cellulose and lignin properties of both cassava peels and sawdust (Frimpong-Manso et al., 2011) (Horisawa et al., 1999). The results also confirmed Stanley & Nyenke's (2011) findings that cassava stimulated luxuriant mycelial growth rate and extension

Table 3. Morphological characteristics of oyster mushroom in different treatments.

Substrate Mixtures	S-CSR 1,ns	S-PHF 2,ns	PHF-FBF 3,ns	FBF-MP 4,ns
100% Sawdust (Control)	35.33	38.67	3	3
100% cassava peels	33.33	37.33	3	3
80% cassava peels + 10% coconut residue + 10% coffee waste	34.67	38.00	3	3
70% cassava peels + 20% coconut residue + 10% coffee waste	35.00	38.00	3	3
50% cassava peels + 40% coconut residue + 10% coffee waste	35.33	40.00	3	3
CV (%) =	2.68	2.52	0.00	0.00

can be comparable to that of a commonly used substrate such as sawdust.

ns=not significant

1 =S-CSR (Days from Spawning to Complete Spawn Run)

2 =S-PHF (Days from Spawning to Pinhead Formation)

3 =PHF-FBF (Days from Pinhead Formation to Fruiting Body Formation)

4 =FBF-MP (Days from Fruiting Body Formation to Full Maturity)

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Percent Contamination of Oyster Mushroom Bags Prepared With Different Substrate Mixtures

Percent of contamination. The percent contamination of oyster mushroom bags prepared with different substrate mixtures is shown in Table 4. Results of the ANOVA revealed significant differences among treatment means ($p > 0.01$). The least percentage of contamination was recorded in the control bags with 100% sawdust (11.11%), followed by the substrate mixtures of 80% cassava peels + 10% coconut residue + 10% coffee waste and 50% cassava peels + 40% coconut residue + 10% coffee waste comparable percent contamination of 37.78% and 35.56%, respectively. Higher contamination was observed in substrate with 100% cassava peels with 71.11% contaminated bags. While the highest contamination was recorded in substrate mixtures with 70% cassava peels + 20% coconut residue + 10% coffee waste with 84.44% contamination.

Common contaminants found in the contaminated fruiting bags were presented in Table 5. The fungal contaminants observed were as follows: *Trichoderma spp.*, *Aspergillus spp.*, *Fusarium spp.*, *Neurospora spp.*, and *Penicillium spp.* These results suggest that 100% sawdust as substrate for *P. ostreatus* had the lowest percent of contamination. Hence, it can be the ideal substrate for oyster mushroom to lower contaminant population. Moreover, cassava mixed with coconut residue and coffee waste can enhance contaminant population. These may be due to the fungal contaminants usually associated with higher percentage of cassava-based substrate like *Aspergillus spp.* (Obadina, 2006), and coconut-based substrate *Fusarium spp.* (Manimekalai, 2010). Number of

Table 4. Percent of contaminated oyster mushroom bags in different substrate mixtures.

Substrate Mixtures	Replication			Total	Mean**
	I	II	III		
100% Sawdust (Control)	6.67	13.33	13.33	33.33	11.11[a]
100% cassava peels	66.67	73.33	73.33	213.33	71.11[c]
80% cassava peels + 10% coconut residue + 10% coffee waste	33.33	40.00	40.00	113.33	37.78[b]
70% cassava peels + 20% coconut residue + 10% coffee waste	86.67	86.67	80.00	253.33	84.44[d]
50% cassava peels + 40% coconut residue + 10% coffee waste	33.33	33.33	40.00	106.67	35.56[b]

CV (%) = 8.02; **=significant at 1% level, THSD.

Table 5. Contaminants found in contaminated fruiting bags

Substrate Mixtures	Image of Contaminations	Morphological Characteristics	Fungal Contaminants
100% Sawdust (Control)		Green-mold. Color yellow to green, gradually creeping into the black substrate (Druzhinina et al., 2011)	*Trichoderma spp.*
100% cassava peels		Produced white to yellowish or crusty on the substrate, foul-rotten smell. (Lopez - Arevalo et al. 1996))	Aspergillus spp
80% cassava peels + 10% coconut residue + 10% coffee waste		Fusarium mold, can appear pale or brightly colored, with a cottony surface. (Manimekalai, 2010)	*Fusarium spp.*
70% cassava peels + 20% coconut residue + 10% coffee waste		Produced bright neon orange to reddish substrate. (Suada et al.2015)	*Neurospora spp.*
50% cassava peels + 40% coconut residue + 10% coffee waste		Produced initially white and become blue-green, gray-green, olive-gray, yellow or pinkish with time substrates. (Lopez - Arevalo et al. 1996))	*Penicillium spp.*

Fruiting Bodies of Oyster Mushroom Grown in Different Substrate Mixtures

Number of fruiting bodies produced in different substrate mixtures is presented in Table 6. Results of ANOVA revealed significant effects of these substrates to fruiting bodies of oyster mushroom on its first, second, and total flushes. Effects of the number of fruiting bodies is shown in Fig. 1.

First Flush. Results of the study showed that fruiting bodies (43.15) were significantly higher in substrates with 70% cassava peel + 20% coconut residue + 10% coffee waste which was comparably higher as compared to substrate with 80% cassava peel + 10% coconut residue + 10% coffee waste with 37.25 fruiting bodies formed, and was followed by the substrate with 100% cassava peels with 34.30 fruiting bodies. Lower number of fruiting body formation was observed in substrate with 50% cassava peel + 40% coconut residue + 10% coffee waste with 30.70 fruits formed. Lowest number of fruiting bodies on the other hand, was recorded in control (100% sawdust) with only 23.21 fruits formed.

Second Flush. Results showed that 50% cassava peel + 40% coconut residue + 10% coffee waste had the highest number of fruiting bodies with 50.09 fruits formed, which is comparably higher as compared to substrate with 80% cassava peel + 10% coconut residue + 10%

coffee waste and 100% cassava peels with 46.56 and 35.00 fruits formed. Seventy percent of cassava peels + 20% coconut residue + 10% coffee waste and the control (100% sawdust) had the lowest number of fruiting bodies (19.75 and 21.49 fruits, respectively).

Total Flush. Results showed that 80% cassava peel + 10% coconut residue + 10% coffee waste and 50% cassava peel + 40% coconut residue + 10% coffee waste had the highest number of fruiting bodies with 83.81 and 80.78 fruits, respectively. These were followed by the substrate with 100% cassava peels and 70% cassava peel + 20% coconut residue + 10% coffee waste having corresponding fruit formations of 69.30 and 62.90. Lowest number of fruiting bodies was recorded in the control (100% sawdust) with only 44. 70 fruits.

Generally, the above results suggest that the substrate mixtures containing 80% cassava peel + 10% coconut residue + 10% coffee waste and 50% cassava peel + 40% coconut residue + 10% coffee waste can increase the production of fruiting bodies of oyster mushroom for about 36.08 - 39.11. These corresponds to the outcomes of the study of Sonnenberg et al. (2015) that demonstrated the potential of cassava peel as a substrate ingredient for the production of oyster mushrooms, with a yield comparable to that of sawdust. These results were further explained by Youri (2003), who showed that

sawdust and cassava peel are lignocellulosic material which consist of three main components, namely: cellulose, hemicellulose, and lignin that serve as a suitable substrate for mushrooms as it degrades lignocellulosic substrates through lignocellulosic enzyme production and utilize the degraded products to produce their fruiting bodies, and were also backed up by the studies of Sá´nchez (2009), and Grim & Wosten (2018). Furthermore, Ginterova and Janotkova (1998) discovered that plant oil with a high total fat content, such as that found in coconut residue,

stimulates the formation of biomass in *P. ostreatus*. As a result, adding the appropriate amount of coconut residue can be used as supplemental substrate, increasing the fruiting bodies and yield of oyster mushroom (Sopit, 2007). Besides, Ropciuc et al. (2016), also observed that mushrooms grown on coffee waste had a respectable percentage of biological efficiency. As a result, combining these substrates can contribute to achieving the highest yield in weight parameters of oyster mushroom.

Table 6. Yield of oyster mushroom in different substrate mixtures.

Substrate Mixture	No. of Fruiting Body (Per flush)			Stipe Height (cm) **	Pileus Diameter (cm) *	Fresh Weight (g. per flush)	
	1st *	2nd **	Total**			1st**	2nd**
100% Sawdust (Control)	23.21[d]	21.49[c]	44.70[c]	19.45[a]	21.15[a]	37.99[bc]	38.10[b]
100% cassava peels	34.30[b]	35.00[b]	69.30[b]	11.35[bc]	13.90[ab]	50.00[ab]	51.00[a]
80% cassava peels + 10% coconut residue + 10% coffee waste	37.25[ab]	46.56[ab]	83.81[a]	12.17[b]	14.34[ab]	63.63[a]	53.50[a]
70% cassava peels + 20% coconut residue + 10% coffee waste	43.15[a]	19.75[c]	62.90[b]	11.00[c]	13.40[ab]	52.10[ab]	32.25[b]
50% cassava peels + 40% coconut residue + 10% coffee waste	30.70[c]	50.09[a]	80.78[a]	9.44[d]	10.78[b]	32.18[c]	34.25[b]
CV (%) =	19.84	14.34	12.31	2.91	21.88	13.97	7.69

*=significant at 5% level, THSD; **=significant at 1% level, THSD.

Figure 1. Number of fruiting bodies of oyster mushroom grown in different substrate mixtures.

Stipe Height (cm) and Pileus Diameter (cm) of Oyster Mushroom Grown in Different Substrate Mixtures

Stipe height (cm) and pileus diameter (cm) of oyster mushroom grown in different substrate mixtures is

also presented in Table 6. Results of ANOVA revealed significant differences on both parameters.

Stipe height (cm). Results showed that the control (100% sawdust) has the longest stipe height with 19.45 cm. This was followed by the substrate with 80% cassava peel + 10% coconut residue + 10% coffee waste with 12.17 cm. height which is comparable with 100% cassava peels with 11.35 cm. stipe height. Shorter stipe height (11.00 cm.) was observed in 70% cassava peel + 20% coconut residue + 10% coffee waste which as followed by the substrate with 50% cassava peel + 40% coconut residue + 10% coffee waste with 9.44 cm.

Pileus Diameter (cm). Results of the study showed control (100% sawdust) still showed the widest pileus with 21.15 cm. Comparably, wider pileus were observed in substrate mixtures such as 100% cassava peels, 80% cassava peel + 10% coconut residue + 10% coffee waste and 70% cassava peel + 20% coconut residue + 10% cof-fee waste with corresponding pileus diameter of 13.90 cm., 14. 34 cm., and 13.40 cm. Smallest pileus was recorded in substrate containing 50% cassava peel + 40% coconut residue + 10% coffee waste with 10.78 cm. wide.

The above results imply that 100% sawdust has the longest stipe and widest pileus with 19.45cm. and 21.15 cm., respectively. Comparable pileus size was also observed in substrates such as 100% cassava peels, 80% cassava peel + 10% coconut residue + 10% coffee waste, and 70% cassava peel + 20% coconut residue + 10% coffee waste. These results inclined to the study of Sanjel et al. (2021), that discovered that the size of the

mushroom is dependent on substrates, and that poor in cellulose, hemicelluloses, and lignin materials can constitute physical barriers that are difficult to be broken down without the presence of lignin-degrading enzymes. In addition, major ecological factors that affects the stipe height, and pileus diameter in oyster mushroom includes the compactibility or the compressibility of material used as substrate. Because of the particle size, it increases the void ratio as moisture plays an important role in bonding because of sawdust and cassava peel's cohesive or lignin characteristics (Azhar et al., 2015). It has been supported by several studies that substrate type such as its lignin characteristic contributes significant differences in stipe height, and pileus diameter of oyster mushroom (Besufekad et al., 2020; Nkwonta, 2013; Tsegaye & Tefera, 2017; Onyeka et al., 2018; Dubey et al., 2019).
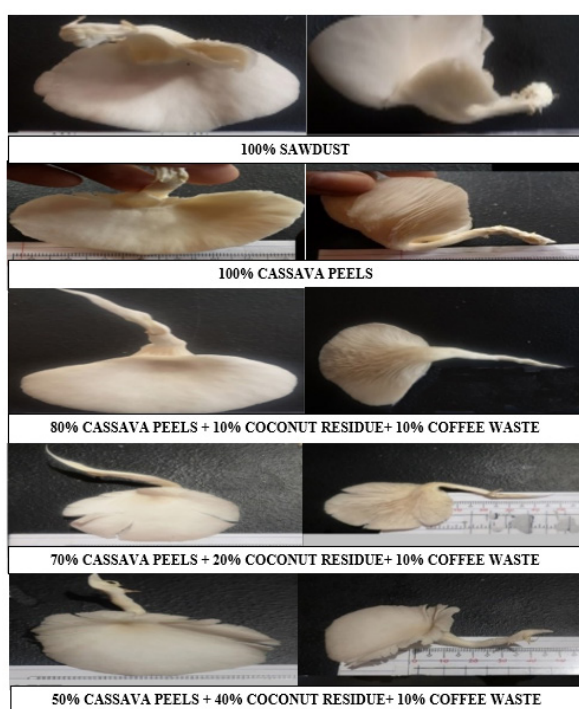

Figure 2. Pileus diameter (left), and stipe height (right) of oyster mushrooms harvested from different substrate mixtures.

Fresh Weights (g) of Oyster Mushroom as Influenced by Different Substrate Mixtures

Fresh weights (g) of oyster mushroom grown in different substrate mixtures is presented in Table 6. Results of the ANOVA revealed significant effects in the fresh weight of oyster mushrooms in first and second flushes.

First Flush. Heaviest fresh weight (63.63 g) was recorded in oyster mushroom grown from substrate with 80% cassava peel + 10% coconut residue + 10% coffee waste. Comparable effects were observed in substrates containing 100% cassava peels, and 70% cassava peel + 20% coconut residue + 10% coffee waste with 50.00 grams and 52.10 grams, respectively. Lighter fresh weights on the other hand, were recorded in substrates with 100% sawdust (37.99 g.) and 50% cassava peel + 40% coconut residue + 10% coffee waste (32.18 g.).

Second Flush. Heaviest fresh weight of oyster mushroom on the second flush were observed in 100% cassava peels and 80% cassava peel + 10% coconut residue + 10% coffee waste with weights of 51.00 grams and 53.50 grams, respectively. Lighter fresh weights were recorded in the other substrate mixtures such as 100% sawdust, 70% cassava peel + 20% coconut residue + 10% coffee waste, and 50% cassava peel + 40% coconut residue + 10% coffee waste with weights that ranged from 32.25 grams – 38.10 grams.

Based on these results, it suggests that 80% cassava peel + 10% coconut residue + 10% coffee waste can produce a heavy fresh weights of oyster mushroom. Moreover, comparable results can be obtained using 100% cassava peels as substrate. These results confirmed the study of Sonnenberg et al. (2015) that cassava peels showed to be a potential ingredient in mushroom substrate for it produced more than 100% BE which is comparable to that the usual sawdust substrate. Moreover, several reports showed that the adding of coconut residue (Sopit, 2007), and coffee waste (Ropciuc et al., 2016) increases yield and gained a respectable percentage of biological efficiency in oyster mushrooms. Therefore, combination of these substrates can contribute to gaining the significant yield in weight parameters of oyster mushroom.

CONCLUSION

Based on the above results, it can be concluded that various substrate mixtures do not significantly influence the oyster mushroom's colonization, pinhead formation, fruiting body formation, and the period of maturation. Among other substrate mixtures, the common substrate (100% sawdust) can produce the least number of contaminated fruiting bags, as well as the widest mushroom pileus and longest stipe. Moreover, the substrate mixture with 80% cassava peels + 10% coconut residue + 10% coffee waste can produce the greatest number of fruiting bodies while also being the most viable in terms of weight yield. Thus, different mixtures of cassava peels, coconut residue, and coffee waste were an effective substrate mixture to produce viable yields and, therefore, can be used as a successful substrate for oyster mushroom cultivation. The findings of this study proved that cassava peels and/ or a combination of coconut residue, and coffee wastes can be use as substrate option for mushroom growers due to its high yield potential. Aside from their nutritional, medicinal, and economic value, oyster mushrooms (*P. ostreatus*) may aid in agricultural waste management in the country. These can help bridge environmental issues and advance climate change response. However, further research on the effects of these substrate mixtures to oyster mushrooms as well as its varying proportions, are recommended.

REFERENCES

Adebayo, G.J., Omolara, B.B., and Toyin, A.E. (2009). Evaluation of yield of oyster mushroom (*Pleurotus pulmonarius*) grown on cotton waste and cassava

peel. 8(2):215–218. https://doi.org/10.5897/AJB2009.000-9039.

Azhar, A., Abdul A., Muneer, A., Shoukat, A., Rashid, T., and Umer. (2015). Compaction characteristics of sawdust, cotton stalks, maize straw and rice straw briquettes. *Journal of Energy, Environment & Carbon Credits.* 5. 1-9.

Besufekad, Y., Mekonnen, A., Girma, B., Daniel, R., Tassema, G., Melkamu, J., Asefa, M., Fikiru, T., and Denboba, L. (2020). Selection of appropriate substrate for production of oyster mushroom (*Pleurotus ostreatus*). *Journal of Yeast and Fungal Research,* 11(1), 15-25. https://doi.org/10.5897/JYFR2019.0187.

Cabrera, C.P.C. (2018). The role of nitrogen sources and caffeine for growth of *Pleurotus ostreatus* (oyster mushroom). *A thesis submitted in fulfilment of requirements of the degree of doctor of philosophy. School of life and environmental sciences faculty of science. The University of Sydney.* (P4, P176). https://Core.Ac.Uk/Download/Pdf/212694161.Pdf.

Caglarirmak, N. (2007). The nutrients of exotic mushrooms (*Lentinula edodes and Pleurotus species)* and an estimated approach to the volatile compounds. *Food Chem.,*105:1188–1194. DOI: 10.1016/j.foodchem.2007.02.021.

Druzhinina, I.S., Sidi-Seiboth, V., Herrera-Estrella, A., Horwitz, B.A., Kenerley, C.M., Monte, E., Mukherjee, P.K., Zeilinger, S., Grigoriev, I.V., and Kubiecek, C.P. (2011). *Trichoderma: The Genomics of Opportunistic Success. Review. Microbiology* 9: 749-759.

Dubey, D., Dhakal, B., Dhami, K., Sapkota, P., Rana, M., Poudel, N. S., and Aryal, L. (2019). Comparative study on effect of different substrates on yield performance of oyster mushroom. DOI:10.24105/2319-.

Erkel, E. (2009). Yield performance of *Ganoderma lucidum* (Fr.) karst cultivation on substrates containing different protein and carbohydrate sources. *African Journal of Agricultural Research* Vol. 4 (11): 1331 – 1333.

Frimpong-Manso, J., Obodai, M., Dzomeku, M., and Apertorgbor, M.M. (2011). Influence of rice husk on biological efficiency and nutrient content of *Pleurotus ostreatus. Kummer' s International Food Research Journal* 18: 249–54. DOI:10.3362/2046-1887.2015.007.

Ginterova, A., and Jaotkova, O. (1981). Utilization of fat and degradation of cholesterol by Pleurotus sp. Folia Microbiol. (Praha), 26:228-231.

Grimm, D., and Wösten, H. A. (2018). Mushroom cultivation in the circular economy. *Applied Microbiology and Biotechnology,* 102(18), 7795-7803.

Guanzon, Y.B., and Holmer, R.J. (2015). Composting of organic wastes: a main component for successful integrated solid waste management in Philippine cities. *Publication at:* https://www.researchgate.net/publication/27279283.

Hayes, S. (1978). Ecology, resources and mushroom cultivation. mush. *Journal.* 84: 515-525.https://www.academia.edu/27417045.

Holmer, R.J., Potutan, G. E., and Schnitzler, W. H. (1999). Urban horticulture in the Philippines: Its impact on food security, health, gender and urban environment. *Proceedings of The Sub-Regional Workshop on Constraints and Opportunities for The Development of Urban and Peri-Urban Horticulture in South-East Asia, Kunming, Yunnan, China. Ministry of Agriculture, People's Republic of China, Food and Agriculture Organization of The United Nations, Rome, Italy,* Pp.39- 48.https://www.researchgate.net/profile/Robert- Holmer/publication/265148443.

Horisawa, S., Sunagawa, M., and Tamai, Y. (1999). Biodegradation of nonlignocellulosic substances II: Physical and chemical properties of sawdust before and after use as artificial soil. Journal Wood Sci 45, 492–497 (). https://doi.org/10.1007/BF00538959.

Khan, M.W., Ali, M.A. Khan, N.A., Rehman, A., and Jave, N. (2013). Effect of different levels of lime and pH on mycelial growth and production efficiency of oyster mushroom (Pleurotus spp.) *Article in Pakistan Journal of Botany.* https://www.Researchgate.Net/Publication/287905250.

Kurtzman, R.H. Jr. (1976). *Nutrition of Pleurotus sapidus*: effects of lipids, mycologia, 68:2, P286-295, DOI:10.1080/00275514.1976.12019911.

Lee, H.Y., Won-Rok, K., and Min, B.H., (2002). Automation of solid-state bioreactor for oyster mushroom composting. *Microbiol.* 30:228–232. DOI 10.1007/s11274-016-2152-y.

Lopez-Arevalo, A., Huerta-Palacios, G., and Sanches-Vazquez, J.E. (1996). Contamination encountered during various phases of cultivation of *Pleurotus ostreatus* in tropical Mexico. *In Mushroom Biology and Mushroom Product, Royse (ed.) Penn State Univ.* ISBN 1-883956-01-3. P:1-5.

Manimekalai, R., and Nagarajan, P. (2010). Bulk line analysis in coconut (*Cocos nucifera* l.) for inferring relationship between talls, dwarfs and niuleka dwarf forms. *Indian J. Plant Genet. Resources* 23.7781.

Nkwonta, C. (2013). Correlation of stipe length, pileus width and stipe girth of oyster mushroom (*Pleurotus ostreatus*) grown in different farm substrates. *Journal of Agricultural Biotechnology and Sustainable Development.* 5. 54-60. 10.5897/JABSD2013.0197.

Obadina, A., Oyewole, O., Abiola, S. and Sanni, L. (2006). Short communication—fungal enrichment of cassava peels proteins. *African journal of Biotechnology,* 5, 302-304.

Obodai, M., Ofori, H., Dzomeku, M., Takli, K., Komlaga, G., Dziedzoave, N., Mensah, D., Prempeh, J., and Sonnenberg, A. (2014). Heavy metal and proximate composition associated with the composting of cassava (*Manihot esculenta*) peels used in the cultivation of mushrooms in Ghana. *African Journal of Biotechnology*

13(22): 2208–14. DOI:10.5897/AJB2014.13733.

Onuoha, C.I., Uchechi, U., and Onuoha, B.C. (2009). *Cultivation of Pleurotus pulmonarius (mushroom)*: Using some agro-waste materials. *Agricultural Journal* 4(2):109–12. DOI:10.3362/2046-1887.2015.007.

Onyeka, U., Udeogu, E., Okehie, M., Umelo, C. (2018). Effect of substrate media on growth, yield and nutritional composition of domestically grown oyster mushroom (*Pleurotus ostreatus*). *African Journal of Plant Science*. 12. 141-147. 10.5897/AJPS2016.1445.

Peng, J.T., Lee, C.M. and Tsai, Y.F. (2000). Effect of rice bran on the production of different king oyster mushroom strains during bottle cultivation. *Jour. Agric. Res. China* 49 (3):60-67. https://www.researchgate.net/publication/283117219.

Quimio, T.H. (1976). Cultivation Ganoderma the "*Pleurotus-way*" mushroom. *Newsletter for Tropics,* 6: 12-130.

Ropciuc, S., Leahu, A., Oroian, M., and Damian, C. (2016). Researches on *Pleurotus ostreatus*: Mushroom's quality cultivated on coffee grounds. *Scientific Papers Animal Science and Biotechnologies.* Vol. 49, o 2.https://spasb.ro/index.php/spasb/article/view/2203.

Royse, D.J. (2013). Trends in mushroom production worldwide. in: Sales-Campos C, Abreu RLS, Vianez BF, Urben AF (eds) Anais do VII Simpo´sio Internacional Sobre Cogumelos No Brasil, 6th Edn. Embrapa, *Brasilia*, pp 38–47.

Sa´nchez, C. (2009). Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotechnol Adv* 27:85–194.https://doi.org/10.1016/j.biotechadv.2008.11.001.

Sa´nchez, C. (2010). Cultivation of *Pleurotus ostreatus* and other edible mushroom. Appl. *Microbiol Biotechnol* 80:1321–1337. DOI: 10.1007/s00253-009-2343-7.

Sanjel, P., Shrestha, R.K., and Shrestha, J. (2021). Performance of oyster mushroom (*Pleurotus ostreatus*) grown on different finger millet husk substrates. *Journal of Agriculture and Natural Resources*, vol. 4, no. 1, pp. 291–300.

Shah, Z.A., Ashraf, M. and Ishtiaq, M. (2004). Comparative study on cultivation and yield performance of oyster mushrooms on different substrates. *Pakistan Journal of Nutrition 3 (3): 158-160*. University College of Agriculture

Rawalakot(UAJ&K), Azad Kashmir, Pakistan. http://guerillagreen.wagn.org/files2686.pdf.

Sonnenberg, A. J., Baars, J., Obodai, M. and Asagbra, A. (2015). Cultivation of oyster mushrooms on cassava waste. *Article in Food Chain*. DOI: 10.3362/20461887.2015.007.

Sopit, V. (2007). The feasibility of using coconut residue as a substrate for oyster mushroom cultivation. *Biotechnology.* 6. DOI:10.3923/biotech.2007.578.582.

Stanley, H. O. and Nyenke, C. U. (2011). Cultural studies on mycelia of Pleurotus pulmonarius (*Oyster Mushroom*) *In Selected Culture Media. International Journal of Science and Nature*. ISSN 2229 – 6441. I.J.S.N., VOL. 2(2) 2011: 183- 185.

Suada, I. K., Sudarma, I., Kim, B. S., Cha, J. Y., & Ohga, S. (2015). Fungal contaminant threatens oyster mushroom (*Pleurotus ostreatus*) cultivation in Bali. *Journal of the Faculty of Agriculture, Kyushu University,* 60(2), 309-313.

Subramanian, T.R. (1986). Nutritive value. *Mushroom Extension Bulletin. Indian Institute of Horticulture Research, India,* 8: 36.

Syed, A.A., Kadam, J. A., Mane, V. P., Patil, S. S., and Baig, M. M. V. (2009). Biological efficiency and nutritional contents of *Pleurotus florida* (mont.) singer cultivated on different agro- wastes, natural Science. 7 (1): 44-48. https://www.researchgate.net/publication/267238203.

Tsegaye Z, and Tefera, G. (2017). Cultivation of Oyster Mushroom (*Pleurotus ostreatus*) Kumm, (1871) Using agro- industrial residues. *J Appl Microb Res* Vol: 1, Issu: 1 (15- 20).

Youri, M. R. (2003). Formulation of media for the production of *Pleurotus ostreatus* using agro-processing waste.*Doctoral dissertation, M. Phil. Thesis, Department of Nutrition and Food Science, University of Ghana.*

Zadrazil, F. (1978). Cultivation of *Pleurotus*. In the biology and cultivation of edible mushroom (S.T.Chang and W.A. Hayes, Eds). *Academic Press, New York*, p512-558 https://doi.org/10.1016/C2013-0-10484-9.