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Comparative yield, yield related parameters and elemental composition of oyster mushroom (*Pleurotus ostreatus*) grown on different substrates

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ABSTRACT

This study examined the yield and nutritional composition of white oyster mushrooms (*Pleurotus ostreatus*) cultivated on wood shaving, rice straw, sugarcane bagasse and coconut coir as substrates. Rice straw and sugarcane bagasse produced the maximum pileus diameter, fresh and dry weight of the fruiting bodies. Sugarcane bagasse produced fruiting bodies with the highest percentage of nitrogen and phosphorus followed by wood shaving, rice straw and the coconut coir. The fruiting bodies grown on rice straw had maximum potassium content while coconut coir had the least. The study confirmed that the yield and nutritional value of mushrooms is affected by the chemical composition of the substrate. *P. ostreatus* produces ligninolytic enzymes that can degrade the organic compounds in the substrates into inorganic compounds that they use for metabolic functions and as energy.

Keywords: Oyster mushroom, wood shaving, rice straw, sugarcane bagasse, coconut coir

Mushrooms are fleshly fungi that produce fruiting bodies either above ground (epigeous) or below ground (hypogeous) (Kamthan, *et al.*, 2017). Mushrooms have two parts: the mycelium which exists underground, and the macroscopic fruiting structures composed of spores, stem, gill, hypha, volva, ring and cap. Mushrooms may be edible or poisonous (Chang, 1999). Edible mushrooms are defined as edible fruiting bodies with the absence of any poisonous effects on human body (Chang, 1999). These include several species of macro fungi, one of which is the oyster mushroom (*Pleurotus ostreatus*).

P. ostreatus belongs to the family Pleurotaceae (Paudel and Dhakal, 2020) and is cultivated all through the world including Japan, China, Asia, Europe and North America. Oyster mushrooms are recorded as the second most cultivated species in the world (Sanchez, 2010). The fruiting bodies of oyster mushrooms are soft and fleshy, and range in shape and colour from white to gray, brown or black (Mensah, 2015). Oyster mushrooms contain many nutrients such as proteins, carbohydrates, vitamins and minerals like phosphorus, iron, calcium, etc. (Utami and Susilawati, 2017).

Oyster mushrooms are easy to cultivate and have a short cropping cycle (Randive, 2012). They require carbon, nitrogen and inorganic compounds as their nutritional sources such as carbon sources like cellulose, hemicellulose and lignin (Sharma *et al.*, 2013). These nutrients are found in different agricultural waste materials, which make them ideal substrates for mushroom production (Chukwurah, *et al.*, 2013). Agricultural wastes are produced as a result of agricultural activity, forestry and industrial activities (Chang, 1999). Some agricultural wastes amply available in Guyana are wood shaving, sugarcane

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bagasse, coconut coir and rice straw. Wood shaving is a by-product from woodwork and timber industries. Dry wood is composed of cellulose, lignin, hemicelluloses, and minor amounts of extraneous materials (Phonphuak and Chindaprasirt, 2015). Sugarcane bagasse is an agro by-product obtained from sugar industry after the juices have been extracted and contains cellulose, hemicellulose and lignin (Carvalho, *et al.*, 2009; Melati, *et al.*, 2017) while coconut coir is middle fibrous material found between the outer coat and the inner shell (Harish *et al.*, 2009) containing lignocellulosic substances (Zafar, 2018; Mothé and de Miranda, 2009). Rice straw is another waste containing ash, insoluble ash, cellulose, hemicellulose and lignin (Jin & Chen, 2007; Utami and Susilawati, 2017). This study tried to explore whether these agricultural wastes (wood shaving, sugarcane bagasse, rice straw and coconut coir) are suitable for cultivating oyster mushroom by measuring the growth parameters and determining the nutrient content (N, P, K).

MATERIALS AND METHODS

The four substrates used in this study to cultivate *P. ostreatus* were wood shaving, sugarcane bagasse, rice straw and coconut coir. Mushroom cultivation experiments were conducted in the mushroom house at University of Guyana, Turkeyen Campus, Georgetown, Guyana. The analysis of nutrient content of substrate material and mushroom fruiting bodies were conducted at the Guyana Sugar Corporation (GUYSUCO), located in La Bonne Intention, Guyana. This study followed the method of Siddiqui (2002) and Seecharran *et al.* (2018).

Preparation of Mushroom mother culture

39.0 g of readymade PDA media was dissolved in 1L of purified water in a conical flask. The mixture was heated to dissolve and autoclaved for 15 minutes at 121^{o}C. It was then cooled to 50-55^{o}C. Ethanol (70%) was used to sterilize the work area. Amoxicillin (antibiotics) was added to the agar at a ratio of 100 mg to 1L PDA to prevent bacterial growth. The solution was poured into Petri dishes and left to solidify. Petri dishes were placed in the incubator to remove excess moisture. Petri dishes were labelled to indicate the species type, date prepared and type of media. A sterilized 0.8-1cm cork-borer was used to cut mycelial discs from previously prepared pure cultures of *P. ostreatus*. Each Petri dish was inoculated with one mycelial disc using a sterilized tweezer and inverted onto the agar. The edges of the Petri dishes were sealed and these were incubated at room temperature. The mycelia colonized the agar medium and appeared thick, woolly and off white in colour.

Preparation of Stock Spawn

500 g of white millet birdseed was soaked for 12 hours. The seeds were boiled in water for 10-15 minutes, strained and allowed to air dry for 12 hours. The seeds were then filled in glass bottles, sealed with cotton wool and gauze, and autoclaved at 121^{o}C for 2 hours and left to cool to room temperature. The work area was sterilized with 70% ethanol. After the bottles were cooled, the cotton plugs were carefully removed, and a sterilized spatula was used to inoculate the birdseed with 3-4 mycelial disks from the mother culture. The bottles were shaken to distribute the discs and were stored in a clean, dark area until mycelia were fully colonized in the bottles. Approximately 3 days after inoculation, the white mycelia were seen gradually covering the seeds. The mycelia had completely occupied the bottle after three weeks.

Mushroom cultivation

A total of four substrates, i.e. rice straw, sugarcane bagasse, coconut coir and wood shaving, were used for cultivation of mushroom. Each substrate was exposed to sunlight for about 2 days to dry and was cut into smaller pieces of approximately 2-3 inches, washed and soaked for 24 hours in water and

pasteurized by boiling in water for one hour at 65-70°C. It was allowed to cool to room temperature. Two gram of calcium carbonate was dissolved in 60ml water and mixed with 200g substrate. A small amount of substrate was removed and wrapped in aluminium foil for further analysis. The substrates were filled in polyethylene bags and autoclaved for 1 hour and then left to cool. The colonized bird seeds in the stock spawn bottles were loosened and added to the substrate. The bags were loosely closed and stored in a dark place to promote mycelial growth. The bags were opened after 14 days to expose the substrate to air and dim light for 12-hour periods. After mycelia had colonized the substrate, slits were made on the sides of the bags to allow the fruiting bodies to emerge. Bags were watered daily and observations recorded.

Harvesting

Fruiting bodies were harvested 4-5 days after they appeared by gently twisting the base of the stipe. The fruiting bodies were ready to be harvested when the pileus/cap of the mushrooms just turned upwards and were white in colour. After harvesting, the bags were watered daily, and the substrate was aerated to allow two more harvests (second and third flushes). Colour and texture of fruiting bodies, number of clusters, number of fruiting bodies, stipe length, stipe thickness, cap diameter and cap thickness were recorded. Fresh and dry weight of mushrooms was recorded. Some of the fruiting bodies were prepared for elemental analysis by drying in the oven at 45-50 ^{o}C until a constant weight was achieved. The dried fruiting bodies were crushed using a mortar and pestle and were stored in polyethylene (Ziploc) bags. It was sent for further analysis. At least 5 g of dried fruiting body was required per sample. Dried substrates were also prepared for elemental analysis, specifically nitrogen (N), phosphorus (P) and potassium (K). The soil substrates and fruiting bodies of the mushrooms were analysed using dry ashing and Atomic Absorption methods at GUYSUCO Central Laboratory.

RESULTS AND DISCUSSION

P. ostreatus is a species of edible mushrooms, which has high nutritional content and health benefits to humans. This research examined the yield and N, P, K content of *P. ostreatus* cultivated on four different substrates i.e. rice straws, sugarcane bagasse, coconut coir and wood shaving. Agricultural wastes make the most ideal form of materials for mushroom production; however, composition of different substrates affects the yield performance (Chukwurah, 2013). The fruiting bodies of oyster mushroom differ with respect to stipe length and pileus diameter when grown on different agricultural substrates (Shah *et al.*, 2004). In this study, *P. ostreatus* fruiting bodies produced in all four substrates were cream to off white in colour and rubbery in texture. On an average, rice straw substrate produced the maximum bunches and fruiting bodies with larger cap diameter with better stipe length. This was followed by sugarcane bagasse and coconut coir substrates. The wood shaving substrate produced the smallest number of bunches, fruiting bodies and smaller stipe length but the fruiting bodies had greater cap diameter and thickness than those from the coconut coir substrate. The rice straw substrate produced fruiting bodies with the highest fresh and dry weight, followed by sugarcane bagasse, wood shaving and coconut coir (Table 1). Also, the fruiting bodies produced on different substrates showed a similar trend with respect to number of clusters, number of fruiting bodies, diameter of pileus (cm), length of stipe (cm), thickness of stipe (cm), fresh weight (g) and dry weight (g). Zhang *et al.* (2002) found that rice straw yielded about 10% more mushrooms than wheat straw under the same cultivation conditions. Similarly, Radwan (2005) reported that the highest cap diameter of mushroom fruiting bodies produced by rice straw substrate, compared to other substrates such as sugarcane bagasse and bermuda grass. In contrast Neupane *et al.* (2018) found that saw dust produced the maximum fruiting bodies of *Pleurotus* sp compared to other

Table 1. Parameters of Oyster mushroom grown on rice straw, sugarcane bagasse, coconut coir and wood shaving

Parameters (Mean ± SD)	Rice straw	Sugarcane bagasse	Coconut coir	Wood shaving
No. of clusters	7.00±0.47	6.00±0.82	3.67±0.94	3.00±0.00
No. of fruiting bodies/bag	32.00±7.79	15.67±2.87	9.33±1.70	9.00±2.16
Diameter of pileus (cm)	14.12±1.01	7.84±1.95	4.23±0.48	4.65±1.46
Length of stipe (cm)	12.45±3.00	5.02±1.15	3.92±1.75	2.85±1.14
Thickness of stipe (cm)	1.80±0.17	0.70±0.13	0.43±0.08	0.51±0.24
Fresh weight (g)	115.5±5.38	55.05±16.47	26.88±2.92	33.11±21.73
Dry weight (g)	36.90±4.38	15.64±2.81	10.25±1.82	10.39±6.49

agricultural waste products. Shah *et al.* (2004) also found that saw dust had the highest yield and number of fruiting bodies when compared to other substrates. The variation in reported results can be attributed to the fact that different agricultural waste contains different types and amount of nutrient needed for proper growth and development of mushrooms (Chukwurah, 2013).

The fruit bodies grown on different substrate had different water content. Sugarcane bagasse had the highest water content (72%), followed by wood shaving (69%), rice straw (68%) and coconut coir (62%) (Table1). This result is similar to the findings of Hoa and Wang (2015) who found that sugarcane had the highest moisture content. They had attributed this to the water holding capacity of substrate and its effect on nutritional composition of fruiting body.

The N, P, K content in the oyster mushrooms produced by each substrate was analyzed. Mushrooms produced by sugarcane bagasse contained the highest percentage of N and P, while those from the rice straw substrate had the highest K content and second highest N content but the lowest P content. Fruiting bodies from the wood shaving substrate had slightly higher N and K content than those from the coconut coir substrate, which had slightly higher P content (Table 2). Hoa and Wang (2015) found that sugarcane bagasse had the highest N and P content while Arisha (2006) found that rice straw substrate produced fruiting bodies with high K content.

It is reported by earlier workers that the substrate used had influences on chemical composition and nutritional value of the mushrooms (Hoa and Wang 2015). The N, P, K contents of the initial and final substrates was also analyzed during the study. Coconut coir substrate had the highest N, P and K content in the initial and final samples while rice straw substrate had the second highest N and K content. Initially, coconut coir and sugarcane bagasse substrates were highest in P content, which decreased in the final samples. Phosphorus content remained same in the initial and final samples from the rice straw and wood shaving substrates (Table 3). Sugarcane bagasse was the only substrate with higher K content in the final sample. Radwan (2005) found

Table 2. N, P & K analysis of fruiting bodies of oyster mushrooms grown on different substrates

Content analysis	Rice straw	Sugarcane bagasse	Coconut coir	Wood shaving
N (%)	3.42±0.21	4.36±0.37	3.01±0.31	3.46±0.43
P (%)	0.90±0.11	1.26±0.15	0.95±0.07	0.94±0.09
K (%)	2.79±0.23	2.75±0.23	2.35±0.12	2.40±0.11

Table 3. N, P & K analysis of initial and final substrates

Content analysis		Rice straw	Sugarcane bagasse	Coconut coir	Wood shaving
N (%)	Initial	0.38	0.42	0.69	0.30
	Final	0.56	0.36	0.66	0.37
P (%)	Initial	0.05	0.10	0.27	0.07
	Final	0.05	0.05	0.10	0.07
K (%)	Initial	0.36	0.17	0.80	0.27
	Final	0.31	0.29	0.55	0.21

that substrates had higher N, P and K content after fruiting bodies were harvested. Mushrooms secrete extracellular enzymes on to their substrate to degrade complex materials into smaller chemical substances that can be used for metabolic functions and for energy (Manzi *et al.*, 1999; Cotter, 2014; Mondal *et al.*, 2010; Bustillos *et al.*, 2016).

Pleurotus sp can produce a broad spectrum of enzymes to meet their metabolic needs and increase the nutrient availability for other organisms (Cotter, 2014). The enzymes like laccases, versatile peroxidases, manganese dependent peroxidases, and aryl alcohol oxidases can oxidize the lignin polymers releasing aromatic radicals (Cotter, 2014; Bustillos *et al.*, 2016). Other mushroom species are not able to colonize on variety of substrates because they produce a narrow spectrum of extracellular enzymes, which gives an advantage to *Pleurotus* spp. to grow on a variety of substrates. It is also reported that using variable substrate, mushrooms prevent weakening of the strain or senescence, which occur when the fungi overuse and overproduce a particular combination of enzymes (Manzi *et al.*, 1999; Cotter, 2014).

CONCLUSION

Mushroom cultivated on the different substrates (wood shaving, sugarcane bagasse, coconut coir and rice straw) vary in yield and nutrient contents. Rice straw was the most suitable substrate for oyster mushroom cultivation as it produced fruiting bodies with the highest cap diameter, stipe length and thickness as well as the highest fresh and dry weight. This was followed by the sugarcane bagasse, wood shaving and coconut coir substrates. Sugarcane bagasse produced fruiting bodies with the highest N, P and K content, followed by wood shaving, rice straw and coconut coir. The yield and nutritional value of mushrooms is influenced by the chemical composition of the substrate.

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