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Cultivation of Oyster mushroom (*Pleurotus ostreatus)* and Button mushroom (*Agaricus bisporus)* on autoclaved coffee grounds and rice straw

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ABSTRACT

This research investigated used coffee grounds, rice straw, and the combination of used coffee grounds and rice straw as substrates to grow *Pleurotus ostreatus* (oyster mushroom) and *Agaricus bisporus* (button mushroom). The procedure consists of four main phases: preparation of mushroom mother culture with potato dextrose agar, preparation of stock spawn, preparation of substrates and cultivation. The elemental analysis (nitrogen, phosphorous, and potassium) of fruiting bodies, initial and final substrate was done by the Guyana Sugar Corporation (GUYSUCO). *P. ostreatus* and *A. bisporus* could be successfully cultivated on the autoclaved rice straw as substrate but not on the used coffee ground and the combination of used coffee grounds and rice straw as substrate.

Keywords: Oyster mushroom, Button mushroom, substrate, coffee ground, rice straw, chemical analysis

Mushrooms have contributed significantly and sustainably as a food source, medicine, and a form of livelihood of the modern world (Boa, 2004). Mushrooms have a fundamental niche of degrading plant and animal waste materials to restore soil fertility (Paulraj and Francois, 1995). Mushroom's mycelium feeds on lignocellulosic materials and decomposed organisms in order to produce its fruiting bodies (Miles and Chang, 2004). Lignocellulosic materials contain cellulose and lignin (Rowell, 1991). The substance to grow mushroom mycelium can be derived from agricultural and domestic waste products such as rice straw, wood chips, sawdust and compost (Oei and Nieuwenhuijzen, 2005). Carbon, nitrogen, sulphur, phosphorus, potassium, calcium, manganese, and trace elements are necessary for mushroom growth and can be found in organic waste products along with hemicellulose, cellulose, and lignin (Brenneman and Guttman, 1994).

Pleurotus ostreatus or oyster mushroom is a saprophytic and lignocellulolytic mushroom. It often has a soft, fleshy fruiting body with caps that range from shell-shaped to semi-circular (Mensah, 2015). It produces ligninolytic laccase, manganese-dependent peroxidase, versatile peroxidase, and aryl-alcohol peroxidase in order to use its substrate (Seecharran *et al.*, 2018). *Agaricus bisporus* (button mushroom) is fleshy and firm in texture with a round cap that expands in a broad convex to an almost flattened shape (Kuo, 2018). It requires high nitrogen and grows in well composted material derived from mixtures of straw and manure (Chang, 2009). Button mushrooms contain enzymes that degrade polysaccharides (xylan, cellulose, pectin, and protein), including carbon-hydrate acting enzymes (CAZymes), lignin-related oxidoreductases and proteases (Morin *et al.*, 2012) at different stages of its growth. Mushrooms can grow on variety of organic lignocellulosic materials,

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including straw, sawdust, cotton waste and used coffee beans (Chang, 2009). Coffee is an unconventional substrate for mushroom growth, and only recently there have been attempts for utilizing spent coffee beans as the substrates (Gluck Thaler, 2012). Coffee beans are lignocellulosic with a composition of hemicellulose, lignin and cellulose (Mano *et al.,* 2017). Used ground coffee is a waste material that can be used to sustainably cultivate mushrooms in Guyana. This research explored used coffee grounds and rice straw as substrates to grow the edible mushroom species *A. bisporus (*button mushroom) and *P. ostreatus* (oyster mushroom). The primary objectives were to compare the substrate suitability and mineral composition of the mushroom species.

MATERIALS AND METHODS

Rice straw, coffee beans and a combination of straw and coffee beans were used as primary substrates to cultivate *A. bisporus* and *P. ostreatus*. A method described by Seecharran *et al.* (2018) was used for the cultivation of mushroom, which included mother culture preparation on potato dextrose agar (PDA), spawn preparation, substrate preparation, and cultivation using the three substrates. The preparation of mother culture and stock spawn was done in the Biology laboratory, while the mushroom cultivation and harvesting experiments were done in the Mushroom House at the University of Guyana, Turkeyen Campus. Analysis of the elemental composition of substrate material and mushroom fruit bodies was conducted at The Guyana Sugar Corporation (GUYSUCO), located in La Bonne Intention (LBI), Guyana.

Preparation of mushroom mother culture (Potato Dextrose Agar)

Readymade 39.0 g PDA was mixed in one litre of water, autoclaved at 121^{o}C for 15 minutes and poured into Petri plates. Mycelial discs (5 mm) of pure cultures of button and oyster mushrooms made using a sterilized cork borer were transferred to Petri dishes. The edges of the Petri dishes were sealed and were incubated at room temperature.

Preparation of stock spawn

White millet birdseed (500g) was soaked for 12 hours, 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 & gauze, autoclaved at 121^{o}C for 2 hours and left to cool to room temperature. The glass bottles with autoclave birdseed were inoculated with 3-4 mycelia disks from the mother culture under sterile conditions. The bottles were incubated in a clean and dark area until mycelia fully colonized in the bottles.

Preparation of substrate

The sun-dried straw 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. It was allowed to cool down to room temperature. The coffee grounds were boiled in water, strained with a sieve and allowed to cool at room temperature. The following combinations were used for the cultivation of mushrooms.

Table 1: Substrates and their combination used

	Substrate 1 (kg)	Substrate 2 (kg)	Substrate 3 (kg)
Rice straw	100		50
Coffee grounds		100	50
Vermicompost	50	50	50
Lime	01	01	01

Samples of the substrate mixtures were taken and wrapped in aluminium foil for further analysis. The remaining substrate mixtures (1.5 kg dry substrate) were placed in autoclavable polypropylene bags and were autoclaved for 1 hour. The substrates were allowed to cool and colonized bird seeds were added to these bags. After mycelia colonization, slits were cut on the sides of the bags with a sharp and sterile razor blade. The bags were watered daily, and emerging fruiting bodies were observed.

Harvesting

Mature fruiting bodies were harvested by gently twisting. After the first flush, bags were kept watered daily, and the substrate was aerated to allow more harvests (second and third flushes). Measurements were taken for the different parameters. Some of the fruiting bodies were used for elemental analysis after 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 (zip lock) bags. At least 5g of dried fruit body was required per sample for elemental analysis. The dried substrate was also prepared for elemental analysis. The substrates and fruiting bodies of the mushrooms were analysed using Dry Ashing and Atomic Absorption methods at GUYSUCO Central Laboratory.

RESULTS AND DISCUSSION

Fruiting bodies of *A. bisporus* and *P. ostreatus* were only produced in the rice straw substrate. The colour of the oyster mushrooms harvested for the straw substrates was cream with golden brow-n edges. Oyster mushrooms harvested were rubbery in texture with some containing a soft/hard stipe. An average of nine oyster mushrooms was harvested (Table 2). The average stipe length of oyster mushrooms was 2.69 cm, and the average stipe thickness was 0.19 cm. The diameter of the oyster mushroom cap had an average of 1.99 cm. The average thickness of the oyster mushroom cap harvested was 0.12 cm. The fresh weight was 21.26 g. The dry weight was 2.24 g.

The fruiting bodies of the button mushrooms were better in quality based on the stipe and cap parameters. The color of the button mushroom varied, from cream to off-white. Some were golden brown around the edges, under the cap, and along the stipe due to drying. The texture of the button mushrooms was mostly soft with rubbery edges. However, some were rubbery and hard, which was also due to drying. The mean numbers of fruiting bodies were 7.86 for each trial of the straw substrate (Table 3). The cap and stipe were generally larger than the oyster mushroom grown on the same straw substrate. There was a significant decrease in the weight of the mushrooms after being dried. The average fresh weight was calculated to be 12.35g. The average dry weight of button mushrooms was 1.79g.

Table 2: Parameters of Oyster mushroom growth in the straw substrate for the first harvest

Parameters		Straw
Color		Cream with golden brown edges
Texture		Rubbery cap, soft stipe/Hard
Number of fruiting bodies/bag (1.5 kg dry straw) (mean ± standard deviation)		9 ± 2.45
		Mean Size (cm) ± Standard Deviation
Stipe	Length	2.69 ±1.10
	Thickness	0.19±0.12
Cap	Diameter	1.99±0.99
	Thickness	0.12±0.06
Average fresh weight (g) (mean ± standard deviation)		21.26±0.75
Average dry weight (g) (mean ± standard deviation)		2.24±0.44

Table 3: Parameters taken for Button Mushroom in the straw substrate for two harvests

Parameters		**Straw substrate**
Color		Cream with golden brown edges/off-white/white, brown under the cap, and along stipe/cream-brown
Texture		Soft, rubbery edges/smooth/rubbery and hard
Number of fruiting bodies/bag (1.5kg dry straw) (mean ± standard deviation)		7.86 ± 4.28
		Average size (cm) (mean ± standard deviation)
Stipe	Length	4.13±1.41
	Thickness	0.31±0.17
Cap	Diameter	2.08±1.34
	Thickness	0.28±0.54
Fresh weight (g) (mean ± standard deviation)		12.35±3.82
Dry weight (g) (mean ± standard deviation)		1.79±1.15

A. bisporus contained larger mushrooms and the longest average stipe length of 4.13 cm. In contrast, oyster mushrooms contained an average stipe length of 2.69 cm. Button mushroom's average stipe thickness was 0.31 cm, and in oyster mushroom, it was 0.19 cm.

Oyster mushrooms cultivated on the rice straw substrate contained more nitrogen (4.13%) and potassium (2.61%) than button mushrooms. There was 2.88% nitrogen and 1.91% potassium in the button mushrooms harvested. Button mushrooms contained more phosphorous (0.66%) than oyster mushrooms (0.55%) (Table 4). There was an increase in nitrogen, phosphorous, and potassium in a majority of the substrates (Table 5). There was also an increase in nitrogen in all substrates, except for the rice straw substrate used to cultivate oyster mushrooms, which had a -0.65% decrease of nitrogen in the rice straw substrate. Similarly, most of the substrates increased in phosphorous (P) content. However, the coffee substrate used to cultivate button mushrooms had a decrease of -10.71% from the initial phosphorous content of the substrate. Similarly, the coffee substrate for button mushroom was the only substrate that had a decrease in potassium content. The other substrates increased in phosphorous content. The coffee substrate for button mushroom decreased by -2.17% than the initial substrate. Oyster mushrooms increased the most in phosphorous and potassium content with 33.33% and 25.98% change. At the same time, button mushrooms contained a 13.81% increase in nitrogen composition, a 6.67% increase in phosphorous composition, and a 9.52% change in potassium composition. More nitrogen was found in the rice straw substrate for button mushrooms than in oyster mushrooms, where there was a decrease in nitrogen content. There was no significant difference elemental composition of the different substrates (ANOVA p-value 0.53).

Table 4: Element composition, N, P, K of oyster and button mushroom grown in straw substrate

Treatments	**Parameter (mean±standard deviation)**		
	N (%)	**P (%)**	**K (%)**
Oyster mushroom	4.13±0.11	0.55±0	2.61±0.13
Button mushroom	2.88±0.47	0.66±0.09	1.91±0.21

Cultivation of *P. ostreatus* and *A. bisporus* was not successful in used coffee grounds and the combination of used coffee grounds and straw

Table 5: Elemental composition; Nitrogen (N), phosphorous (P), and potassium (K) of initial and final used coffee grounds, straw, and the combination of used coffee grounds and straw substrate and change in composition for oyster and button mushroom

Treatment	Elements								
	N (%)			P (%)			K (%)		
	Initial	Final	% change	Initial	Final	% change	Initial	Final	% change
Oyster mushroom									
Rice straw	0.78	0.77	-0.65	0.11	0.22	33.33	0.71	0.98	15.98
Ground coffee	1.56	2.06	13.81	0.28	0.32	6.67	0.95	1.15	9.52
Ground coffee + rice straw	1.51	1.66	4.73	0.2	0.24	9.09	1.11	1.35	9.76
Button mushroom									
Rice straw	0.75	0.79	2.66	0.12	0.16	14.89	0.71	0.963	15.12
Ground coffee	1.58	2.26	17.71	0.31	0.25	-10.71	1.41	1.35	-2.17
Ground coffee + rice straw	1.69	2.31	15.5	0.2	0.31	21.57	1.12	1.37	10.04

(Negative) value means a decrease; + (Positive) value means an increase

substrate. However, *P. ostreatus* and *A. bisporus* were successfully grown in the rice straw substrate. Mycelial growth was present initially when introduced into the substrates containing used coffee grounds. However, after some time, the mycelial growth declined as the consistency and appearance of the substrates changed. The used coffee ground substrate changed from black to dark brown. The porosity of the used coffee grounds also decreased, resulting in a compact, almost solid structure.

Chang & Miles (1984) found that mushrooms, including *P. ostreatus* and *A. bisporus* utilize agricultural wastes and industrial discards as growth substrates that are often inedible for human consumption. Cereal straw, including rice straw, was even used as the sole substrate for various mushrooms. Straw is enriched with carbon, found in cellulose, hemicelluloses, lignin, and some soluble carbohydrates. Seecharran *et al.* (2018) found that rice straw, supplemented with agriculture wastes were suitable for growing oyster mushrooms. Mamiro & Mamiro (2011) highlighted that oyster mushroom contains the ability to degrade lignocellulosic and convert it into biomass enriched with protein. *P. ostreatus* secretes enzymes that degrade rice straw and other crop residues by degrading the cell wall components, cellulose, and lignin of rice straw and other crop residues (Mamiro & Mamiro, 2011; Adedokum, 2014).

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