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Growth of oyster mushroom using sawdust and agriculture waste as substrates

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ABSTRACT

Mushroom culture is an important micro-industrial source of protein in developing countries such as Guyana. This study investigated the growth and mineral composition of *Pleurotus ostreatus* (oyster mushroom) using rice straw, grass clippings [Bermuda grass (*Cynodon dactylon*)] and sawdust as substrate materials. Growth was only successful in the treatment that contained rice straw as substrate, whereas no growth was obtained from substrates that contain grass clippings and sawdust. N, P, K, Ca, Fe, Cu, Mn and Mg were present in the oyster mushroom samples and the spent mushroom substrate.

Keywords: Oyster mushroom, cultivation, sawdust, vermicompost

Mushroom cultivation started in the ancient times, and were likely used by the Egyptians over 4500 years ago, and it was preserved as food for the royals and as such, no commoner was supposed to eat mushroom (Abdel-Azeem 2010). Mushrooms, a highly priced delicacy for more than two thousand years, are now consumed by many people (Daba *et al*., 2008; Josephine 2014). The Chinese were the first to grow mushrooms for human consumption (Daba *et al*., 2008). Mass production of oyster mushrooms started in the late 1960's using a straw-based substrates (Tisdale 2004).

The *Pleurotus* spp. are widely cultivated throughout the world due to their low-cost production technology, efficiency in degrading a range of agriculture wastes, and its ability to grow in a wide range of temperatures (Mane *et al*., 2007). There are over 40 species of *Pleurotus* and new species are still being identified (Ahmed *et al*., 2016). The lignocellulolytic *Pleurotus ostreatus* (oyster mushroom) is the second most important cultivated mushroom for food purposes (Deepalakshmi *et al*., 2014; Adebayo and Martinez-Carrera 2015). *Lentinula* and *Pleurotus* are the most cultivated species contributing about 26% and 21% of the world's mushroom production, respectively (Singh *et al*., 2020). Mushrooms, as a result of their saprophytic and lignocellulosic ability, are well adapted to grow on lignocellulosic substrates (Ahmed *et al*., 2013; Adebayo and Martinez-Carrera 2015). Composted wheat and paddy straw, banana leaves, sugarcane bagasse and leaves, wheat bran, rice husk, cotton waste, wheat straw and sawdust can be used as substrates for growing mushroom (Ashraf *et al*., 2013; Josephine 2014). Mushroom production indirectly provides materials that are used to improve soil nutrient and structure for production of other crops (Brenneman, and Guttman 1994).

Pleurotus excrete hydrolyzing and oxidizing enzymes, such as peroxidases, laccases, cellulases,

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hemicellulases and xylanases, through the tips of their hyphae (Pathmashini *et al*., 2010). These enzymes can break down complex organic compounds in agriculture waste and industrial by-products (Ahmed *et al*., 2013; Josephine 2014). They degrade cellulose, hemicellulose and lignin to produce fruiting bodies that contain essential amino acids, carbohydrates, vitamins and minerals that are suitable for human consumption (Mane *et al*., 2007).

Hoa *et al*., (2015) compared the effects of different agriculture wastes on the growth, yield and nutritional composition of *P. ostreatus* and *P. cystidiosus*. There were seven substrate formulas that included corn-cob, sawdust and sugarcane bagasse. Substrates with 100% corncob and 100% sugarcane bagasse were the most suitable substrates for oyster mushroom cultivation, producing the best growth parameters, mineral content and biological efficiency (Hoa *et al*., 2015). Ashraf *et al*., (2013) cultivated *P. sajor-caju, P. ostreatus*, and *P. djmor* on cotton waste, wheat straw and paddy straw. *P. ostreatus* and *P. sajor-caju* produced the highest protein and fiber contents, and cotton waste was found to be the most favorable substrate for mushroom cultivation. Josephine (2014) studied the growth of King Oyster mushroom (*Pleurotus eryngii*) on sawdust and rice straw in Bangladesh and found that sawdust showed the highest biological efficiency (73.5%). Although the sawdust substrate produced better yield and efficiency, rice straw produced the mushrooms with larger fruiting bodies. Seecharran *et al*., (2018) found that rice straw was a suitable substrate for *P. ostreatus.*

Sawdust substrate, due to its lignocellulosic content, is also very efficient in mushroom cultivation provided that it is composted and can be supplemented with nitrogen sources, such as wheat bran or vermicompost (Josephine 2014; Viriato *et al*., 2021). Mushroom cultivation is common in Guyana but there is limited knowledge about its nutritional benefits. This study investigated whether sawdust, grass clippings and rice straw are suitable substrates for the growth of *P. ostreatus*, and determined the nutritional and mineral content of the mushroom produced.

MATERIALS AND METHODS

This study focused on the growth of oyster mushroom (*Pleurotus ostreatus*) on three substrates: rice straw, grass clippings, and sawdust. This research was conducted from January to September, 2017, at the mushroom house, University of Guyana Turkeyen Campus, Guyana. Four trials were conducted with twenty-one bags for each trial; 7 bags per substrate. Analysis of the mineral composition of the (substrate material and oyster mushroom samples were carried out at the Central laboratory of the Guyana Sugar Corporation (GUYSUCO).

This study adopted the method by Jaikaran *et al*., (2017) and Seecharran *et al*., (2018), which included five phases. Phase one was preparation of the mother culture. The media was prepared by mixing 39.0g of Potato Dextrose Agar (PDA) with purified water, heating the mixture until the PDA dissolved, and autoclaving the mixture at 121°C for 15 minutes. After the mixture was cooled to about 50-55°C, amoxicillin (antibiotic) was added at a ratio of 5g amoxicillin to 1litre PDA to prevent bacterial growth. The mixture was poured into Petri dishes, covered and allowed to solidify. Mycelial discs from pure *P. ostreatus* cultures were transferred to the prepared Petri dishes, which were then sealed and incubated at room temperature (28 - 32°C). After two weeks, mycelial growth was observed as a thick white wool network covering the entire medium.

Phase two involved the preparation of the stock spawn in sterilized glass bottles. The substrate for the stock spawn was prepared by soaking 500g of white millet (bird seed) for 12 hours. After soaking, the seeds were boiled in water for 10-15 minutes, allowed to air dry for 12 hours and added to glass bottles. The bottles were sealed with gauze and autoclaved at 121°C for 2 hours. After the bottles were cooled, they were inoculated with 3-4 *Pleurotus ostreatus* mycelia

discs from the mother culture. The inoculated bottles were stored in a clean, dark area at 28 - 32 °C until the mycelia fully colonized the bottles observed as a thick mass of white feather-like mycelia, which took approximately 2 weeks.

Phase three involved preparation of substrates and inoculation with mushroom mycelia. Rice straw and grass clipping were sun dried for 2 days, cut into pieces of approximately 2-3 inches, washed and soaked in water for 24 hours. These substrates were pasteurized by boiling in water for 1 hour and allowed to cool. The following formula was used to make the substrate materials: 200g substrate material → 100g vermicompost → 2g lime dissolved in 60 ml water. Small amount of the substrate mixtures was removed for further analysis. The remaining substrate mixtures was placed in autoclavable polyethylene bags and labelled accordingly. The bags were autoclaved for 1 hour and then cooled. The sawdust substrate was combined with vermicompost and left to compost in heaps for 30-50 days. It was turned and moistened with water every four (4) days. The composted substrate was transferred to autoclavable bags and sealed. The autoclavable bags were placed in the laminar airflow cabinet under ultra violet (UV) for 20 minutes. After the bags cooled, approximately one - third of the mycelia colonized bird seeds from the stock spawn bottle were added to each bag. The bags were loosely sealed to allow air flow but prevent insects from entering, and stored in the sterile, dark mushroom room and left for full mycelium colonization. The substrate bags were watered twice daily by gently misting distilled water on the surface. After about 14 days, small slits were made on the outside of the bags to allow for air exchange and fruiting bodies to develop. Sawdust treatments were incubated for 4 -6 weeks. The treatments were observed daily for the appearance of fruiting bodies.

Harvesting was done in Phase four. Once the fruiting bodies matured, they were harvested by gently twisting the base of the stipe. The substrate was continued to be watered and aerated to allow for one-two more harvests (second and third flushes). Parameters measured included color and texture of pins, number of pins, number of fruit bodies, size of fruit bodies, stipe length, stipe thickness, cap diameter, cap thickness and fresh weight of fruiting bodies. Fruiting bodies were dried at 60-70°C until constant weight was achieved; dried weight was recorded. Dried fruiting bodies were crushed and prepared for elemental analysis. The initial and final substrate were also prepared (dried) for elemental analysis.

Phase five involved elemental analysis. Fruiting bodies of *Pleurotus ostreatus* and spent substrates were analyzed for N, P, K, Ca, Mg, Cu, Fe and Mn. Dry Ashing and Atomic Absorption Spectrometry was conducted at the GUYSUCO Central Laboratory. The method for Atomic Absorption Spectrometry was referenced from Isaac and Kerber (1971), Wagner (1994); Dima *et al*., (2006); and Petisleam *et al*., (2007). In the present study saw dust composted with vermi-compost for 30-50 days did not proved good. Autoclaved substrate was used in the study.

RESULTS AND DISCUSSION

Mushrooms are used as both food and medicine (Josephine, 2014). Edible mushrooms have fleshy fruiting bodies and are consumed globally as they provide a variety of tastes, flavor and texture, and contain valuable minerals such as iron, potassium, phosphorus, calcium and copper, as well as carbohydrate, protein and fat (Alananbeh *et al*., 2014; Deepalakshmi *et al*., 2014; Josephine 2014). *P. ostreatus* is easy to grow, requires few environmental controls, has the ability to grow on a wide variety of substrates, produce high yields, and their fruiting bodies are not as susceptible to pests (Tisdale 2004; Josephine 2014). In this study, oyster mushroom growth was successful in two treatments (T1-rice straw with vermicompost and T-2 rice straw alone). All treatments with the grass clippings (T3) and sawdust substrates (T4) experienced contamination and did not produce any fruiting bodies. There was significant difference between growth parameters

from the two successful trials (ANOVA single factor p-value of 0.0065), which may be due to the two harvests from trial 1 and one from trial 2. No fruiting bodies were produced in trails 3 and 4. Trial 1 produced the highest yield with larger fruiting bodies, and greater cap diameter and stipe thickness, but trial 2 had the longest stipe length (Fig. 1). Higher yields from trial 1 first harvest can be attributed to the higher nutrients composition of the substrate. Although the

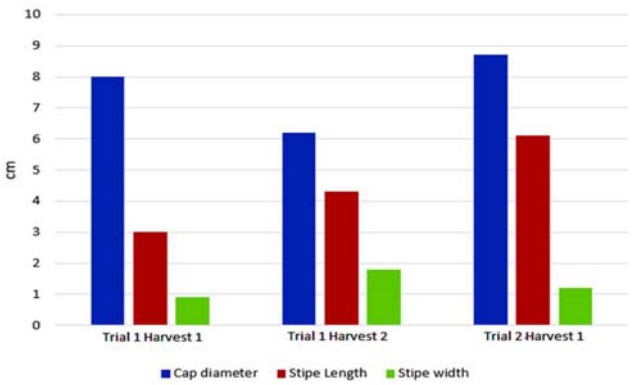

Fig. 1. Growth parameters of mushrooms

substrates for each trial was prepared following the same procedure, the initial substrates for trial 1 had higher concentration of N, P, K, Ca, Fe, Cu, Mn and Mg (Table 1 and 2), which translated into higher yields for trial 1. The rate of production of oyster mushroom fruiting bodies from the mycelia stage is influenced by the carbon and nitrogen sources, the mineral content of the substrate, as well as the pH of substrate, light availability, incubation period and the control of contamination (Ibekwe *et al*., 2008). Bhattacharjya *et al*., (2015) found that different sawdust substrates, including Fig tree, Mahogany tree, Rain Tree, Ipil ipil tree, Eucalyptus tree and a mixture of these substrates, can be used for *P. ostreatus* production. Seecharran *et al*., (2018) experimented with several substrates and founds that rice straw supplemented with vermicompost and agriculture wastes (cattle dung and duck manure) were suitable substrate for cultivation of *P. ostreatus.* Viriato *et al*., (2021) found that eucalyptus bark had potential as a substrate for production of *P. ostreatus,* producing higher yield, fruiting bodies and biological efficiency than the traditional eucalyptus sawdust material. In the present study, the approach of composting the sawdust for 50 days as described above was not a suitable approach for cultivation of *P.ostreatus*. Pasteurized rice straw has been successfully used for cultivation of *P.ostreatus* (Singh and Upadhyay, 2015) instead of autoclaving it as done in the present study.

Table 1. Concentrations of N, P, K levels in the initial and final substrates

Treatment	**Parameter (%)**		
Rice straw +Vermicompost +$CaCO_3$ (T1)	**N**	**P**	**K**
Trial 1 (Initial substrate)	1.52	0.85	0.79
Trial 1 (Final substrate)	1.87	0.33	0.71
Trial 2 (Initial substrate)	0.41	0.16	0.45
Trial 2 (Final substrate)	1.15	0.27	0.41

Mushroom samples from both trials were analyzed for N, P and K, which are macronutrients that are important for the growth and development. The higher macronutrients levels in trail 1 (Fig. 2) can be due to the mineral composition of the substrate. Nitrogen was

Table 2. Concentrations of Ca, Fe, Cu, Mn and Mg levels in the initial and final substrates

Treatment	**Parameters (mg/kg)**				
Rice straw +Vermicompost +$CaCO_3$ (T1)	**Ca**	**Fe**	**Cu**	**Mn**	**Mg**
Trial 1 (Initial substrate)	5.65	1139	5.21	2.05	400
Trial 1 (Final substrate)	10.1	1774	9.09	3.96	762
Trial 2 (Initial substrate)	3.96	338	2.13	1.84	526
Trial 2 (Final substrate)	9.59	704	6.21	5.10	821

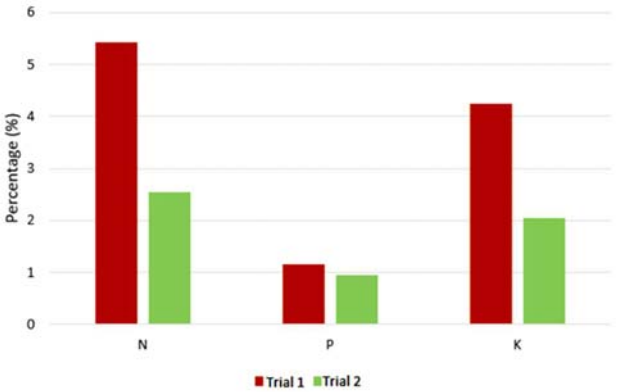

Fig. 2. Concentration of N, P and K in oyster mushroom samples

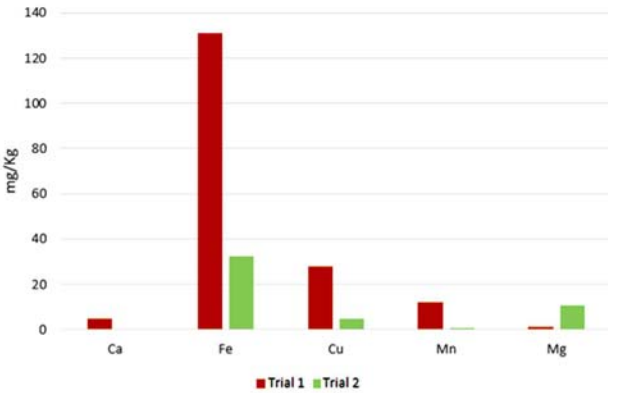

Fig. 3. Concentration of Ca, Fe, Cu, Mn and Mg in oyster mushroom samples

highest (5.42 %) in fruit bodies of trial 1 in first harvest. Higher concentrations of Ca, Fe, Cu, and Mn were also found in trial 1, while Mg was higher, 10.6 mg/kg, in trial 2 (Fig. 3). The variation can be attributed to the mineral composition of the substrate. Kalaè (2010) and Ouzouni *et al*., (2009) reported similar results of Mn content varying between 10.0 and 60.0 mg/kg and 7.2 – 55.6 mg/kg in mushroom species.

Nutrients are essential for saprobiotic colonization of cultivated mushrooms. (Campos 2009). Nitrogen is an important nutrient for mushrooms, which is needed for protein, nucleic acid, purine, pyrimidine and polysaccharide synthesis, and supplementing with nitrogen can increase mushroom productivity and biomass (Nunes *et al*., 2012). P, K and Mg are the necessary for fungal growth (Campos 2009). Minerals, such as Na, Mg and Ca, stimulate mycelium growth, and promote fruiting body formation (Kurtzman and Zadrazil 1982). Copper induces laccase transcription, accounting for its increase in the post substrate material. Laccase is a copper-containing phenol oxidase, degrades lignin, and oxidizes phenol/phenolic lignin like substructures (Ahlawat 2007). Kurtman and Zadrazil (1984) reported that the presence of Mg stimulates mycelial growth, and fruiting body formation. Fe, Mn, Cu, are among the most essential micro- nutrients (trace elements) for the growth of many species of fungi (Campos 2009).

There was a decrease in the phosphorous and potassium levels of the substrate after the mushrooms were harvested, whereas nitrogen level increased after harvesting. Generally, there was an increase in the nutrient and mineral content of the post-harvest substrates compared to the mineral content of the input substrates (Table 1 and 2), which can be attributed to the biodegradation and utilization of the substrate by the enzymes produced by the mushroom mycelia as a result of which there is decrease in weight of the substrate but only little change in the total mineral contents. The initial chemical profile of the substrate is important to the growth and nutritional content of *Pleurotus.* Lignocellulosic materials are usually low in protein content, and may not be sufficient for the cultivation of mushrooms, which requires nitrogen, phosphate and potassium. Since the nitrogen plays an important role in the growth of fruiting body, supplementing the substrate with nitrogen can increase mycelial growth, yield and biological efficiency (Nunes *et al*., 2012;). For this study, vermicompost was one of the components of the substrate. Vermicompost is produced by the decomposition of organic material by earthworms to produce humus-like material (Ansari 2010; Viriato *et al*., 2021). Since the earthworms would have already started the decomposition process, it is expected that abundant nutrients would be readily available for the mushroom to utilize. Seecharran *et al*., (2018) found that vermicompost substrates produced mushrooms

with the highest nutrient content. The substrates remaining after mushrooms are harvested have high nutritional values, including mushroom mycelia, degraded cellulosic fibers, degraded lignin, proteins, and minerals. Based on the final mineral content, the substrate is valuable as an organic fertilizer and a soil conditioner for plant growth (Brenneman, and Guttman, 1994).

In conclusion, rice straw is a suitable substrate for the cultivation of *P. ostreatus,* whereas the sawdust and grass clippings substrates were not successful due to contamination. Generally, post-harvest substrates had higher mineral content compared to the initial substrates, which can be attributed to the degradation of the substrate by enzymes produced by the oyster mushroom. These spent mushroom substrates can be utilized as organic fertilizer since it is a good source of nitrogen and nutrients.

After successful cultivation from small scale practices and knowledge gained from previous experiments, upscaling would be beneficial. It would also be useful to explore other methods of preparation of substrate.

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