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Effect of different organic substrates on growth and development of edible oyster mushroom (*Pleurotus ostreatus*)

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Abstract

Pleurotus ostreatus is among the most commonly cultivated edible mushrooms with many nutritional and medicinal benefits. It is able to grow on a wide variety of substrates and inexpensive to cultivate. This study investigated the effects of organic substrates (saw dust (T1) substrate supplemented with the vermicompost of rice straw (T2), jamun leaves (T3) or neem leaves + cattle dung (T4)) on the yield of *P. ostreatus* mushrooms. High yield (weight and number of fruiting bodies) were recorded for T3 and T4 while least for T1 and T2. However, according to rank analysis T2 produced mushrooms with the best growth parameters. Significant differences were found for several parameters (weight and number of fruiting bodies, stipe thickness, cap diameter and cap thickness) with the exception of stipe length. The yields from T3 and T4 indicated that jamun and neem leaves have the potential of being suitable substrates for oyster mushroom cultivation.

Key words : *Pleurotus ostreatus,* Mushroom, Vermicompost, Substrate

Introduction

Mushrooms belong to the kingdom fungi and phylum Basidiomycota (Atilia *et al*., 2017). They are heterotrophic organisms, and though they can be mistaken for plants, they do not possess chlorophyll and therefore are not involved in photosynthesis (Chang and Miles, 2004). Mushrooms fall into the categories of edible and non-edible mushrooms. Edible mushrooms have many nutritional and medicinal benefits; properties which attract health-conscious consumers. They are a good source of protein, carbohydrates, unsaturated fatty acids, vitamins and minerals, and low in calories, and calcium (Chang and Miles, 2004; Manikandan, 2011; Thu, *et al*., 2020). The moisture content present in mushrooms is also of high percentage (90%) (Sánchez, 2010). Medicinal value includes anti-obesity, anti-diabetes, anticancer, antibiotic and anti-tumor properties (Chang and Miles, 2004; Friedman, 2016; Singh *et al.,* 2021; Adams *et al.,* 2022).

The most cultivated of mushrooms species produced worldwide include *Agaricus bisporus* (button mushroom) and *Pleurotus ostreatus* (oyster mushroom). These edible mushrooms are commonly cultivated on lignocellulosic substrates such as sawdust, rice straw, tea leaves and other agricultural wastes, along with various animal manure; horse, chicken and cattle manure (Kamthan *et al*., 2017; Siwulski *et al*., 2018; Singh *et al*., 2021; Adams *et al*., 2022). The ability of mushrooms to obtain their energy from lignocellulosic substrates results from their highly efficient enzymatic system. Lignin-degrading enzymes, and hemicellulose and cellulose-degrading enzymes are produced which breaks down the complexity of the lignocellulosic substrates to simpler forms,

enabling access to nutrients (Sánchez, 2009). In this study, neem and jamun leaves were selected as substrates for the growth of oyster mushrooms. Both of these organic materials are associated with high nutrient content and medicinal benefits, factors which make them suitable potential substrates for the cultivation of mushrooms. Positive results of increased yield of crops were experienced by farmers of India, with neem as the source fertilizer for their food and cash crops (Ayyanar, and Subash-Babu, 2012; Amruta *et al*., 2015).

In recent years, the introduction of vermi-technology was made in the cultivation of crops and mushrooms. Vermi-technology involves the usage of earthworms to decompose organic materials from which nutrient rich substrates are obtained. The product formed is known as vermicompost, a humus-like material. (Ansari *et al.,* 2016; Seecharran *et al*., 2018). The process of vermicomposting is done to allow nutrients to be readily available to crops that are cultivated (Zafar, 2016). Using the technique of vermi-technology, vermicomposting of the different organic wastes was done to produce vermicompost and subsequently used as substrates to test their effectiveness in increasing the yields of fruiting bodies and level of nutrient content of oyster mushrooms. The suitability of substrate for the cultivation of mushrooms on a large scale is determined by best yields and nutrient content.

Materials and Method

The experiment was conducted at Mushroom laboratory, Department of Biology, University of Guyana, Guyana, South America in year 2020. *Pleurotus ostreatus* was cultivated on substrates of sawdust or sawdust + vermicompost of (neem/ jamun leaves/rice straw +cattle dung), a total of four treatments. The cultivation of mushrooms involves four (4) important stages: the preparation of mushroom mother culture, preparation of spawn culture, preparation of substrate and harvesting. The technique of vermicomposting was incorporated to obtain the required substrates (Method was extracted from Ansari *et al*., 2016; Seecharran *et al*., 2018)

Vermicomposting

Vermicomposting units were created using several layers of materials. The first layer (bottom layer), 1/5 of container were of stones. The same height of sand and garden soil was added as the second and third layer, respectively. 100g of dried crushed neem/jamun leaves/ rice straw, 100 g of cattle? and 100g the dried leaves were added as the fourth, fifth and sixth layer respectively. Earthworms were added to the units to allow for decomposition. Dried leaves and cattle dung were continuously added two days per week for one month. The units were watered regularly to maintain moisture content and agitation of mixture for aeration. The completion of vermicomposting was indicated by the black granular appearance of the compost which was then harvested for use as mushroom substrate.

Preparation of the Mushroom Mother Culture

The culture media was prepared by combining 39.0g of powdered Potato Dextrose Agar (PDA) and 1 litre (L) of purified water. A homogenous mixture was created by heating and continuous stirring of the mixture (PDA + water) in a conical flask. The mixture was then autoclaved for approximately 15 minutes at 121^{o}C then allowed to cool to 50-55 ^{o}C. For the prevention and inhibition of any bacterial growth, 5 g of amoxicillin was added to the 1 L of PDA solution. The mixture was then poured into several petri dishes and allowed to solidify for 24 hours in a sterile environment. With the usage of a sterilized cork borer, mycelial discs were cut from pure cultures of *P. ostreatus* and transferred to the petri dishes. The inoculated petri dishes were sealed and incubated at room temperature to allow for mycelial colonization.

Preparation of Spawn Culture

The preparation of mushroom spawn culture was done using white millet bird seeds. 500g of white millet bird seeds were soaked for 12 hours, then boiled in water for 10-15 minutes. The seeds were strained and allowed to air dry for 12 hours, then placed in transparent glass bottles and sealed. The sealed bottles were autoclaved at 121^{o}C for approximately 2 hours, cooled to room temperature. Mycelial discs from the pure culture of *P. ostreatus* were transferred to the seeds in the bottles and shaken to ensure even distribution of

discs. The inoculated and sealed bottles were stored in a clean, dark area until the mycelia fully colonized the seeds. Full colonization occurred within 1- 2 weeks.

Preparation of Mushroom Substrate

Approximately 600g of sawdust was soaked for 24 hours, pasteurized by boiling for 1 hour, allowed to cool to room temperature and 6g of calcium carbonate ($CaCO_3$) mixed in 180 ml water was added to form substrate for control treatment (T1). Treatments 2-4 each contained 600g sawdust, 6g of $CaCO_3$ mixed in 180 ml water,300g vermicompost of rice straw (T2)/neem leaves(T3)/ jamun leaves (T4) (Table 1). The mixture was thoroughly mixed anda small amount of the initial substrate was removed for elemental analysis. The remaining substrate was equally divided into three parts and placed into autoclavable polyethylene bags, sealed, labelled and autoclaved for approximately 1 hour, then allowed to cool to room temperature. A sterilized spatula was used to loosen the colonized bird seeds in the stock spawn bottles which were then added to the substrates. The bags were loosely secured and plugged with cotton wool to prevent insects while allowing air to enter. The inoculated bags were stored in a dark room and left for mycelial growth. After 14 days, slits were made on the bags to allow for additional entry of air. The substrates were water twice daily by gently misting distilled water on the surface until mushrooms were fully matured.

Table 1. An outline of the different mushroom substrates used in different treatments.

Treatment	Substrate formula
T1	600g SD + 6g $CaCO_3$
T2	600g SD + 6g $CaCO_3$ + 300g VRS+CD
T3	600g SD + 6g $CaCO_3$ + 300g VJL+CD
T4	600g SD + 6g $CaCO_3$ + 300g VNL+CD

Abbreviations: Sawdust (SD), Vermicompost (V) of rice straw (RS), jamun leaves (JL), neem leaves (NL), cattle dung (CD), calcium carbonate ($CaCO_3$)

Harvesting

After maturation, mushrooms were harvested removing them from base of the stipe. After first harvest, the substrates were continually watered to allow for 2nd and 3rd harvests. For each fruiting body, parameters were measured including; colour, texture, stipe length and thickness, cap diameter and thickness. Total weight of fruiting bodies wastaken per harvest as well as total yield (Fig.1a-d).

Results and Discussion

The *Pleurotus* species is the second most cultivated of mushroom species. It is one of the most adaptive of all species, being able to grow on a wide range of substrates and under varying climatic conditions (Chang and Miles, 2004; Singh *et al.,* 2021; Adams *et al.,* 2022). The *Pleurotus* species is known for their many medicinal and nutritional properties, as well as having a favourable taste. *Pleurotus ostreatus*, in particular is widely cultivated. Growth of this species has been successful on many substrates tested over the years (Chang and Miles, 2004; Kamthan *et al*., 2017; Siwulski *et al*., 2018; Singh *et al.,* 2021; Adams *et al.,* 2022). For this study, growth of mushrooms was successful on sawdust substrate supplemented with vermicompost of neem and jamun leaves.

Physico-chemical components of substrates

The mineral and chemical composition of substrates utilized in mushroom cultivation play an important role in the growth and development of fruiting bodies. Carbon and nitrogen are two important components that mushroom depend on for growth in addition to small amounts of minerals and vitamins (Chang and Miles, 2004; Singh *et al.,* 2021; Adams *et al.,* 2022). Plant materials, which are the most used substrate for cultivation, possess cellulose, hemicellulose and lignin, all of which are important sources of carbon. Nitrogen is also obtained from the plant material but can be supplemented with animal manure to increase nitrogen level (Chang and Miles, 2004; Singh *et al.,* 2021; Adams *et al.,* 2022).

In this study, mushrooms cultivated on saw dust and vermicompost supplemented with rice straw, jamun leaves or neem leaves. The substrates produced varying yields because of difference of nutrient composition. Sawdust which is common in oyster mushroom cultivation has the main

chemical components; carbon (60.8%), hydrogen (5.2%), oxygen (33.8%), nitrogen (0.9%) and comprises of cellulose, lignin, and hemicelluloses (Phonphuak and Chindaprasirt, 2015). The nutrient value of rice straw includes an estimate of 40% nitrogen, 30-35 % phosphorus, 80- 85 % potassium, 40-50 % sulfur, micronutrients zinc and silicon, cellulose (39-41%), lignin (12-14%) and hemicellulose (27%) (Dobermann and Fairhurst, 2002; Kamthan and Tiwari, 2017).

Mushrooms also utilize organic acids and amino acids to gain nutrients, components that are rich in neem and jamun leaves (Chang and Miles, 2004; Singh *et al.*, 2021; Adams *et al.*, 2022). As a result, there is a high probability of these substrates producing good yields if used to cultivate mushrooms on a large scale. The leaves of neem (*Azadirachta indica*) have high nutrient content of protein, carbohydrates, minerals, calcium, phosphorus, vitamin C and carotene (Shukla, 2018). They are rich in glutamic acid, tyrosine, aspartic acid, alanine, praline, glutamine and cysteine, and several fatty acids (dodecanoic, tetradecanoic, elcosanic) (Shukla, 2018). The leaves of Jamun plant (*Syzygium cumini*) possess a range of alkaloids, flavonoids, glycosides, steroids, phenols, tannins, saponins and cardiac glycosides (Jagetia, 2017). The inclusion of cattle dung provided additional nutrients being composed of cellulose, lignin and hemicellulose, nitrogen, potassium, along with trace amount of sulphur, iron, magnesium, copper, cobalt and manganese (Gupta *et al.*, 2016).

The vermicomposting of these substrates produced substrates rich with nutrients, and increased nutrient availability (Ansari *et al.*, 2016). With varying nutrient composition of the different plant material and animal manure, differences in yields can be seen, as indicated in Table 2**.**

Growth Parameters of harvested mushrooms

Yield (weight + number) of fruiting bodies

In this study, T1 and T2 recorded the least yields while T3 and T4 recorded the highest yields (both in number of fruiting bodies and weight) (Table 2). The yield of fruiting bodies harvested from substrates is an important indicator to de-

Fig 1a. T1

Fig 1a. T2

Fig 1a. T3

Fig 1a. T4

Table 2. Parameters measured for harvested mushrooms on different vermicompost substrate

Parameters	Treatments			
	T1	T2	T3	T4
No. of fruiting bodies	4	7	33	29
Length of Stipe (mm)	28 ± 15.7	37.28 ± 17.5	29.75 ± 10.6	28.62 ± 10.7
Stipe Thickness (mm)	10.5 ± 5.74	10 ± 2.71	5.56 ± 2.75	5.90 ± 3.57
Cap Diameter (mm)	84.5 ± 54.0	86.7±29.4	53.6 ± 24.2	56.2 ± 22.4
Cap Thickness (mm)	2 ± 0.9	2.35 ± 0.8	1.8 ± 0.8	1.4 ± 0.6
Fresh weight (g)	13.4 ±4.10	20.4 ± 9.72	64.8 ± 34.4	41.7 ± 9.40
Total fresh weight (g)	40.06	61.29	194.47	125.14

termine the suitability of a substrate for the cultivation of mushrooms. Nutrient and moisture content of substrate, and temperature, are some of the factors that affect yield of fruiting bodies (Hoa *et al*., 2015; Bellettinia *et al*., 2019).

Hoa *et al*., (2015) and Colmenares-Cruz *et al*., (2017), found that yield of mushrooms was influenced by the difference of the physical and chemical composition of the substrate formulas and more so their C/N ratio. A lower C/N ratio increased yields and higher C/N ratio decreased yields. The addition of other substrates to sawdust increased the nitrogen content which can account for increased yields (Hoa *et al*., 2015).

Sawdust has a high percentage of carbon and low percentage of nitrogen, an estimate of 60.8 % and 0.9 % respectively, and can vary with plant species; affecting yield accordingly (Phonphuak and Chindaprasirt, 2015; Bhattacharjya *et al*., 2014). The addition of the different vermicompost substrates which have varying amounts of nitrogen and carbon, created a more suitable C/N ratio thereby resulting in more yields compared to the sawdust substrate alone (Hoa *et al*., 2015; Bellettinia *et al*., 2019). There was a significant difference in the weight and number of fruiting bodies produced for the different substrates, p value~0.0361 and 0.000269 respectively. This suggests that that the varying vermicompost substrate materials have different levels of nutrient content which influenced the growth and development of fruiting bodies (Bellettinia *et al*., 2019).

While Hoa *et al*., (2015) recorded the trend of higher yields after other substrates were added, many other researchers found that sawdust as control produced the highest yields. However, there were variances in yield according to the ratio of sawdust to other substrate which supports the fact that varying nutrient content of substrate affects yields (Shah *et al*., 2004; Buah, 2010; Rambey *et al*., 2019; Rambey *et al*., 2020). Another factor affecting growth of fruiting bodies is the moisture content of the substrate. There is the likelihood that there was a high moisture content in the T1 and T2 which affected mycelium development thereby affecting yields (Bellettinia *et al*., 2019).

Diameter and thickness of pileus/cap

Sawdust and rice straw substrates gave the lowest yields, however, fruiting bodies were found to have larger and thicker caps compared to the other treatments. There was a record of numerous small-sized fruiting bodies present in the substrates that gave higher yields. Variations of fruiting bodies cap size harvested were noted between all substrates. T1 however, produced fruiting bodies with largest variations in cap diameter size as indicated by the standard deviation of ± 54.0mm. The largest in cap diameter was found in T1 (160mm) while smallest in T3 and T4 (23mm). There was a significant difference in the cap diameter of fruiting bodies harvested from the different substrates (p value- 0.00606). A decrease in size of cap diameter while there is an increase in number of fruiting bodies can be explained by the lack of space for the fruiting body to expand further (Samuel and Eugene, 2012; Rambey *et al*., 2019). There was also statistical difference in the cap thickness of mushrooms harvested (p value-0.0117). Thickness of cap is more likely to be affected by the availability of nutrients. A greater number of fruiting bodies requires more distribution of nutrients, thus the treatments with lower yields produced fruiting bodies with thicker caps. While those with higher yields produced fruiting bodies with thinner caps.

Length and thickness of stipe

The average length of stipe of mushrooms harvested ranged from 28 mm to 37.28 mm, while thickness 5.56 to 10.5 mm (Fig. 2). There was no significant difference in stipe length of mushrooms harvested from the different treatments (p value~0.359), however there was significant difference in stipe thickness of fruiting bodies (p value~0.0013). While the different substrates have little to no effect on stipe length/height, there is a probability that thickness can be affected. Factors that affect stipe length and thickness include air temperature, humidity, fresh air, and compact material (Sher *et al*., 2010; Bellettinia *et al*., 2019). The competition for space and nutrients can also affect stipe thickness, as it is noted that the treatments that gave the highest yields had the smallest diameter in average than those that gave lower yields.

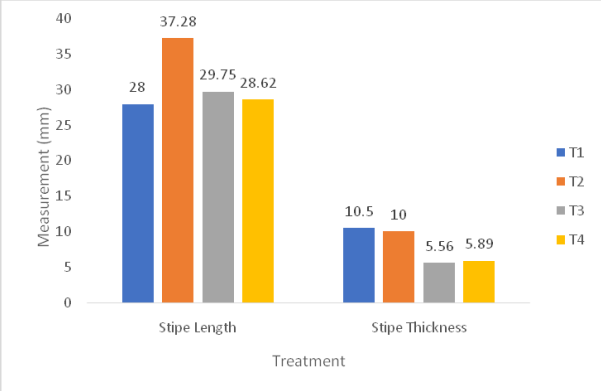

Fig. 2. Stipe length and thickness of fruiting bodies obtained from different treatments

Rank analysis

Despite T3 and T4 producing the highest yield in the number and fresh weight of fruiting bodies, rank analysis showed that overall T2 produced the better results in growth parameters as indicated in Table 3. T2 was followed by T3, T1 then T4. Based on rank analysis, T2 was most productive in producing mushrooms with favorable characteristics/ growth parameters. Two (2) studies done by Sharma *et al*., (2013) and Rajapaskse *et al*., (2007) give similar results where paddy/rice straw was used as a substrate. In the study done by Sharma *et al*., (2013), all rice straw substrates gave better results in yield than sugarcane bagasse and sawdust substrate. While in the study done by Rajapaskse *et al*., (2007), the rice straw based substrate produced the best yield among five (5) treatments.

Table 3. Rank analysis of growth parameters of harvested mushrooms

Parameters	T1	T2	T3	T4
No. of fruiting bodies	4	3	1	2
Length of Stipe (mm)	3	1	2	4
Stipe Thickness (mm)	1	2	4	3
Cap Diameter (mm)	2	1	4	3
Cap Thickness (mm)	2	1	3	4
Total fresh weight (g)	4	3	1	2
Total	16	11	15	18
Rank	**3**	**1**	**2**	**4**

Nutrient content of *Pleurotus ostreatus* mushrooms

Mushrooms are known for their high nutrient content. Different species may have slight variances in composition but maintain a high percentage in nutrient composition. Variances are related to substrate chemical and mineral composition (Chang and Miles, 2004; Patil *et al*., 2010; Hoa *et al*., 2015). Hoa *et al*., (2015) supported this in their research findings. Protein content increased with an increase in the amount of supplemental substrates. Nitrogen content of substrate was found to directly affect protein content as it is important in protein synthesis. Carbohydrates and fat content were also affected. The range of nutrient composition of *P. ostreatus* in different substrate formula include; protein (19.52~29.70%), fat (1.32~2.78%), Fiber (22.00~29.75%), carbohydrate (30.78~47.62%) ash (5.90~7.10). Patil *et al*., (2010), recorded nutrient content of *P. ostreatus* as protein (20.33~24.66%), fat (2.56~2.82%), carbohydrate (50.50~56.20%), ash (5.90~6.70%). Emiru *et al*., (2016); Tolera and Abera (2017) and Jin *et al*., (2018) also recorded similar values for nutrient content. Table 4 gives the average nutrient content of mushrooms obtained from different substrates of several experimental exercises.

Mineral content also varied according to substrate formula for the two studies (Patil *et al*., 2010; Hoa *et al*., 2015), establishing that mineral and nutrient content varies according to the composition of the substrate. Seecharran *et al*., (2018) further established a strong negative relationship

Table 4. Nutrient content (protein, fat, fibre, carbohydrate) of *P. ostreatus* cultivated on different substrates

Study No.	Author	Protein (%)	Fat (%)	Fiber (%)	Carbohydrate (%)
1	Hoa *et al*., (2015)	24.13	2.16	26.43	40.70
2	Patil *et al*., (2010)	22.84	2.67	7.46	53.73
3	Emiru *et al*., (2016)	15.05	6.04	22.53	39.73
4	Tolera *et al*., (2017)	28.85	2.47	12.87	48.16
5	Jin *et al*., (2018)	22.05	2.45		66.6

between soil nutrients and mushroom nutrients; an increase in nutrient content of mushroom with a decrease in nutrient content of soil. It can therefore be estimated that the nutrient and mineral content of mushrooms harvested in this study is within the similar percentage range to the studies listed. Based on Seecharran *et al*., (2018) substrate formula with vermicompost supplement is likely to have a higher percentage in nutritional values as compared to saw dust alone, as nutrients would be more readily available.

Moisture content of mushrooms

Mushrooms have a high percentage of moisture content, an average of 90% for most species (Sánchez, 2010). Moisture content of mushrooms can however fluctuate depending on moisture content of substrate or the water holding capacity of the substrate, temperature and humidity (Chang and Miles, 2004; Bellettinia *et al*., 2019). Seecharran *et al*., (2018) found that moisture content of *P. ostreatus* range from 85.83 % to 93.70 % when cultivate in cattle dung, duck manure and vermicompost substrates. Hoa *et al*., (2015) recorded 89.37 to 91.56% for *P. ostreatus* while 87.14 to 92.45 % for *P. cystidiosus*; both grown on same substrate formulas. While moisture content of substrate can influence moisture content of fruiting bodies it can also be influenced by mushroom species. It can be estimated that the results of moisture content of this study will fall within the range of 85 % to 93 %, a combination of Hoa *et al*., (2015) and Seecharran *et al*., (2018) as substrates in use were similar (Table 5).

Table 5. The average percentage of moisture content of *P.ostreatus* grown on different substrates

Author	Moisture content (%)
Hoa *et al*., (2015)	90.36
Patil *et al*., (2010)	89.03
Tolera *et al*., (2017)	88.75
Jin *et al*., (2018)	88.62
Seecharran *et al*., (2018)	89.63

Conclusion

The vermicompost of neem leaves and jamun leaves with cattle dung provided beneficial supplement to saw dust substrate for the cultivation of oyster mushrooms. Treatments 1 and 2 produced less yield in number of fruiting bodies which may be due to too much moisture in substrates or any environmental factor that may have hindered growth. T2 however, ranked first in producing the best growth parameters.

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