

Growth and nutritional content of *Pleurotus ostreatus* using different substrates (rice straw, vermicompost and vermiwash)

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ABSTRACT

Vermiculture is a significant part of sustainable agriculture and organic farming techniques that lead to a decrease in organic materials through practicing vermitechnology which includes vermicomposting and vermiwash. Vermicompost and vermiwash can be used as suitable substrates for cultivation of nutritious edible mushrooms. Mushrooms acts as decomposers in the environment by degrading complex organic materials or any type of lignocelluloses substrates in plants and animals. The study investigated the growth and nutritional content of oyster mushrooms (*Pleurotus ostreatus*) using different types of substrates. *Pleurotus ostreatus* showed significant growth when subjected to five treatments of vermicompost, vermiwash and rice straw (in different combinations) compared to control substrate. Among the different treatments, there was no significant effect of the fresh ($p=0.469$) and dry weight ($p=0.385$). The results showed that the vermicompost (T_2) was the best substrate both in fresh and dry weight, producing mushrooms with significantly better growth parameters. From the study, it can be inferred that vermicompost and vermiwash, rice straw, vermiwash substrates can be effective for the growth of oyster mushrooms.

Keywords: Mushrooms, nutrients, lignocellulose, edible, vermicompost, vermiwash

Fungi belonging to the phylum Basidiomycota seen growing “above or underground” are known as mushrooms (Shepherd and Totterdell, 1988; Chang and Miles, 2008; Ergonul *et al.*, 2013). There are about 2,000 species of mushrooms, but only 25 are cultivated for food commercially (Chang and Miles, 2008; Ergonul *et al.*, 2013). Globally, mushrooms have been considered as a cuisine; especially for their unique flavor, nutritional contents and also their medicinal properties (Boa, 2004). One of the most prominent features of mushroom is that they can degrade any type of lignocelluloses substrates for instance, recycling agriculture wastes that is ecosystem friendly (van der Westhuizen and Eicker, 1994; Chang, 1996; Joh *et al.*, 2004). Additionally, mushrooms cannot manufacture their own food. Therefore, they obtain

their nutrient by degrading complex organic materials in plants and animals (van der Westhuizen and Eicker, 1994). Mushrooms can be cultivated using the agriculture waste products such as agricultural residues and animal dung as substrates. These agriculture waste products are converted into rich proteins and vitamin sources (Chang, 1996).

One of the second most cultivated edible mushrooms is known as *Pleurotus ostreatus* (oyster mushroom) belonging to the class Agaricomycetes (Sánchez, 2010; Kuo, 2011). Oyster mushrooms are capable of degrading complex lignocellulose substrate (Woller, 2007). They produce higher nutritional contents and have the potential to grow on various substrates, for instances rice and wheat straw (in most

cases) (Garcha *et al.*, 1993). Globally, organic waste is considered to be harmful to our environment today and it can contribute to climate change by releasing green –house gases in the process (Ansari, 2008). This organic waste problem can be eliminated by vermitechnology. The vermitechnology includes vermiwash and vermicompost. These are important components of biodynamic farming (Ansari, 2008). Vermiculture is referred to as “sustainable agriculture” and “organic farming” techniques that utilize mainly earthworms species (Ansari and Sukhraj, 2010; Ramnarain *et al.*, 2017). It is a combination of soil micro-organisms and species of earthworms (Ansari, 2008; Ansari and Sukhraj, 2010; Chin *et al.*, 2018). Vermiculture practice produced nutritious food without utilization of expensive equipment (Alexopoulos *et al.*, 1996.). Cultivating mushrooms using this technology can assist in overcoming poverty because it will provide a source of income by quick production of nutritious mushrooms (Anon Biotech, 2010). In the present study, the aim was to investigate the growth and nutritional content of oyster mushrooms (*Pleurotus ostreatus*) using different types of substrate in Guyana. This study used rice straw, vermicompost rice straw and vermiwash as the substrates. Three units of rice straw based vermicomposting and vermiwash was produced that was used to grow the oyster mushrooms.

MATERIALS AND METHODS

This research was carried out at the University of Guyana, Turkeyen Campus. Additionally, the vermiwash and vermicompost units were produced in the mushroom house. After setting up the vermiwash and vermicompost units, they were left for 60 days to stabilize. New organic materials were added two (2) times per week to each of the unit for approximately 60 days as done initially. When the liquid flowed through the tap changed color from chocolate brown to pale yellow, the wash was ready to harvest. The vermicompost was ready to harvest when the organic material in each unit changed

completely in structure (firmed) and a soil-like scent was observed. The sample was collected twice a week from each unit and was used as a substrate for the mushroom.

The preparation of mushroom mother culture and spawn production was done in the Biology Laboratory. The parameters of mushrooms measured included color and texture of fruiting bodies, number of pins and fruiting bodies, size of fruiting bodies, fresh and dry weight of fruit bodies, elemental analysis of fruiting bodies and the nutritional contents of the fruiting bodies. Analysis of the mushrooms nutritional content was compared using literature review for each type of substrates medium comprising of rice straw, vermicompost and vermiwash. *Pleurotus ostreatus* (oyster mushrooms) was successfully grown on the vermicompost soil substrate, rice straw based vermiwash and rice straw as the control. The cultivation of mushroom consisted of three main phases: spawn production, mushroom production and harvesting. The study focused on five treatments in three cycles for which the set up consisted of 15 bags per cycle in triplicate. It was divided into eight phases. The project began on the 19th December, 2019 and concluded on 12th March, 2020.

Procedure for preparation of the vermiwash units

The methods were taken from Ansari (2012). Three square four gallon containers were used to set up the units. A tap was fixed on the lower side of each unit and each unit was placed on a stand for easy flow of the vermiwash. Five cm of broken pebbles were placed at the bottom of each unit after which 5.5 cm of coarse sand was added. Water was allowed to flow through these layers so that the basic filter unit was settled. Four cm layer of loamy soil was added on top of the filter bed. Approximately 20 earth worms were introduced into the units as stepped up in Fig. 1. Four cm cattle dung was added on top of the soil for the earthworm, followed by 7cm rice straw on top of the cattle dung. The tap was left open after

the different materials were layered. Each unit was monitored and organic materials were added in the specified quantity when required. Each unit was watered so as to maintain the moisture content and was drained at every two days period. After the liquid that flowed through turned pale yellow, the vermiwash was collected. The tap was closed and water was allowed to flow overnight. The tap was closed the next day and the vermiwash was collected in a dark container to prevent the sunlight from contaminating the wash.

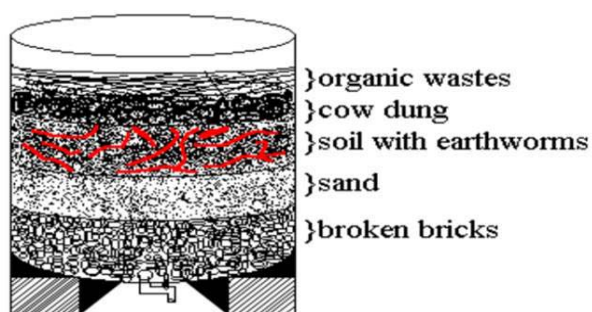


Fig. 1. Set up of the vermiwash unit (Source: Ansari, 2012)

Vermiwash units were set up in triplicate as mentioned in table 1.

Table 1. Treatments and Description of Vermiwash Units

Treatments	Description of Vermiwash Units preparation [VW]
A	5cm brick+ 5.5 cm sand+ 4 cm soil with Earthworms+ 4cm cattle dung +7cm rice straw

Procedure for preparation of the vermicomposting units

The methods were taken from Ansari (2012). Three square four gallon containers were used to set up the compost units. A 4.5 cm layer of broken pebbles were placed at the bottom of each unit, followed by 5cm layer of coarse sand. Six cm layer of loamy soil was placed over and 50 earthworms (*Eisenia foetida*) were introduced in each unit with the soil. 200g layer of damp rice straw was added on top of the soil. Followed by, 100 g of cattle dung to make a layer of 4cm on top of the basic filter. After which, another 200 g of damp rice straw was placed on top the organic materials as in Fig. 2. The units were sprinkled with water to moist all the layers. The units were left to stabilize and organic materials were added twice a week as specified amount. The vermicompost was ready to harvest in about 30 days and the water was addition stopped to the units for 2–3 days to allow the earthworms to settle at the bottom layers. The vermicompost was ready to be stacked in bags that had the proper optimum moisture content. Vermicomposting units were set up in triplicate as mentioned in table 2.

Table 2. Treatments and Description of Vermicompost

Treatments	Description of Vermicompost preparation [VRST]
A	14g soil with 50 Earthworms+ 200g rice straw+ 100g cattle dung+ 200 g rice straw

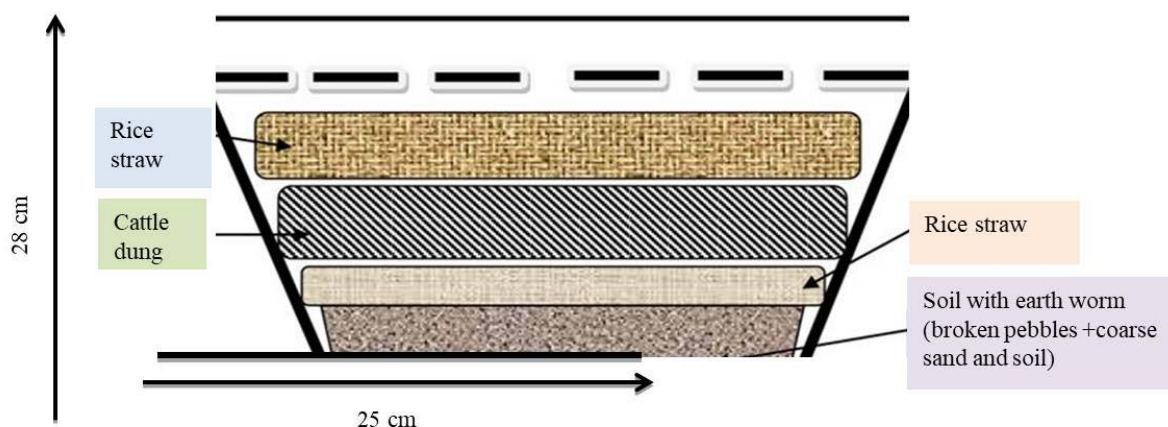


Fig. 2. Set up of the vermicomposting unit

Phase I: Preparation of Mushroom mother culture (Potato Dextrose Agar)

The methods were taken from Diana *et al.* (2018). A quantity of 39.0 g of potato dextrose agar (PDA) was used to prepare 1L of growth medium. The potato dextrose agar (PDA) was added to a conical flask with 1L of purified water and the mixture was mixed thoroughly until evenly distributed. The media was autoclaved at 121°C for 15 minutes and the solution was allowed to cool to approximately 50-55°C. Strict aseptic techniques were used during the inoculation. Antibiotic (Amoxicillin) was added to the agar media @ 30 mg to 1L potato dextrose agar (PDA) to prevent any bacterial growth. The solution was poured into petri dishes, a lid was placed to cover and left to solidify. After solidification, the petri dishes were placed in the incubator to evaporate moisture content. A mycelial bit from pure culture of *Pleurotus ostreatus* of 0.8 to 1.0 cm dia was placed in the centre of the petridish with the help of a sterilized cork borer. The edges of the petri dishes were sealed with scotch tapes and were incubate inverted at room temperature.

Phase II: Preparation of stock spawn

White millet (bird seed) was used as the substrate for the stock pawn. 500g of white millet bird seeds were soaked in water for 12 hours and boiled for 10-15 minutes, after which it was strained and allowed to air dry for 12 hours. The seeds were filled in glass bottles and the mouth of glass bottles were sealed with cotton reinforced with gauze. The glass bottles were autoclave at 121°C for 2 hours and it was left to cool to room temperature. A few mycelial bits of mother culture was transferred aseptically to the sterilized and cooled bottles and incubated at 25 °C until full mycelial colonization.

Phase III: Preparation of mushroom mother culture (Calcium carbonate + substrate (rice straw))

The rice straw was exposed to sunlight for approximately 2 days to ensure it was dried. The rice

straw was cut into smaller pieces of approximately 2-3 inches, washed and soaked for 24 hours in water. The rice straw was pasteurized by boiling in water for one hour and it was allowed to cool to room temperature. The substrate was made using a formula: 200g rice straw and 2g of Calcium carbonate. The rice straw was rendered as the control. 200g of rice straw was measured and placed in a bucket. 2g of Calcium carbonate was mixed with 60ml of distilled water in a beaker until evenly dissolved. The solution was added to the bucket and was mixed thoroughly. A small amount of the substrate mixture was removed and wrapped in aluminium foil for further analysis. The remaining substrate mixture was divided into four equal portions. Each portion was placed in autoclave-able polyethylene bags and labelled accordingly. The polyethylene bags were autoclaved for 1 hour. After finished autoclaving, the substrate mixture was left to cool. A sterilized spatula was used to loosen the colonized bird seeds in the stock spawn bottles. Each of the four (4) substrate portions were opened and colonized bird seed was added to them. The bags were sealed with rubber bands and were stored in a sterile, dark area to allow mycelium growth. Sides of the bag were roll down so as to create hole where the rubber bands were tied. Cotton wool was placed in the opened area of the bag to prevent insects from entering but still allowed air to pass through the bags. The substrate was watered twice daily by gently misting distilled water on the surface. After 14 days, the bags were opened to expose the substrate to air and dim light (30%) for 12- hour periods. After mycelium had finished colonized, a sharp, sterile razor blade was used to create slits on the bags for fruiting bodies to grow. The bags were watered daily and emerging fruiting bodies were observed.

Phase IV: Preparation of mushroom beds (Calcium carbonate + substrate (rice Straw) + vermicompost)

The substrate was made using a formula: 200g rice straw, 2g of Calcium carbonate, 100g

vermicompost and 2g of Calcium carbonate. The solution was added to the bucket and was mixed thoroughly. A small amount of the substrate mixture was removed and wrapped in aluminium foil for further analysis. The remaining substrate mixture was divided into four equal portions. The substrates were used to grow mushroom as in phase-III.

Phase V: Preparation of mushroom beds (Calcium carbonate + substrate (rice Straw) + vermiwash)

The substrate was made using a formula: 200g rice straw, 2g of Calcium carbonate and 100 ml vermiwash. A small amount of the substrate mixture was removed and wrapped in aluminium foil for further analysis. The remaining substrate mixture was divided into four equal portions. The substrates were used to grow mushroom as in phase-III and IV.

Phase VI: Preparation of mushroom beds (Calcium carbonate + substrate (rice Straw) + Vermicompost+ Vermiwash)

The substrate was made using a formula: 200g rice straw, 2g of Calcium carbonate, 100 g vermicompost and 100 ml vermiwash. A small amount of the substrate mixture was removed and wrapped in aluminium foil for further analysis. The remaining substrate mixture was divided into four equal portions. The substrates were used to grow mushroom as in phase-III, IV and V.

Phase VII: Preparation of mushroom beds (Calcium carbonate + substrate (rice Straw) + Vermicompost+ Vermiwash)

The substrate was made using: 200g rice straw, 2g of Calcium carbonate, 50 g vermicompost and 50 ml vermiwash. A small amount of the substrate mixture was removed and wrapped in aluminium foil for further analysis. The remaining substrate mixture was divided into four equal portions. The substrates

were used to grow mushroom as in phase-III, IV, V and VI.

Phase VIII: Harvesting

When the fruit bodies matured, they were harvested by slightly twisting the base of the stipe. The maturity varied depending on the species and consumer preference. After harvested, the substrate was kept on watering and aerated to allow for two (2) more harvests (a second and third cycle). Parameters measured included colour and texture of pins and fruit bodies, number of pins and fruit bodies, size of fruit bodies (stipe length and thickness, cap diameter and thickness), fresh and dry weights of fruit bodies. In addition, elemental analyses of fruit bodies and substrate (Nitrogen (N), Phosphorus (P) and Potassium (K)) and the nutritional content of fruit bodies. Some of the fruit bodies were prepared for the elemental analysis whereby drying them in brown paper bags in the oven at 60-70°C until the constant weight is achieved. The dried fruiting bodies were crushed using mortar and pestle and stored in polyethylene (Ziploc) bag with labelled until they were ready for further analyses at The Guyana Sugar Cooperation. About 5g of dried fruit body was needed per sample. The initial and final substrates were prepared for elemental analysis by drying and storing in paper bags.

Preparation of oyster mushrooms

Mushroom were grown in five different treatments given in Table 3.

Table 3. Treatments and Description of oyster mushrooms preparation

Treatments	Details of Treatments
T ₁ (Control)	200g substrate (rice straw) [S] + 2 g Calcium carbonate
T ₂	2 g Calcium carbonate + substrate (200 g rice straw + 100 g vermicompost [VRST])
T ₃	2g Calcium carbonate + substrate (200 g Rice Straw + 100ml vermiwash [VW])

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Treatments	Details of Treatments
T ₄	2g Calcium carbonate + substrate (200 g Rice Straw + 100 g vermicompost [VRST] +100ml vermiwash [VW])
T ₅	2g Calcium carbonate + substrate (200 g Rice Straw + 50 g vermicompost [VRST] +50 ml vermiwash [VW])

amount of fruiting bodies on harvest along with largest length and thickness of stipe and diameter of cap followed by treatment 1 (T₁) having the greatest thickness of cap. Treatment 3 (T₃) had significantly less fruiting bodies of smaller size, with least length and thickness of stipe, diameter and thickness of cap. Treatment 4 (T₄) had medium number of fruiting bodies followed by treatment 5 (T₅) having moderate growth of fruiting bodies, length of stipe and diameter of cap.

RESULTS AND DISCUSSION

Growth parameters of harvested oyster mushrooms

Fruiting bodies grew well on each treatment comprising vermicomposting, vermiwash as well as on rice straw (control). The total physical growth parameters for the fruiting bodies were observed after the two week of inoculation. It is evident that fruiting bodies were successfully grown on each treatment as seen in Table 4. Treatment 2 (T₂) had the greatest

Results of oyster mushrooms’ average growth at the time of harvest are shown in Table 5. The average fruiting bodies, length and thickness of stipe and diameter of cap of *Pleurotus ostreatus* was highest for treatment 2 (T₂) at 46.67, 122.23, 21.02 and 184.98 respectively and the lowest for treatment 3 (T₃) at 23.33, 57.27, 12.87 and 92.37 respectively. The average thickness of cap was greatest at treatment 1 (T₁) at 11.03, and the lowest for treatment 3 (T₃)

Table 4. Total growth parameters of fruiting bodies at harvest

Treatments	Total Parameters				
	Number of fruiting bodies	Length of stipe (cm)	Thickness of stipe Max. (cm)	Diameter of cap (cm)	Thickness of cap (cm)
T ₁	109	279.9	60.8	418.5	33.1
T ₂	140	366.7	63.6	554.95	25.5
T ₃	70	171.8	38.6	277.1	11.2
T ₄	82	207.9	43.9	363.0	21.4
T ₅	111	318.3	51.5	440.8	22.3

Table 5. Average growth parameters of fruiting bodies at harvest (Mean ±Standard deviation)

Treatments	Parameters				
	Number of fruiting bodies	Stipe length (cm)	Stipe thickness (cm) Max.	Cap diameter (cm)	Cap thickness (cm)
T ₁	36.33±7.57	93.30±12.41	20.27±6.23	139.50±45.60	11.03±3.81
T ₂	46.67±12.90	122.23±25.32	21.20±2.72	184.98±19.83	8.50±2.36
T ₃	23.33±8.96	57.27±19.14	12.87±4.37	92.37±31.06	3.73±1.72
T ₄	27.33±20.50	69.30±36.70	14.63±12.00	121.00±81.51	7.13±7.70
T ₅	37.00±15.87	106.10±23.85	17.17±7.51	146.93±41.64	7.43±4.09

at 3.73. Among all the substrates used, vermicomposting (treatment 2 (T₂)) was the best followed by the combination of vermicompst (50g) + vermiwash (50 ml) (treatment 5 (T₅)) and rice straw (control) for the oyster mushroom growth. Similarly, vermiwash substrate also showed as a supporter of oyster mushroom growth.

Analysis of harvested mushrooms

Analysis of fruiting bodies between the five different treatments using Analysis of Variance (ANOVA) shows that there was no significant effect of substrate media on the number of fruiting bodies (Table 6). This was revealed by the Pr (>F) value (0.425) being greater than the significance level of

Table 6. One-way ANOVA for the number of fruiting bodies in the five different treatments

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	4	163.1	40.79	0.979	0.425
Residuals	63	2623.8	41.65		

0.05, failed to reject the null hypothesis. Fruiting bodies of *Pleurotus ostreatus* was greater in T₂ followed by T₅ and the lowest was seen as T₃ as shown in the graph (Fig. 3).

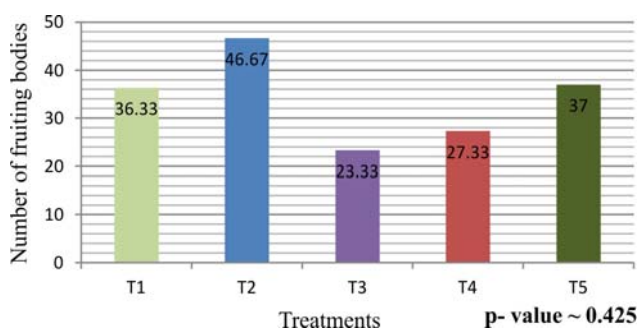


Fig. 3. Number of fruiting bodies of oyster mushrooms in five different treatments

Analysis of the stipe length between the five different treatments using Analysis of Variance (ANOVA) shows that there was significant effect of substrate media on the stipe length among the five (5)

treatments (Table 7). This was revealed by the Pr (>F) value (0.044) which is less than the significance

Table 7. One-way ANOVA for the length of the stipe in the five different treatments

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	4	16.4	4.112	2.469	0.044 *
Residuals	485	807.7	1.665		

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level of 0.05, rejecting the null hypothesis. Fruiting body of *Pleurotus ostreatus* stipe length was greater in T₂ followed by T₅ and the lowest was seen as T₃ as shown in the graph (Fig. 4).

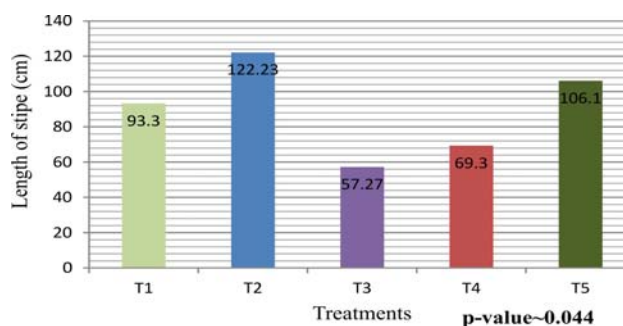


Fig. 4. Length of stipe of oyster mushrooms in five different treatments

Analysis of thickness of stipe between the five different treatments using Analysis of Variance (ANOVA) shows that there was no significant effect of substrate media in the thickness of stipe among the five (5) treatments. This was revealed by the Pr (>F) value (0.747) being greater than the significance level of 0.05, failed to reject the null hypothesis (Table 8). Fruiting bodies of *Pleurotus ostreatus* thickness of stipe was greater in T₂ followed by T₁ and the lowest was seen as T₃ as shown in the graph (Fig. 5).

Table 8. One-way ANOVA for the thickness of stipe in the five different treatments

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	4	0.51	0.1273	0.484	0.747
Residuals	528	138.77	0.2628		

GROWTH AND NUTRITIONAL CONTENT OF *PLEUROTUS OSTREATUS* USING DIFFERENT SUBSTRATES

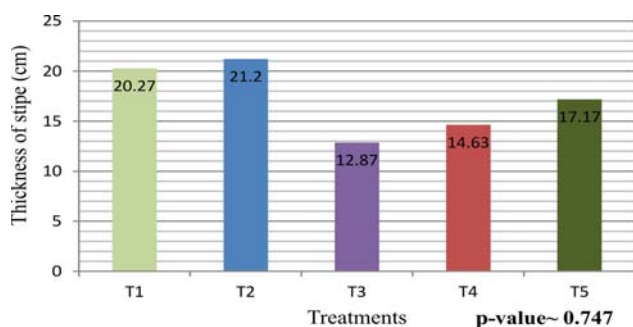


Fig. 5. Stipe thickness of oyster mushrooms in five different treatments

Analysis of diameter of cap between the five different treatments using Analysis of Variance (ANOVA) shows that there was no significant effect of substrate media in the diameter of cap among the five (5) treatments. This was revealed by the Pr (>F) value (0.54) being greater than the significance level of 0.05, failed to reject the null hypothesis (Table 9).

Table 9. One-way ANOVA for the difference of diameter of cap in the five different treatments

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	4	14.4	3.598	0.777	0.54
Residuals	522	2417.2	4.631		

Fruiting bodies of *Pleurotus ostreatus* diameter of cap was greater in T₂ followed by T₅ and the lowest was seen as T₃ as shown in the graph (Fig. 6).

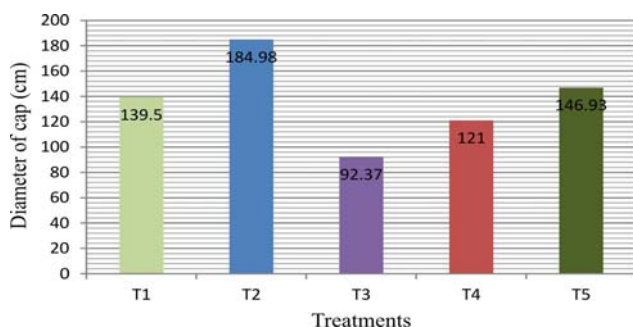


Fig. 6. Stipe thickness of oyster mushrooms in five different treatments

Analysis of thickness of cap between the five different treatments using Analysis of Variance (ANOVA) shows that there was significant effect of

substrate media in the thickness of cap among the five (5) treatments. This was revealed by the Pr (>F) value (4.77e-09) which is less than the significance level of 0.05, rejecting the null hypothesis (Table 10). Fruiting bodies of *Pleurotus ostreatus* thickness of cap was

Table 10. One-way ANOVA for the thickness of cap in the five different treatments

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	4	1.485	0.3712	11.65	4.77e-09 ***
Residuals	493	15.708	0.0319		

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greater in T₁ followed by T₃ and the lowest seen as T₃ as shown in the graph (Fig. 7).

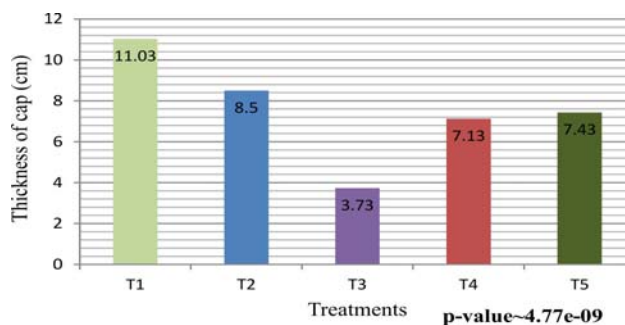


Fig. 7. Thickness of cap of oyster mushrooms in five different treatments

Table 11 showed the results obtained from the overall ranking of the physical growth parameters from different substrate medium; treatment 2 (T₂) was ranked as the best then followed treatment 5 (T₅) and treatment 1 (T₁).

Weight and moisture content of harvested oyster mushrooms

The moisture content varied among the substrates used as shown in Table 12. The percentage of moisture content ranged from 82.24 to 90.17% with the vermicompost substrate (treatment 2(T₂)) showed the highest moisture content compared to the other treatments. The total yield in vermicompost (treatment 2 (T₂)) was the highest at 647.24 g and also dry weight

Table 11. Rank analysis for the physical parameters of fruiting bodies measured at harvest

Treatments	Parameters						Total	Rank
	Number of fruiting bodies	Stipe length (cm)	Stipe thickness (cm) Max.	Cap diameter (cm)	Cap thickness (cm)			
T ₁	3	3	2	3	1	12	2	
T ₂	1	1	1	1	2	6	1	
T ₃	5	5	5	5	5	25	5	
T ₄	4	4	4	4	4	20	4	
T ₅	2	2	3	2	3	12	2	

Table 12. Total weight and moisture content for fruiting bodies at harvest

Treatments	Total weight of mushrooms per harvest		Moisture %
	Fresh weight (g)	Dry weight (g)	
T ₁	287.57	25.61	87.52
T ₂	647.24	66.02	90.17
T ₃	263.1	44.2	82.24
T ₄	325.98	39.02	88.53
T ₅	381.91	44.67	88.10

Percentage moisture= Fresh weight-Dry weight ÷Fresh weight * 100

at 66.02g followed by treatment 5 (T₅) at 381.91g, treatment 4 (T₄) at 325.98, control at 287.57g and vermiwash (treatment 3 (T₃) at 263.1g (Table 12).

The average highest fresh and dry weight was obtained for treatment 2 (T₂) at 215.75g and 22.01 g

Table 13. Weight and moisture content for fruiting bodies at harvest (Mean ±Standard deviation)

Treatments	Weight of fruiting bodies		Moisture % ± SD
	Fresh weight (g)	Dry weight (g)	
T ₁	95.86±56.81	8.54±2.76	87.52±9.47
T ₂	215.75±21.86	22.01±14.56	90.17±5.56
T ₃	87.70±36.22	14.73±12.11	82.24±16.42
T ₄	108.69±23.44	13.01±9.95	88.53±7.73
T ₅	127.30±53.21	14.89±6.00	88.10±1.74

respectively and the lowest for treatment 1 (T₁) at 95.86 g and 8.54grespectively as seen in Table 13.

Analysis of harvested mushrooms

Analysis of fresh weight between the five different treatments using Analysis of Variance (ANOVA) shows that there was no significant effect of substrate media in the fresh weight among the five (5) treatments. This was revealed by the Pr (>F) value (0.469) being greater than the significance level of 0.05, failed to reject the null hypothesis (Table 14).

Table 14. One-way ANOVA for the fresh weight of oyster mushrooms grown in the five different treatments

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	4	1171	292.8	0.896	0.469
Residuals	104	34001	326.9		

Analysis of dry weight between the five different treatments using Analysis of Variance (ANOVA) shows that there was no significant effect of substrate media in the dry weight amongthe five (5) treatments. This was revealed by the Pr (>F) value (0.385) being greater than the significance level of 0.05, failed to reject the null hypothesis (Table 15). Fruiting bodies

Table 15. One-way ANOVA for the dry weight of oyster mushrooms in the five different treatments

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	4	9.73	2.433	1.051	0.385
Residuals	103	238.52	2.316		

of *Pleurotus ostreatus* fresh weight was greater in T₂ followed by T₅ and the lowest was seen as T₃ whereas dry weight were greater in T₂ followed by T₅ and the lowest was seen as T₁ in the graph (Fig. 8).

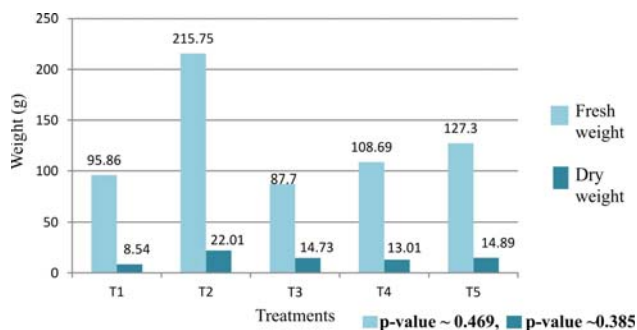


Fig. 8. Comparison of the fresh and dry weight of oyster mushrooms grown in five different treatments

Physico-chemical analysis of soil substrates and mushrooms grown on different substrates

The data for the physico-chemical analysis of the soil substrates and mushrooms grown on different substrates were compiled from the relevant literature review. Therefore, this research is expected to follow similar trends as observed by most studies. Higher levels of nitrogen was observed in the rice straw substrate as compared to the other substrates. Phosphates and potassium were higher in vermicompost soil substrate followed by vermiwash, and rice straw (Table 16) (Ansari, 2012; Hala El-Sayed *et al.*, 2019). There are not many studies conducted on the physico-chemical properties of vermiwash and vermicompost.

Vermicompost indicated an increase in nitrogen, potassium and phosphorous and was ranked with the

highest mineral content obtained for mushrooms harvested from this substrate (Fig.9) (Diana *et al.*, 2018). Zang *et al.* (2002) recorded low nitrogen content but an increase in phosphorous and potassium contents were observed for the harvested mushroom from rice straw (Fig. 9). There are not many studies and researches done using vermiwash and vermicompost with vermiwash nutrient analysis of harvested mushrooms.

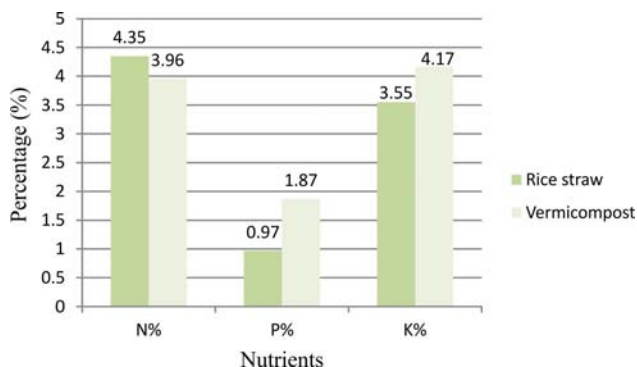


Fig. 9. Comparison of the nutrients from the harvested oyster mushrooms grown using rice straw and vermicompost substrates (Sources: Zang *et al.*, 2002; Diana *et al.*, 2018)

Oyster mushrooms cultivation are becoming more common throughout the world due to their potential to grow at a large range of temperature using different lignocellulose substrates (Pidgeon and Anderson, 1981; Mueller and Gawley, 1983). Diana *et al.*, (2018) reported that nearly all agricultural waste are a form of ideal materials required for the production of mushroom. The results obtained indicated that there are variations in the physical growth parameters of oyster mushrooms (*P. ostreatus*) that are depended on the type of agricultural waste used in the different substrates medium either single or combinations of different

Table 16. Comparison of the soil nutrients of oyster mushrooms substrates grown on the different treatments.

Nutrients	Rice straw	Source	Nutrients	Vermicompost	Vermiwash	Source
N%	1.30	Hala El-Sayed <i>et al.</i> , 2019.	Total nitrogen (%)	1.11	0.02	Ansari,2012.
P%	0.16		Available phosphate (ppm)	597.67	48.86	
K%	0.98		Potassium(ppm)	2428.33	245.67	

agricultural wastes in the preparation process (Chukwurah *et al.*, 2013).

The different agricultural wastes utilized in preparing the substrates yielded mushrooms of significantly different sizes. The highest fresh weight was achieved from the mushroom grown in treatment 2 (vermicompost). Figs. 3-6 indicated that the number of fruiting bodies produced, stipe length and thickness, cap diameter were in support of this treatment with the best growth parameters when compared to the other substrates. Thus, the greater the cap diameter obtained, the higher yield will be produced (Ahmed *et al.*, 2013). The weight of the fruiting body is influenced by the thickness and cap diameter (Ahmed *et al.*, 2013). The yield is correlated to the stalk size and pileus (Samuel and Eugene, 2012). High fresh weights were also recorded from the mushroom grown in the substrate medium of vermicompost with vermiwash, rice straw and vermiwash. The percentage of moisture content ranged from 82.24 to 90.17% with treatment 2 as the highest against the other treatments. The moisture content accounts for the freshness of mushrooms (Gogavekar *et al.*, 2014). Mushrooms contain about 90% water as well as 10% dry matter (Morais *et al.*, 2000; Sánchez, 2010). This was confirmed by Ahmed *et al.* (2013) who reported that *P. ostreatus* grown on wheat bran and sawdust produced fruiting bodies with 90% moisture content (Guillamón *et al.*, 2010). The moisture content of *P. ostreatus* ranged from 88.51 % to 89.88% (Manzi *et al.*, 1999). The mushrooms were cultured and cultivated in a sterilized environment using different agricultural wastes. Mushroom cultivated indoor usually produce higher and more stable yield as indicated on figure 8 (Chang, 1996).

The ANOVA show that there were no significant differences ($p = 0.469$) among the different treatment for the fresh weight, similarly no significant differences ($p = 0.385$) were recorded among the dry weight. Significant differences ($p = 0.044$) and ($p = 4.77 \times 10^{-9}$) with respect to the stipe length and cap thickness

respectively existed between the mushrooms grown in the different treatments, whereas no significant differences ($p = 0.747$) and ($p = 0.54$) with respect to the stipe thickness and cap length respectively existed between the mushrooms grown in the different treatments. Similarly, no significant difference also exists between the number of fruiting bodies yielded among the different treatments whereas the $p = 0.425$ at 5% level. The study accepted the null hypothesis indicating that there is no significant effect of substrate media on growth parameters of oyster mushrooms. However, there are variations of the fruiting bodies of oyster mushroom with respect to its stipe length and cap diameter when grown in different substrates (Shah *et al.*, 2004).

Based on the overall ranking T_2 (vermicompost) was found to be the best substrate followed by treatment T_5 (vermicompost + vermiwash (50 g/ml)), T_1 (rice straw; the control medium) and T_4 (vermicompost + vermiwash (100g/ml)). Vermicomposts contains bacteria, actinomycetes, fungi (Tomati *et al.*, 1987; Edwards and Burrows, 1988) and also cellulose degrading bacteria (Werner and Cuevas, 1996). Cellulose, hemicelluloses and nitrogen are considered as key nutrition needed for oyster mushroom growth and fructification (Diana *et al.*, 2018). Vermicompost contains plant growth promoters such as auxins and cytokinins (Krishnamoorthy and Vajranabhaiah, 1986; Yadav *et al.*, 2017). It comprises of higher nutrients like NPK, soluble calcium, and trace elements necessary to improve plant growth (Sinha *et al.*, 2011; Hussain and Abbasi, 2018). Plant absorbed the essentials micronutrients (manganese, copper, zinc, and iron) from soil sample resulting in producing optimum quantity (Krsihnamoorthy and Vajranabhiah, 1986; Kale, 1998; Ansari and Ismail, 2001; Meena *et al.*, 2015). Vermicompost is known to protect plant without affecting it (Ansari and Jaikishun, 2011; Meena and Yadav, 2015). Similar observations were reported by samba rice cultivation indicated that the addition of vermicompost results in significantly

positive effects on the soil physical, chemical properties and growth parameters of the plant (Tharmaraj *et al.*, 2011). Most researchers have stated that organic inputs (vermicompost) increases crop yield and productivity due to the available nutrients present such as nitrogen, phosphorus and potassium in optimum levels (Ozores-Hampton *et al.*, 1994; Kumar *et al.*, 2017). Diana *et al.*, (2018) reported that highest cap diameter, stipe length and thickness but the smallest cap thickness and number of fruiting bodies was observed on the vermicompost substrates in comparison to the duck manure and cattle dung. In our results, the number of fruiting body produced is not consistent with their findings.

T₁ the control medium had the greatest cap thickness and medium amount of growth parameters. The results obtained are contrasted with Hala El-Sayed *et al.*, (2019) which indicate that there is a significant difference in cap diameter among rice straw, wheat straw, alfalfa hay, bean straw and pea straw. Rice straw had the highest cap diameter, cap weight, fruiting body weight and yield. The chemical constituents of fruiting bodies revealed that rice straw substrates seem to be the best treatment by having increasing percentages of dry matter (DM), phosphorus, potassium and carbohydrates when grown. Zhang *et al.*, (2002) indicated that agriculture wastes (mostly from the rice industry) are ideal substrates for *Pleurotus* cultivation as this is agreement with rice straw substrate as a supporter of oyster mushroom although it did not produce favourable outcomes in this study. Similarly, Bhatti *et al.*, (1987) reported that *P. ostreatus* was successfully grown on wheat straw, cotton waste, maize cobs and rice straw. If a substrate material is used alone for *Pleurotus* mushrooms cultivation, it is sometimes unable to provide sufficient nitrogen and other nutrients become necessary for optimal growth (Siqueira *et al.*, 2012; Soliman, 2011; Soliman *et al.*, 2011). This is in agreement with this current study. Zhang *et al.*, (2002) studied *Pleurotus sajar-caju*

mushroom cultivation in two substrates: rice straw and wheat straw in the forms of grinding and chopping and it was observed that ground straw yielded higher mushroom growth rate than chopped straw. Grinding produced better results because it ruptured the cell walls, making the nutrients in the straw more accessible for mushroom growth. In our study chopped rice straw resulted in the substrates not having good growth parameters. Also, Sharma *et al.*, (2013) reported that rice straw (control) was found as a best substrate with yield followed by rice plus wheat straw, rice straw plus paper waste for the production of mushroom because rice straw contained sufficient amount of necessary nutrients for the growth of *P. ostreatus* and higher degradation of various constituents of the substrate and consumption of high nutrients by *P. ostreatus*. But our study findings are not similar. Even though, its main function is to provide a reservoir of cellulose, hemicelluloses and lignin which is utilized during the growth and fructification, the yield was low in our experiment, although it produced the best cap thickness in comparison to other substrates as shown on Figures 3-8.

Vermiwash contains soluble nutrients that are released during the vermicomposting process by the presence of earthworm (Shield and Earl, 1982; Nath *et al.*, 2009). Although it has rich nutrients, our study showed that the lowest growth parameters were seen in mushroom grown in T₃ along with significantly smaller fruiting bodies (Figures 3-6). Vermiwash is utilized mainly as a liquid nourishment as well as a enzymatic element for nurturing all green plants growth (Gorakh *et al.*, 2009). This contributed to better plant growth and higher yield by releasing nutrients for absorption along with additional nutrients like gibberellin, cytokinin and auxins through the applications of organic materials such as the combination of vermicompost with the vermiwash (Raviv *et al.*, 1998; Subler *et al.*, 1998; Lalitha *et al.*, 2000). Nath and Singh (2012) reported that vermiwash extract from vermicompost of various

mixtures of animal agriculture wastes had significant effect on the growth and productivity of paddy, maize and millet crops at the combination of 10 mg vermiwash with straw and horse dung with gram bran concentration and 30 mg of similar combinations was spray on their crops. Although their study dealt with different substrates, our study is not consistent with their findings. This is probably because they used a low concentration of vermiwash extract from the different animal wastes which was applied as a foliar spray. Vermiwash is found to increased germination rate and improved the growth of seedling at higher dilution in the plants studied. The plants' level of response varied because of the physiology of the plants under consideration and the vermiwash concentration applied has to be standardized to match the plant (Anasri and Sukhraj, 2010). Kumar *et al.*, (2013) reported the application of vermiwash increases growth; flowering and corms yield characters of gladiolus when applied for a long time with recommended fertilizers doses. But this study results were not consistent with our findings. This is probably because spraying the vermiwash twice a week is effective instead of mixing it with the rice straw as it contains the necessary nutrients required for better performance on plant. Vermiwash contains soluble plant nutrients, some organic acids, mucus and microbes, which is effective when used both as a biological fertilizer (foliar spray) and a pesticide (Pramo, 1995; Ismail, 1997; Kale, 1998).

The addition of organic inputs such as vermicompost and vermiwash enhances the soil growth, yield of the plant and improving the soil micronutrient content (Lalitha *et al.*, 2000; Ansari and Sukhraj, 2010). The nitrogen exists in the form of mucus, nitrogenous excretory substances like growth simulating hormones and enzyme (Tripathi and Bhardwaj, 2004). Mushrooms grown using vermicompost with vermiwash (T_5) have good sources of metabolites and vitamins from the B group and pro-vitamin D group which increase the growth

of plant (Tomati *et al.*, 1988; Chauhan and Joshi, 2010). Atiyeh *et al.*, (2001) reported that vermicompost had increased plant productivity when the vermicompost was used at 10-40% of the volume of plant growth medium. The results closely corresponds to the finding by Mahto and Yadav (2005) who reported that the combined use of vermicompost with vermiwash about 5 or 10% produced higher fresh yield and better performance although their study dealt with vegetable pea plant. Also, Esakkiammal *et al.* (2015) observation was similar with our study as they reported that the combination of vermicompost with vermiwash indicated maximum positive effects on the growth and yield even though their study used lablab beans.

Physico-chemical analysis of soil substrates and mushrooms grown on different substrates

There is presence of different types and amount of nutrients and minerals in each of the different agricultural wastes (Diana *et al.*, 2018). Edible mushrooms have the potential to degrade lignocellulosic substrates and even waste produced from agriculture, forest and food processing industries (Baysal *et al.*, 2003; Sánchez, 2010). They produced enzymes like cellulases, amylases, laccases, lignin peroxidase and manganese peroxidase that is released by the fungi to break the large molecules; cellulose and lignin into smaller chemical substances such as organic nitrogen, carbon and mineral which are then simply absorbed by the cell walls as energy and metabolic purpose (Manzi *et al.*, 1999; Mondal *et al.*, 2010; Cotter, 2014; Bustillos *et al.*, 2016).

Vermicompost increases the amount of available water and N (nitrogen), P (phosphorous) and K (potassium), which produced better plant growth (Ismail, 2005a). Vermicompost substrate is formed after the earthworms break down the organic wastes to a humus-like material (Zambare *et al.*, 2008). Thus, the abundant nutrients from the vermicompost are available for the mushroom to use (Diana *et al.*,

2018). Manyuchi *et al.*, (2013) reported that the nitrogen (57%) and potassium (79.6%) contents were observed to be higher in the vermicompost as compared to the vermiwash substrate on corn. Although, this study used a different substrate, similar findings were observed expected with the increased in nitrogen. Similar physiochemical properties of vermicompost and vermiwash were observed by Ismail (2005b) and Lalitha *et al.* (2000). Vermiwash of different animal dung as well as a solid waste contain significant amounts of nitrogen, phosphorus, vitamins and plant hormones, which stimulates the growth of plant (Kaushik and Garg, 2003; Pathak and Ram, 2004; Suthar, 2008).

Zang *et al.* (2002) reported low 35.4 N %, 58.3 P% and 20.1 K % for chopped rice straw substrate as compared to ground substrates nutrient content (42.8 N%, 70.3 P% and 36.7K %), which attributed to our study producing lower yield. The higher absorption of these nutrients from the ground substrate correlated with the higher yield of mushroom. This analysis of rice straw substrate is in agreement by the work done by Hala El-Sayed *et al.* (2019). Mushroom is considered nutritious because it has medicinal properties and help against diabetes, ulcer and lung diseases (Quimio, 1976; Shah *et al.*, 2004). *Pleurotus* are considered a rich source of protein, minerals (Ca, P, Fe, K and Na) and vitamin C, B complex (thiamine, riboflavin, folic acid and niacin) (Çađlarýrmak, 2007; Patil *et al.*, 2010). The fruiting bodies of oyster mushroom are produced by converting the substrate into available nutrient that can be easily utilized by the mushroom (Sánchez, 2010). Mushrooms nutritional value is based on the mineral content (Ahmed *et al.*, 2013).

Diana *et al.* (2018) reported that vermicompost indicated an increase in nitrogen, potassium and phosphorous and based on the rank analysis, the highest mineral content was obtained for mushrooms grown on vermicompost substrate (Fig.9). A higher nutrient content of the mushrooms was because of a

reduction in nutrient content of the soil. The mushrooms harvested from the vermicompost units produced a higher nutrient content will correlated with the reduction in nutrient content of the substrate. Hala El-Sayed *et al.*, (2019) found that the chemical constituents of fruiting bodies revealed that there is an increase in phosphorous and potassium the rice straw substrates but a decrease in the nitrogen as compared to the other substrate when grown and compared in two seasons; 3.31 and 3.02 % in season 2016 and 2017 for nitrogen, 0.93 and 0.98 % in season 2016 and 2017 for phosphorous, 4.00 and 3.96 % in season 2016 and 2017 for potassium. This result do not correlates with the findings observed by Zhang *et al.* (2002) since this study recorded a decrease in the nutrient content for harvested mushroom (Fig. 9). Thus, the lower uptake of these nutrients correlated with low yield of mushroom obtained.

Cultivating mushroom using agricultural waste can play an important role in utilizing organic waste. Globally, there is a built up of organic waste which not only releasing green –house gases but cause pollution as most farmers burn their rice straw. This can be avoided by using vermitechnology that includes vermiwash and vermicompost which are eco-friendly substrates, contains essential nutrients that is necessary for plant growth and less expensive for farmers to use to cultivate mushroom. This technology can also substitute the need for chemical fertilizers by applying vermiwash to plants to help to reduce pests.

CONCLUSIONS

Results suggest *Pleurotus ostreatus* can be successfully grown on any type of agricultural wastes. This study revealed that vermicompost (T₂) substrate showed the best growth parameters (fruiting body, stipe length and thickness and cap diameter) along with better yield and higher nutrients among all the substrates used including the control. This was followed by the combinations of vermicompost with

vermiwash (T₃) and rice straw (T₁) can be substituted for the cultivation of *P. ostreatus* since it produced average amount of growth parameters. Due to the unavoidable COVID pandemic, the nutrient analysis was not conducted at GUYSUCO which would have produced knowledge of the nutrients found in oyster mushrooms and substrates. Difficulties obtained when extracting the nutritional content from literature review since most studies did not use the same treatments like vermiwash and the combinations of vermicompost and vermiwash substrates at different concentrations on mushroom cultivation as our study focused on.

Vermiwash and vermicompost should be prepared using different substrates such as paddy shell, rice bran and wheat straw so as to determine whether these substrates are ideal for the cultivation of mushroom. More research should be conducted to test the effect of vermiwash and vermicompost substrates medium on different types of mushroom species such as the button mushroom (*Agaricus bisporus*). Experiment should be conducted using more than one species of mushroom to provide a comparison of their growth and analysis of their nutritional content using similar treatments. This similar study “Growth and nutritional content of *Pleurotus ostreatus* using different substrates (rice straw, vermicompost and vermiwash) in Guyana” can be repeated to conduct the analysis of the nutritional content of fruiting bodies and soil substrates because this will determine the total amount of nutrients that was utilized in the harvested mushrooms from the substrates.

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