Waste Substrates Used on *Pleurotus spp.* Under Climatic Condition in Zamboanga Sibugay, Philippines

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ABSTRACT

Oyster mushroom in the Philippines was cultivated using paddy rice straw, saw dust and rice hull. The data collected in this study were analyzed using Analysis of Variance (ANOVA) where non-significant data resulted from the Oyster Mushroom average days of pin head initiation to harvest. There were also two significant data and one highly significant. Therefore, it was clearly observed from this study that the comprehensive cropping period of oyster mushroom production has a substantial difference on the different substrates used in growing oyster mushroom. These different substrates used have been proven to be useful for growing oyster mushroom. This will also contribute to an agribusiness-economic initiative for our local farmers, women and youth.

Keywords: Mushroom, *Pleurotus spp*, substrates, farm waste, climate change

1 INTRODUCTION

Mushrooms are fleshy fruiting bodies (Alexopoulos et al., 1996) that are considered one of the delicious fruits, and are commonly produced worldwide (Madbouly and Al-Hussainy, 1996). They are a rich source of carbohydrates, proteins, vitamins, and minerals (Ananbeh, 2003). Mushrooms grow on decayed organic matters rich in lignin, cellulose, and other complicated carbohydrates.

Large quantities of agro-industrial wastes that are produced worldwide often cause environmental and health problems (Garg and Gupta, 2009). In addition, the ever-growing need of cheap nutritious food, and the lack of protein in developing countries led to the development of the mushroom cultivation industry (Sivaprakasam and Kandasawmy, 1981, Levanon et al., 1993, Yildiz et al., 1997, Croan, 2000, Zervakis et al., 2001).

The Oyster mushroom is characterized by its rapid growth on agro-wastes such as olive cake, tomato tuff, pine needles, wheat straw, banana leaves (Ananbeh and Almomany, 2005, Ananbeh and Almomany, 2008 Al-Momany and Ananbeh, 2011), leaf of hazelnut (Yildiz et al., 1997), cotton waste (Oh et al., 2000), maize stover (Fanadzo et al., 2010), palm oil (Rizki and Tamai, 2011) and other wastes. The individual specimens of the oyster mushroom often grow in layers on top of each other (Hoglov, 1999).

Mushroom production technology is not well-established in Saudi Arabia. Very few studies have been conducted to produce mushrooms and other basidiomycetes (Al-Qarawi et al., 2013, Khanaqa, 2006). Different agro wastes are available in huge amount in Saudi Arabia such as windbreak residues, citrus and olive trees pruning, green landscapes, vegetables and fruits, date trees (Sadik et al., 2010), boobialla leaves, sawdust, and wheat straw. Most of these residues are utilized in an inefficient way, but some were successfully used as compost (Al-Barakah et al., 2013, Sadik et al., 2010).

Mushroom plays an important role in the environment by breaking down organic matter to recycle essential nutrients. Mushroom cultivation can also mitigate climate change by sequestering carbon in the so-called “liquid carbon pathway”. According to Colin Averill and Jennifer Bhatnagar (August 03, 2018) “these fungi may not be visible to us, but our research group has found that these mycorrhizal fungi are doing us a huge climate favor behind the scenes. These fungi are climate change warriors, helping forests absorb CO_2 pollution, delaying the effects of global warming, and protecting our planet”.

Mushrooms are also “meat” substitutes”. Kevin Kessler of the *thecookful.com* says that mushrooms also have a flavor known as umami, which is a savory taste found in meat which helps mushrooms be a meat substitute. Mushrooms have been found to be rich sources of protein, lipids, amino acids, glycogen, vitamins and mineral elements (*Okhuoya et al.,2010*). According to *Rambeli (1983*), the mineral salt content of mushrooms is superior to that of meat and fish and nearly twice that of the most common vegetables. The vitamins of mushrooms are not destroyed by cooking, drying and freezing (*Nair, 1982*). Edible mushrooms are recommended by the FAO as food. Mushrooms provide people with high quality proteins, minerals and vitamins. They are highly nutritious and can be compared with eggs, milk and meat. They are easily digestible as they have no cholesterol (*Oei,2003*).

PHILSTAR(2009) reported that the mushroom industry in the Philippines has exacerbated since 1995, and the lowest production volume was 355 metric tons (MT) in 2009. Most of the mushrooms consumed were imported from the different countries of South East Asia like China, Taiwan, Thailand, Malaysia, Korea and Japan. Growing mushrooms in the Philippines is economically feasible due to low-production cost, abundance of cheap substrates from agro-wastes and high demand, which will be profitable to the mushroom growers.

Oyster mushroom in the Philippines were cultivated using paddy rice straw, saw dust and rice hull. These different farm waste used in culturing oyster mushroom offers economic initiatives for our local farmers, farm women and rural young farmers as an introductory step for agribusiness during Covid-19 pandemic and save our mother earth from global warming. For successful mushroom cultivation, three factors must be considered, namely reliable spawn, good substrate and a conducive environment (Rajapakse et al. 2007). According to the review article of Dr. R. Mary Josephine (2014), different kinds of waste have been proven to be useful for oyster mushroom growing. Mushroom cultivation can be a big source of income through rural development program for local farmers if they are aware of its importance, cultivation process and what farm waste substrates could give them higher yields. Therefore, this study used farm wastes in the Island of Olutanga, Zamboanga Sibugay. It aims to assess the production of white oyster mushroom to assess their potentials by using the locally available farm waste substrates.

2 MATERIALS AND METHODS

Study site and Sample Collection

The experiment was conducted at Mabuhay 4-H Club (young farmers) Building, Sitio Hula-Hula, Poblacion, Mabuhay, Zamboanga Sibugay Province,7010. Farm wastes namely: rice straws, rice hulls, weed coconut coir, saw dust were collected from different barangays of Mabuhay, Zamboanga Sibugay. Oyster mushroom spawn was bought at the Mushroom Betinan Research Center of the Department of Agriculture, Region IX, Philippines.

Substrates and Procedures

Treatment 1 (*D.A-1*) is composed of 76 kilograms sawdust mixed with 20 kilograms rice hulls and 1 kilogram of calcium carbonate (agricultural lime). Treatment 2 (D.A.-2) is composed of 70 kilograms rice straw mixed with 26 kilograms sawdust and 1 kilogram lime powder. Treatment 3 (BING) is made of 70 kilograms shredded coconut coir, 25 kilograms sawdust and 2 kilograms agricultural lime powder while the formulation of Treatment 4 (BONG) are 70 kilograms weeds *(30% carabao grass and 70% napier grass*), 26 kilograms sawdust and 1 kilogram lime. Water was added slowly (in the 4 formulated mixtures) until it reaches 40% moisture then the four (4) treatments used were covered with sackolin trapal (lona blue orange) for its fermentation process. Three (3) days after, substrates were remixed to release its excess heat (methane gasses), breakdown the building up of other fungi species, and to assure that all substrates per treatment are well fermented. After remixing of substrates, all treatments are again covered with trapal for continual fermentation. Treatment 1 and Treatment 4 were decomposed for 30 days while Treatment 2 and 3 was decomposed for 7days. After the decomposition process, each treatment was mixed with 2kilograms rice bran and 1-kilogram brown sugar diluted in a 1-liter water as its supplement. One (1) kg of mixed substrates was placed into a polypropylene bag of dimension 7 x 14 with thickness of 0.02mm.

According to Viziteu Gabriel of Romania in his Mushroom Grower's Handbook (2004), oyster mushrooms need substrates abundant in polysaccharides (cellulose, and hemicelluloses) and lignin for their growth. The mycelial growth of oyster mushrooms makes use of soluble carbohydrates, glucose, molasses, organic, nitrogen sources like wheat bran, barley, oat, maize, among others, as well as mineral sources such as ammonium sulphate. In addition, Viziteu Gabriel (2004) added that nutritious substances for oyster mushroom can be categorized into two categories; staples- which are the base of nutritional materials, and additives- which are protein and nitrogen sources. In this study, staples used are rice straws, sawdust, coconut coir, and weeds which are rich in cellulose and hemicellulose. Additives used in this study are rice bran and molasses. The optimal substrate pH value for mycelial growth is 5-6.5, though mycelium can survive between pH 4.2 and 7.5 (Viziteu Gabriel, 2004). Viziteu Gabriel (2004) added that mycelium grows slowly as the pH lowers and stops growing at pH 4.0. Calcium carbonate or known as the agricultural lime was incorporated with all treatments to be able to neutralized acidity during its decomposition process.

Pasteurization and Incubation of substrates

All treatments were pasteurized for 8 hours using a galvanized drum. Inoculation was done inside the isolation room using inoculating needle, alcohol lamp and denatured alcohol. Each bag was inoculated with one (1) tablespoon of oyster mushroom planting spawn and its polypropylene mouth of the bags were closed with clean bond paper and tied with rubber band. Inoculated bags were then kept in the incubation room at a room temperature of 22-28 degree Celsius.

After the four (4) treatments hve been fully colonized with oyster mushroom mycelia, the polypropylene bags were then hanged in the mushroom fruiting area. Tips of the fruiting bags were slashed using clean and disinfected blades to allow oyster mushroom to grow out. Misting was done twice a day - every 9:00 o'clock in the morning and 3 o'clock in the afternoon.

Oyster mushroom mycelial growth (for all treatments used) from the day of inoculation was recorded on a weekly basis. Days to primordial pinhead initiation, three (3) flushing periods and oyster mushroom yields per treatment, percent (%) survivability and percent (%) contamination of *Pleurotus spp* were also documented.

Harvesting

Mushrooms were harvested by twisting the fruiting body from its base. Gross weight was gathered and so with its net weight. Sorted and graded mushrooms were cut using scissor or clean knife and placed in a tray for packaging. Mushrooms were vended through online selling due to the enacted Covid-19 protocols by the Local Government in Mabuhay.

3 RESULTS AND DISCUSSION

The requisite time for the formation of pin-heads is comparable with other studies of (*Ahmed,1998*) which reported that pin-head formation of oyster mushroom cultivated in different substrates is to be between 23 and 27 days from spawning, while (*Fan et al., 2000*) reported it to be 20–23 days after spawning. It was clearly observed from this study that the comprehensive cropping period of oyster mushroom production has a substantial difference on the different substrates used in growing oyster mushroom.

According to (*Khanna and Garcha, 1981*), it may take up-to 104 days to harvest yield from oyster mushroom grown on paddy straw. Another study (*M. Masevhe et al., 2016*), oyster mushroom harvesting will start at 66 days after planting. Above study was inconsistent with the findings of this present analysis. Oyster mushroom using different farm waste substrates will be harvested 21-41 days after spawning based on the study conducted. It takes 2.5 -3 days for an oyster mushroom to be fully matured after pinhead initiation. Based on the observation of this study, oyster mushroom fruiting bodies should be harvested within three (3) days after its pinhead initiation to avoid shrinking and development of light to brown patches discoloration which seriously decrease its quality. Based on the three (3) flushes recorded, the highest yield was harvested from D.A-2 (treatment 2), followed by D.A-1 (treatment 1), BING (treatment 3), while the least was obtained from BONG (treatment 4).

This study indicated that D.A-1 (treatment 1) and BONG (treatment 3) suppressed contamination throughout the production period. According to *Kumari and Achal (2008)*, contamination can be caused by improper pasteurization of substrates used and the availability of contaminants in a substrate. *Sofi et al.* (2014) indicated that nutrient poor substrates exhibited low mycelial densities, making them prone to contamination especially by green mols

1. Determine which farm residues will enhance growth and productivity of *Pleurotus spp.*

Table 1: Percent Productivity of Fruiting bags. 17 days after incubation

	REPLICATIONS			TOTAL	MEAN	CF
TREATMENTS	1	2	3			780.692
Treatment 1	9.45	8.37	7.75	25.57	8.523333	
Treatment 2	9.45	10	7.75	27.2	9.066667	
Treatment 3	8.94	7.75	8.37	25.06	8.353333	
Treatment 4	6.32	6.32	6.32	18.96	6.32	
Grand Total	34.16	32.44	30.19	96.79	34.16	
Grand Mean					32.26333	

Table 1 shows the (%) productivity of oyster mushroom using different substrates per treatment. Table below shows that the formulated Treatment 2 (D.A-2) which composed of 76 kilograms sawdust mixed with 20kilograms rice hulls got the highest percent of oyster mushroom fruiting bags productivity(9.06 %) followed by Treatment 1(D.A-1) with 8.5 % productivity, Treatment 3 (BING) with 8.35% productivity. The least fruiting bag productivity was recorded at Treatment 4 (BONG) with 6.32%. The percent data was transformed using the Square Root Transformation. (*see Appendix Table 4 for the computation of Analysis of Variance*).

2. Compare which among the farm waste substrate will be the best growing media for Pleurotus sp. production.

Table 2 : Total weight (g) of Pleurotus sp. in different medium of fruiting bags. 15 days after incubation.

	REPLICATIONS			TOTAL	MEAN	CF
TREATMENTS	1	2	3			199176.3
Treatment 1	163.75	151.25	98.5	413.5	137.8333	
Treatment 2	264.75	237.25	116.25	618.25	206.0833	
Treatment 3	134.5	106	131.75	372.25	124.0833	
Treatment 4	40.25	36	65.75	142	47.33333	
Grand Total	603.25	530.5	412.25	1546		
Grand Mean					515.3333	

Table 2 shows the total weight of oyster mushroom fruiting body per treatments used. Treatment 2 shows the highest yield with 618 grams followed by Treatment 1 (413g) and Treatment 3 (372g). The least yield was recorded at treatment 4 with 142grams (*see Appendix Table 3 for the computation of its Analysis of Variance).*Average weight of of *Pleurotus sp* in the different medium of fruiting bags can be

seen in the Table 2 of the Statistical Analysis and Appendix Table 2.

4 Statistical Analysis

The results obtained were statistically analyzed using analysis of variants (ANOVA)

Table 1: Average number of Days from Pinhead formation to harvest of Pleurotus sp. 9 days after incubation

	REPLICATIONS			TOTAL	MEAN	CF
TREATMENTS	1	2	3			93.26975
Treatment 1	2	2.75	3	7.75	2.583333	
Treatment 2	2.67	3.625	3	9.295	3.098333	
Treatment 3	3	3	2.33	8.33	2.776667	
Treatment 4	2.75	3	2.33	8.08	2.693333	
Grand Total	10.42	12.375	10.66	33.455		
Grand Mean					11.15167	

Table 2 : Average Weight (g) of Pleurotus sp. in different Medium of Fruiting Bags. 15 days after incubation.

	REPLICATIONS			TOTAL	MEAN	CF
TREATMENTS	1	2	3			5235.118
Treatment 1	18.19	25.21	15.91	59.31	19.77	
Treatment 2	29.42	26.31	23.25	78.98	26.32667	
Treatment 3	19.21	21.2	21.96	62.37	20.79	
Treatment 4	10.062	18	21.92	49.982	16.66067	
Grand Total	76.882	90.72	83.04	250.642		
Grand Mean					83.54733	

Table 3 : Total weight of Pleurotus sp. In different medium of fruiting bags. 15 days after incubation.

	REPLICATIONS			TOTAL	MEAN	CF
TREATMENTS	1	2	3			199176.3
Treatment 1	163.75	151.25	98.5	413.5	137.8333	
Treatment 2	264.75	237.25	116.25	618.25	206.0833	
Treatment 3	134.5	106	131.75	372.25	124.0833	
Treatment 4	40.25	36	65.75	142	47.33333	
Grand Total	603.25	530.5	412.25	1546		
Grand Mean					515.3333	

Table 4: Percent Productivity of Fruiting bags. 17 days after incubation

	REPLICATIONS			TOTAL	MEAN	CF
TREATMENTS	1	2	3			780.692
Treatment 1	9.45	8.37	7.75	25.57	8.523333	
Treatment 2	9.45	10	7.75	27.2	9.066667	
Treatment 3	8.94	7.75	8.37	25.06	8.353333	
Treatment 4	6.32	6.32	6.32	18.96	6.32	
Grand Total	34.16	32.44	30.19	96.79	34.16	
Grand Mean					32.26333	

*Table 5 :*Percent survivability of Pleurotus sp.
15 days after incubation

TREATMENTS	REPLICATIONS			TOTAL	MEAN	CF
	1	2	3			1079.393
Treatment 1	10	10	10	30	10	
Treatment 2	10	10	9.49	29.49	9.83	
Treatment 3	10	10	10	30	10	
Treatment 4	7.01	8.37	8.94	24.32	8.106667	
Grand Total	37.01	38.37	38.43	113.81		
Grand Mean					37.93667	

5 CONCLUSION

This study revealed that D.A-2 (treatment 2) has superior mycelia growth, faster spawn run and better yield among four (4) treatments used. This was followed by D.A-1 (treatment 1) and BING (treatment 3) composed of coconut coir and sawdust. The least mycelial growth and yield was observed in BONG (treatment 4). Moreover, cultivation of oyster mushroom using different farm waste substrates provides multi-disciplinary advantages for our ecosystem as well as the higher living animals on earth. These different substrates used have been proven to be useful for growing oyster mushroom. Therefore, it was clearly observed from this study that the comprehensive cropping period of oyster mushroom production has a substantial difference on the different substrates used in growing oyster mushroom. Lastly, this will also give our local farmers, women and youth an economic initiative for agribusiness to use these farm wastes as a valuable resource to agriculture and in climate change mitigation.

6 APPENDICES

Fig. 1: Experimental Plot Layout and Randomization using Draw Lots.

Lot 1 T3R2	Lot 2 T2R3	Lot 3 T2R2
Lot 4 T1R2	Lot 5 T3R1	Lot 6 T2R1
Lot 7 T1R1	Lot 8 T1R3	Lot 9 T3R3
Lot 10 T4R2	Lot 11 T4R1	Lot 12 T4R3

Fig.1. Above figure shows the CRD Experimental lay-out

Table 1. Average number of Days from Pinhead formation to harvest of Pleurotus sp.
9 days after incubation

Analysis of Variance						
Source of Variance	DF	SS	MS	Computed F	Tabular F Value 5%	1%
			0	0	4.07	7.59
Treatment		0.441856	0.147285	0.764763		
Error		1.540717	0.19259			
Total		1.982573				
	cv	3.935289				

Table 2: Average Weight (g) of Pleurotus sp. in different Medium of Fruiting Bags. 15 days after incubation.

Analysis of Variance						
Source of Variance	DF	SS	MS	Computed F	Tabular F Value 5%	1%
			0	0	4.07	7.59
Treatment	4	146.1269	48.70896	2.723936		
Error	8	143.0547	17.88183			
Total	11	289.1816				
	cv	5.061432				

Table 3. Total weight of Pleurotus sp. in different medium of fruiting bags.
15 days after incubation.

Analysis of Variance						
Source of Variance	DF	SS	MS	Computed F	Tabular F Value 5%	1%
			0	0	4.07	7.59
Treatment	4	38140.13	12713.38	6.399065		
Error	8	15894.04	1986.755			
Total	11	54034.17				
	cv	8.649359				

Table 4. Percent productivity of Fruiting bags. 17 days after incubation

Analysis of Variance						
Source of Variance	DF	SS	MS	Computed F	Tabular F Value 5%	1%
			0	0	4.07	7.59
Treatment		13.02469	4.341564	7.030303		
Error		4.9404	0.61755			
Total		17.96509				
	Cv	2.435717				

Table 5. Percent survivability of Pleurotus sp. 15 days after incubation

Analysis of Variance						
Source of Variance	DF	SS	MS	Computed F	Tabular F Value 5%	1%
			0	0	4.07	7.59
Treatment	4	7.647825	2.549275	9.530594		
Error	8	2.139867	0.267483			
Total	11	9.787692				
	cv	1.363293				

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