Alternatives Substrates for the Production of Pleurotus ostreatus (Oyster Mushroom)

ELIZABETH ANGELI PORTILLO[1], LEONILA ROSE BENIGNO[1], MA. SOPHIA BUENAOBRA[1] and CATHERINE JOY MEDIODIA[1]

[1] *Philippine Science High School Western Visayas Campus - Bito-on, Jaro, Iloilo City 5000, Department of Science and Technology, Philippines*

Abstract –This study aims to use agricultural wastes rice stalk, bran and husk as alternative substrates for Pleurotus ostreatus. The study was done at Western Visayas Integrated Agricultural Research Center, in ratios of 50:50 and 100:0, wastes to sawdust respectively. After harvest, the masses were weighed, the caps counted, and the biological efficiency was measured. Rice stalk treatments had the highest biological efficiency while rice bran and hull treatments did not produce any caps for the first flush. The dry mass post hoc results showed significant difference amongst all substrates, proving that rice stalk treatments have the highest biological efficiency and are most compatible with *P. ostreatus.*

Introduction. – Oyster mushrooms are commonly cultivated due to its rich nutrient content and various medicinal properties. It needs shorter growth time and utilizes industrial and agricultural wastes as substrates. It is low cost production as it does not require much resources [1].

Pleurotus ostreatus is the second most widely cultivated edible species worldwide. *P. ostreatus* takes a relatively shorter time to grow than other edible mushrooms. Along with it having little requirements for growing and a lesser chance of being diseased makes growing *P. ostreatus* easier and cheaper [2].

In most Asian countries rice is a major crop, and rice bran, rice husk and rice stalk are easy to purchase and are cheap as these are considered wastes. Mushrooms breakdown organic materials which other organisms cannot decompose. The mycelia produce enzymes that breakdown and use lignocellulosic wastes, usually used for biofuel, including agricultural wastes such as corn husks, rice stalks, wheat, and other organic matter [3].

This study aims to investigate alternative mushroom substrates using the agricultural wastes rice stalk, bran, and husk for mushroom production. The study aims to: (i) compare the growth of *Pleurotus ostreatus* based on fresh and dry mass, and number of caps and (ii) obtain the biological efficiency, of each alternative substrate.

Significance. This is a sustainable project that can be done at home, as rice production wastes rarely used for other than compost and feeds are utilized to reduce total waste and add to income by growing food. *Pleurotus* sp. are low-maintenance and require carbon sources provided by substrates that can be found in agricultural wastes.

Limitations. Limitations. This study will be limited to the use of alternative media from the agricultural wastes in the production of rice. The mushrooms to be grown will be *Pleurotus ostreatus*, the spawn obtained from West Visayas Agricultural Research Center.

Methods. – The study aims to use agricultural rice wastes as alternative substrates for production of *Pleurotus ostreatus* mushroom. This includes the procedures from preparing the substrates until the disposal of spent bags and statistical analysis.

Materials and equipment used. For the preparation of substrates, we have the wastes: rice stalks (RS), rice bran (RB), rice husk (RH), and sawdust (SD) with plastic bags as substrate bags and inoculated corn kernels.

Acquisition of mushroom spawn. The mushroom spawn was obtained from West Visayas Integrated Agricultural Research Center in Hamungaya, Jaro, Iloilo City.

Preparation of the area. The room was sprayed with disinfectant spray and the temperature was controlled using an air conditioning unit.

Preparation of substrates. For the preparation of substrate mixture, the total mass of each fruiting bags was 750 grams, comprised 98 percent of the alternative substrate with 1 percent molasses and 1 percent lime. Each treatment had five replicates to account for mushroom mortality.

There are 7 treatments: (i) the control, (ii) 100% rice stalk, (iii) 100% rice bran, (iv) 100% rice husk, (v) 50% rice stalk and 50% sawdust, (vi) 50% rice bran and 50% sawdust, and (vii) 50% rice husk and 50% sawdust. The control was based on substrate mixture ratio used by WESVIARC.

Bagging of substrates and sterilization. After mixing, the substrates were placed in plastics bags using a cylindrical metal mold. After filling the bag, the mold was removed and then a piece of polyvinyl chloride pipe was placed there to be a pathway for the spawn to go inside the bag. Cotton was then placed in order to avoid contamination, and another piece of plastic was used to cover it.

Inoculation of spawn. The spawn was divided equally among all the substrates and their trials and mixed thoroughly.

Observation period. The mushrooms are ready for harvest if their caps turn up from the outer corners. To remove the mushroom, it should be twisted from the base and pulled.

The parameters measured were the caps, fresh and dry mass. The biological efficiency was also calculated.

Caps. The number of caps in each fruiting bag was counted manually.

Fresh and dry mass. After harvesting, the mushroom was weighed using a weighing scale and the gram unit. The mushrooms were then air dried for 2 days and the dry mass was obtained.

Biological efficiency. Determined by the following equation [4]: BE =(dry weight of harvest / weight of dry substrate)x100

Disposal. After the data gathering, the substrates are disposed by vermicomposting.

Statistical analysis. The ANOVA test was used to compare all the means of the samples of each trial for every substrate to find out if there is a significant difference in terms of physical data gathered from the experiment.

Results. – This chapter contains the data obtained from the experiment, its statistical analysis, and significance. Using agricultural wastes such as rice stalk, bran, and hull as alternative substrates for *P. ostreatus* culture, the biological efficiency of each set-up was determined.

Table 1: Mean Summary of Results from *P. ostreatus* Harvested from Treatments (N=15)

Treatment	Fresh Mass (in grams) $^{\alpha}$	Dry Mass (in grams)	Number of Caps $^{\alpha}$	Biological Efficiency (%) $^{\alpha}$
Control	5.7	2.5	2.7	22.5
Rice Straw	42.0 $^{\beta}$	9.7	18.4 $^{\beta}$	35.0 $^{\beta}$
Rice Hull	0	0	0	0
Rice Bran	0	0	0	0
Rice Straw and Sawdust	22.8	6.7	11.2	33.3
Rice Hull and Sawdust	0	0	0	0
Rice Bran and Sawdust	0	0	0	0

$^{\alpha}$ - ANOVA test significant at 0.05
$^{\beta}$ - LSD Post-Hoc test significant at 0.05

One reason that rice straw has the highest fresh mass is due to the number of substrate bags that fruited. There are 15 fruiting bags in each treatment and none of which were contaminated, resulting in more yield produced, compared to the other treatments wherein there are fewer substrate bags. Rice straw is the most effective among the three substrates since it has the greatest mushroom growth based on the masses of the harvest and the amount of fruiting bags that has growth in it.

The ANOVA statistical test showed that the treatments have a significant difference with each other since the p-value is less than the alpha value 0.05, this means that the weight of the harvest per treatment differs and is not near equal with the others.

Since there is a significant difference between the treatments, a post hoc test was conducted. With the p-value of 0.03, rice straw was significantly different compared to the other treatments. It is the most effective among the three followed by sawdust and rice straw and then the control treatment.

Dry mass. The pure rice straw has the highest mean with 9.7 grams, followed by the rice straw and sawdust with 6.7 grams, and the control with the lowest mean of 2.5 grams.

The mean dry mass of the rice straw substrate is slightly higher than the other treatments since this treatment has the highest amount of fruiting bags that bloomed and most of the harvest outweighs the other mushrooms in the study. Based on the results in the fresh mass, the rice straw substrate will also have the highest dry mass since its fresh mass is also higher than others. It is then followed by the rice straw and sawdust substrate and then the control treatment. Since the p-value is higher than the alpha value, there is no significant difference in the dry masses of the treatments, this means that the dry masses of the harvest are almost the same with each other.

Number of caps. The pure rice straw has the highest mean with 9.7 grams, followed by the rice straw and sawdust with 6.7 grams, and the control with the lowest mean of 2.5 grams.

The mean dry mass of the rice straw substrate is slightly higher than the other treatments since this treatment has the highest amount of fruiting bags that bloomed and most of the harvest outweighs the other mushrooms in the study. Based on the results in the fresh mass, the rice straw substrate will also have the highest dry mass since its fresh mass is also higher than others. It is then followed by the rice straw and sawdust substrate and then the control treatment.

The null hypothesis was accepted as the p-value is higher than the alpha value, there is no significant difference in the dry masses of the treatments. The dry masses of the harvest are almost the same with each other because they do not have a great difference.

Biological efficiency. The mass of the fresh harvest was divided over the mass of the dry substrate multiplied by 100 to calculate the biological efficiency of the the substrate treatment. Pure rice straw has the highest biological efficiency mean with 35%, followed by rice straw and sawdust with 33.3%, and the lowest with 22.5% from the control.

Based on the average biological efficiencies of the treatments, the rice straw treatment, which is the pure rice straw treatment, has the highest biological efficiency out of all the substrates, followed by the rice straw and sawdust treatment. Sawdust as part of a substrate is lacking since it is generally low in protein which is necessary in the cultivation of mushroom and should have undergone composting to effectively breakdown its cellulose and lignin components that aids in the releasing of essential nutrients for the mushroom [5].

This is also supported by the study of Girmay et al. (2016), which used sawdust as part of their treatment and garnered the lowest harvest weight and biological efficiency out of all their treatments [6].

The ANOVA results showed the p-value to be 0.032, which is less than the significant value of 0.05, meaning there is a significant difference in the biological efficiency of the treatments. This means that different substrates produce different biological efficiencies, therefore those with lower yields are less compatible in growing P. ostreatus and those with higher yields are more compatible with growing these species.

The post hoc test results showed that treatment B has a significance level of 0.009 which is lower than the alpha value, therefore the pure rice straw substrate has a significant difference from the other treatments. Comparing their compatibility, treatment B has the highest compatibility followed by treatment E and then the control.

Out of the 105 fruiting bags prepared, 50 of those were contaminated with green, yellow, and black molds. Trichoderma spp. is known to be the cause of the green mold disease in mushroom, mostly on the spawn running period. These molds usually affect the lignocellulosic substrates because it degrades cellulosic materials, examples of these are the substrates used in the study: rice stalk, rice bran, and rice hull [7].

Only three substrate treatments bore fruiting bodies: the control group, rice stalk and sawdust treatment, and the pure rice stalk treatment. The data shows that both rice stalk treatments have higher biological efficiency than the control.

Discussion. – After 2 days of air-drying, at least 70% of the total weight of the harvest was lost and this was the result of the drying up of water or moisture from the mushroom.

Treatment of rice hull and sawdust was fully wiped out by the mold with no visible signs of the inoculated mycelium spreading in the substrate, while treatments of pure rice hull, pure rice bran, and rice bran and sawdust showed no signs of primordial growth at all. The rice bran groups must have been too compact for the inoculated corn kernels to grow. The absence of growth can be possibly due to the rice bran and rice hull having too little cellulose in them for the mushrooms to feed on compared to the rice straw. Rice straw has been used in previous studies in growing P. ostreatus, such as in the studies of Kungu n.d.[7], Dundar et al 2008 [8], Girmay et al 2016 [6], Moonmoon et al 2010[1], and Yang et al 2013[9].

Rice hull has been used effectively as an additive when growing P. ostreatus in the study conducted by Frimpong-Manso et al 2011[10]. It was recommended to be used only as an additive as it was quick to dry up and lose moisture, and this is a possible reason as to why there was no observed growth in both the sawdust treatments.

According to the study of Starnets and Chilton 1983, airborne contaminants are the primary cause of culture that uses agar or grain by which corn kernels are considered grain culture [11]. In the case of the treatments, pure rice bran and rice bran and sawdust, that did not show any signs of growth, one of the possible causes could be the lack of airing. The fruiting bags contents were compact with the inoculated kernels not able to penetrate the material, and due to the brans powdery property, the mycelia did not survive. A study by Yildiz et al. (2002) stated that once the concentration of the rice bran exceeds 25%, the risk of contamination is increased [12]. This a possible reason for the causes of contamination of the substrates that used rice hull and why there was no growth.

Conclusion. – With the use of one-way ANOVA test, it was proven that there is a significant difference in the biological efficiency which measures the compatibility of the substrates with the mushroom. The rice stalk groups (B and E) proved to have the highest biological efficiency and thus the best compatibility for growing *P. ostreatus*. Meanwhile, the rice bran treatments (D and G) and rice hull (C and F) barely went past the spreading of the mycelium and were unable to produce fruits in time with the first flush.

* * *

We would like to thank the following for their help in the conduct of our study:

Department of Agriculture Regional Office,

Western Visayas Integrated Agricultural Research Center especially to Mr. Eden Panisales and the Mycology Department,

and to our paper reviewers, Ms. Lovie Aguaras, Mrs. Catherine Joy Mediodia, Mrs. Virna Navarro, Mrs. Rowena Labrador, Ms. Justine Golingay, Mr. Harold Mediodia, and Mr. Oliver Fuentespina.

REFERENCES

[1] Moonmoon M, Uddin N, Ahmed S, Shelly NJ, and Khan A., *Cultivation of different strains of king oyster mushroom ()Pleurotus erynjii) on sawdust and ricestraw in Bangladesh*, Vol. **17** (Saudi Journal of Biological Sciences) 2010, p. 341345.

[2] , *Cultivation of(Pleurotus ostreatus) and other edible mushrooms on sawdust and ricestraw in Bangladesh*, Vol. **85** (Appl Microbiol Biotechnol) 2010, p. 13211337.

[3] Dundar A, Acay H, and Yildiz A., *Effect of using different lignocellulosic wastes for cultivation of (Pleurotus ostreatus) on mushroom yield, chemical composition, and nutritional value*, Vol. **8(4)** (African Journal of Biotechnology) 2008, p. 626666.

[4] Liang C-H. et al., *Biological efficiency and nutritional value of the culinary-medicinal Auricularia cultivated on sawdust basal substrate supplement with different proportions of grass plants.*, Vol. **85** (Saudi Journal of Biological Sciences) 2016

[5] Obodai M, Sawyerr LCB, and Johnson PNT., *Yield of seven strains of oyster mushrooms ((Pleurotus ostreatus)) grown on composted sawdust of Triplochiton scleroxylon.*, Vol. **40** (Tropical Science) 2010, p. 9599.

[6] Girmay Z, Gorems W, Birhanu G, and Zewdie S, *Growth and yield performance of (Pleurotus ostreatus) Kumm (oyster mushroom) on different substrates.* (AMB Express) 2016

[7] Kungu J., *Trichoderma species. Canada: Mold and Bacteria Consulting Laboratories.* (https://www.moldbacteria.com/mold/trichoderma.html) 2018

[8] Dundar A, Acay H, and Yildiz A. , *Yield performances and nutritional contents of three oyster mushroom species cultivated on wheat stalk.*, Vol. **7(19)** (African Journal of Biotechnology) 2008, p. 34973501.

[9] Yang WJ, Guo FL, and Wan ZJ., *Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cottonseed hull.* , Vol. **20** (Saudi Journal of Biological Sciences) 2013, p. 333338.

[10] Frimpong-Manso J, Obodai M, Dzomeku M, and Apertorgbor MM., *Influence of Rice Husk on Biological Efficiency and Nutrient Content of (Pleurotus ostreatus) (Jacq. ex. Fr.) Kummer*, Vol. **18** (International Food Research Journal) 2011, p. 249254.

[11] Starnets P and Chilton J.S. 1983. , *The Mushroom Cultivator.* (http://library.uniteddiversity.coop/Permaculture/Mushroom_Cultivator- A_Practical_Guide_to_Growing_Mushrooms_at_Home.pdf)

[12] Yildiz S, Yildiz UC, Gezer ED, and Temiz A. , *Some lignocellulosic wastes used as raw material in cultivation of the Pleurotus ostreatus culture mushroom.*, Vol. **38** (Process Biochemistry.) 2002, p. 301306.