AGRICULTURAL CROP RESIDUES: ITS POTENTIAL AS NUTRIENT MEDIA IN *IN-VITRO* CULTURE OF OYSTER MUSHROOM (*Pleurotus ostreatus)*

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Abstract—

The disposal of agri-waste is one of the most pressing problems facing the Philippines today. Farmers have chosen to burn agricultural waste as a disposal method, which pollutes the environment and leads to climate change. A study was conducted to determine the potential of different crop residues as agar-based nutrient media for the in-vitro culture of Oyster Mushroom (*Pleurotus ostreatus).* The study was lay-outed using Completely Randomized Design (CRD) replicated three times with 7 treatments include Water Hyacinth Agar (WHA), 50% Banana Leaves and 50% Bracts Agar (BLBA), 50% Rice Straw and 50% Hull Agar (RSHA), Palm Oil Empty Bunch Agar (POEBA), 50% Corn Cobs and 50% Husk Agar (CCHA), Coconut Coir Agar (CCA) with Potato Dextrose Agar (PDA) as control. The different media were prepared aseptically in the laboratory, and isolation and data collection took place 7-14 days later. Results showed that crop residues from Water Hyacinth, Banana Leaves and Bracts, Rice Straw and Hull, Palm Oil Empty Bunch, and Coco Coir were found to be a good substitute media for oyster mushroom development, as the substrate promotes oyster mushroom growth and colonization in in-vitro culture. However, since it colonized the media in such a short time, Palm Oil empty bunch may be one of the best alternatives for PDA.

Keywords: **Crop Residues, Nutrient Media, Oyster Mushroom**

I. INTRODUCTION

The disposal of agri-waste is one of the most pressing problems facing the Philippines today. Farmers have chosen to burn agri-waste as a means of disposal. Major abandoned biomass producing regions were identified for the different biomass resources namely, corn cobs, rice hull, sugarcane bagasse, coconut residues, animal manure, forestry wastes, urban refuse and other cellolusic materials (Baconguis, 2007).

Biomass burning is considered a carbon-neutral operation, it does produce the toxic greenhouse gases CH4 and N2O (Gadde et al., 2009) and up to 100% nitrogen (N) is lost, 25% phosphorous (P) is lost, 20% potassium (K) is lost, and 5–60% sulfur is lost (Romasanta et al., 2017).

SOCKSSARGEN Region is known to grow rice, corn, coconut, banana and palm oil. In addition, it has lakes where water hyacinth is fast growing. According to Zafar (2020) almost 70% of the fresh fruit bunches are turned into wastes in the form of empty fruit bunches, fibers and shells, as well as liquid effluent. While 158.797 tons waste of rice hull and 377.01 tons of coco-coir in a year in the Philippines (Baconguis, 2007).

These agricultural wastes can be recycled by making the most of their ability as artificial media in mushroom cultivation. While Potato Dextrose Agar is the most popular media for isolating mushrooms, there are many agri-wastes in the area that could be suitable for mushroom growth.

Mushrooms are edible fungi known as vegetables that are high in riboflavin, potassium, vitamin D, selenium, and other nutrients that are good for human health. The findings of clinical and preclinical research on edible mushroom consumption indicate that eating them can help with good immunity, weight loss, and overall health. Furthermore, mushroom consumption may lower the risk of diseases like prostate cancer and breast cancer. (Market Research Report, 2018).

Hence, this study was conducted to determine the potential of different crop residues as agar-based nutrient media for the in-vitro culture of Oyster Mushroom (*Pleurotus ostreatus).*

II. METHODOLOGY

A. Experimental Design and Treatment

The study was laid out in a Complete Randomized Design (CRD) with three replications. The treatments were as follows:

T1- Control Potato Dextrose Agar (PDA)
T2- Water Hyacinth Agar (WHA)
T3- 50% Banana Leaves and 50% Bracts Agar (BLBA)
T4- 50% Rice Straw and 50% Hull Agar (RSHA)
T5- Palm Oil Empty Bunch Agar (POEBA)
T6- 50% Corn Cobs and 50% Husk Agar (CCHA)
T7- Coconut Coir Agar (CCA)

B. Media Preparation

The various agri-wastes were obtained from various farm areas in Lutayan, Sultan Kudarat, Philippines. These were washed with tap water, and 300 g of media was used per treatment, with 150 g for treatment combinations, for a total of 300 g. After that, they were boiled for 15 minutes in a liter of distilled water. The liquid was sieved and 20 g agar and 20 g white sugar was added. To achieve the desired fineness of the agar, these mixtures were thoroughly mixed in a blender. The extract was cooked until it became sticky. This was then moved to a bottle with a cotton plug and foil covering. After that, the bottles were sterilized in a 15 psi autoclave for 15 minutes. Three duplicates of each treatment were then dispensed onto petri plates.

C. Isolation of Pleurotus ostreatus

The *P. ostreatus* were sliced into small squares and each square was isolated at the center of the petri plates with different media. These were held in a dark room temperature until they were completely colonized. After three days of isolation, the diameter of the mycelium expansion was measured every day before complete colonization.

D. Measurement of Mycelial Growth

The mycelial growth of *P. ostreatus* in the different media were measured using Vernier caliper starting from 3 days after isolation until full colonization.

III. RESULTS AND DISCUSSION

E. The zone of growth (mm) of P. ostreatus in different nutrient media

The mean diameter (mm) of zone growth of *P. ostreatus* on different nutrient media during 3rd, 6th, 9th and 12th days after incubation is presented in Table I.

TABLE I.
MEAN DIAMETER (MM) OF ZONE GROWTH OF *P. OSTREATUS*

Treatments	3rd	6th	9th	
T1-Control (PDA)	0.23^{bc}	1.73^{a}	3.30^{a}	4.50^{a}
T2-WHA	0.28^{bc}	1.23^{ab}	2.76^{c}	4.50^{a}
T3-BLBA	0.26^{bc}	1.71^{a}	3.57^{a}	4.50^{a}
T4-RSHA	0.19^{bc}	1.08^{ab}	2.07^{d}	3.57^{b}
T5-POEBA	0.62^{ab}	1.86^{a}	3.25^{ab}	4.47^{a}
T6-CCHA	0.00^{c}	0.35^{b}	0.51^{e}	0.51^{c}
T7-CCA	0.79^{a}	1.69^{a}	2.91^{bc}	3.62^{b}
CV (%)	2.1	2.2	0.6	0.1

After three days of incubation, significant difference among treatment was observed with T7 (Coco Coir Agar-CCA) obtained the largest zone growth with a mean of 0.79 mm, which was statistically comparable to T5 (Palm Oil Bunch Agar-POEBA) with a mean of 0.62 mm. This was followed by T2 (Water Hyacinth Agar-WHA); T3 (Banana Leaves and Bracts Agar-BLBA); T1 (Potato Dextrose Agar-PDA); and T4 (Rice Straw and Hull Agar-RSHA) with mean diameter of 0.28, 0.26, 0.23, and 0.19 mm, respectively. No growth was observed in T6 (Corn Cobs and Husk Agar-CCHA).

At 6th days of incubation, result showed significant difference from each treatment used. The T5 obtained the largest mycelial growth with a mean of 1.86 mm, which was comparable with T1 the standard check; T3 and, T7, T2 and T4 with a mean of 1.73, 1.71, 1.69, 1.23 and 1.08 mm, respectively. The T6 had the smallest mycelial growth with a mean of 0.35 mm.

On the 9th day of incubation, result showed highly significant difference among treatment tested. *P. ostreatus* in T3 obtained the largest mycelial growth with a mean diameter of 3.57 mm, which was comparable with that of T1 Control and T5 with a mean of 3.30 and 3.25 mm, respectively. This was followed by T7 with a mean of 2.91 mm. The *P. ostreatus* isolated in the T6 obtained the smallest mycelial growth with a mean of 0.51 mm.

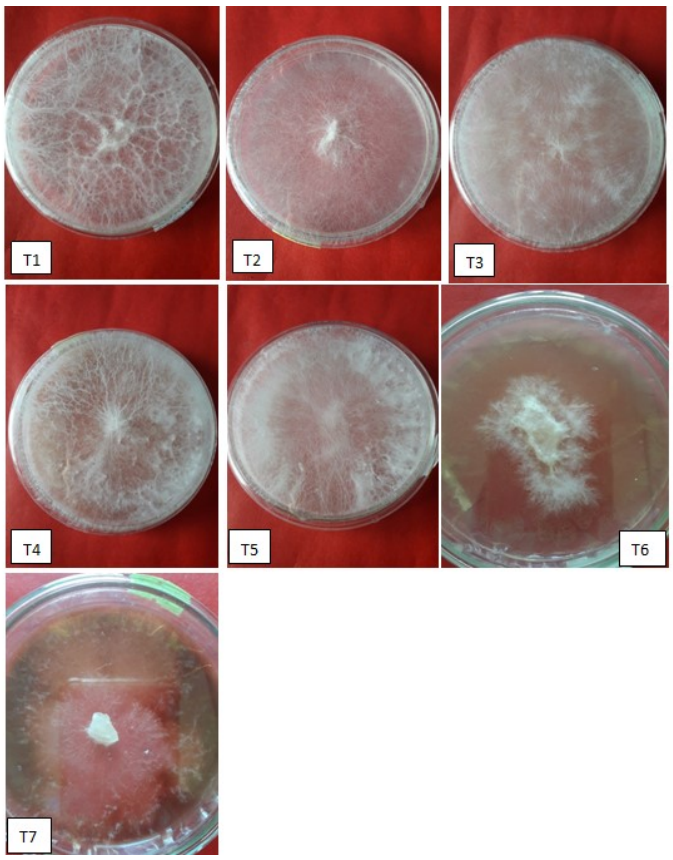
During the 12th days of incubation, there was highly significant difference observed among treatments in terms of mycelial growth. T1 (Potato Dextrose Agar), T2, T3 obtained the largest growth with 4.50 mm, which was comparable with T5 with a mean of 4.47 mm. This was followed by T7 with a mean of 3.62 mm, which was comparable to Treatment 4 with a mean of 3.57 mm. The shortest zone of mycelial growth was observed in T6 with a mean diameter of 0.51 mm (Figure 1).

This implies that using either the five different crop residues media like Water Hyacinth Agar, Banana Leaves and Bracts Agar, Rice Straw and Hull Agar, Palm Oil Empty Bunch Agar, Coco Coir Agar, is a good substitute media for PDA (Potato Dextrose Agar) in In-vitro culture of *P. ostreatus.*

This result is in conformity with the study of Adjapong et al. (2015) who mentioned that mushrooms are cultivated successfully using lignocellulosic wastes as substrates. According to Hoa and Wang (2015) the main factors that affected mycelium growth of mushroom include culture media, temperature, carbon and nitrogen sources, grain sources and lignocellulosic substrate sources. Moreover, oyster mushroom can be grown in various substrates including paddy straw (Adjapong, et al., 2015), abundant wastes from the oil palm plantation (Sudirman, et al., 2011), coconut coir substrate (Amin, et al., 2010), water hyacinth (Quimio, 1995), banana leaves (Tuazon, 2013). Thus, it is thought that combining some parts of each agri-wastes can add to the favorable growth of mushroom.

However, Corn Cobs and Husk Agar in T6 unfavorable growth conforms to the study of Hancock (2009) who stated that corn stover (stalks, leaves, cobs, shucks and left over grain after harvesting) are quite low in quality and has low digestible energy and protein.

FIGURE 1
MYCELIAL GROWTH OF *P. ostreatus* 12th DAYS AFTER INCUBATION IN DIFFERENT NUTRIENT MEDIA



F. Number of days to full colonization of P. ostreatus

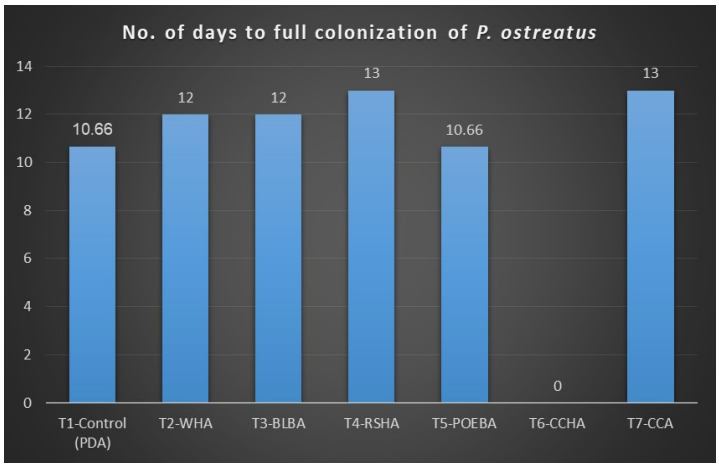
A result on the number of days to full colonization of P. ostreatus is presented in Graph 1.

Results showed highly significant differences among treatment means with T1 (PDA) and T5 (POEBA) had the shortest days to full colonization with a mean of 10.66 days. These were followed by T2 and T3 with a mean of 12 days; and T4, T7 with a mean of 13 days. No colonization observed in T6 (CCHA) until 12 days of observation period.

This implies that T5 (POEBA) is a best substitute media of T1 (PDA) in growing *P. ostreatus.* This can be attributed to the composition of palm oil residual biomass which has fibers and substances that are rich in protein, lipids, carbohydrates and minerals that can nourish edible mushrooms, this is according to Marcos Enê Oliveira (Brazillian Agricultural Research Corporation, 2018)

On the other hand, the zero colonization in CCHA in T6 can be attributed to the low quality and low digestible energy and protein on corn stover Hancock (2009).

GRAPH 1
NUMBER OF DAYS TO FULL COLONIZATION OF *P. ostreatus* IN THE DIFFERENT



IV. CONCLUSION

Different agar-based nutrients from agricultural crop residues, such as Water Hyacinth, Banana Leaves and Bracts, Rice Straw and Hull, Palm Oil Empty Bunch, and Coco Coir, can be a good substitute nutrient media for oyster mushroom production, since the substrate favors the growth and colonization of oyster mushroom in in-vitro culture. Furthermore, this is less costly than using a PDA. Palm Oil Empty Bunch, on the other hand, could be one of the best alternatives for Potato Dextrose Agar because it colonized the media in the shortest amount of time.

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