Sea Lettuce *(Ulva Lactuca)* as Potential Substrate Conditioner in Oyster Mushroom *(Pleurotus Ostreatus)* Cultivation

FAJARDO, ERBORNE G.[1], RAVAL, PRECIOUS ADRIANNA MARIE C.[2], BASSAR, SHARMAINE G.[3], BOOK, RENZ JERVY A.[4]

[1]*Fajardo, Erborne G., Ateneo de Zamboanga City*

[2]*Precious Adrianna Marie C, Ateneo de Zamboanga City*

[3]*Bassar, Sharmaine G., Western Mindanao State University*

[4]*Book, Renz Jervy A., Western Mindanao State University*

Abstract*— An experimental research study using the completely randomized design aimed to determine the potential of sea lettuce (Ulva lactuca) extract as a substrate conditioner in oyster mushroom (Pleurotus ostreatus) cultivation. Specifically, it sought to find out the significant difference among the different treatments as substrate conditioner in terms of the number and weight of oyster mushroom fruiting bags and was compared to water. There were four (4) experimental treatments [T1(1:1), T2(2:1),T3(1:2) of sea lettuce extract is to water and T4(pure sea lettuce extract)] and one control set-up T0(water) prepared which were sprayed equally to the fruiting bags containing 5 spawns of oyster mushroom with 5 replicates. The findings indicated that T3 obtained the highest Mean value in terms of number and weight of oyster mushroom. One Way Analysis of Variance (ANOVA) revealed that the decision is to reject the Null hypothesis; means that there is a significant difference among the four (4) treatments. The result implies that the treatments have varying effects as substrate conditioner. Post-Hoc Test (Tukey) identified T3 as the most significant variant. Whereas independent samples T-test revealed that there is a significant difference between T3 compared to T0 as substrate conditioner. The result implies that T3 is better than T0 as substrate conditioner in oyster mushroom cultivation in terms of number and weight of oyster mushroom fruiting bodies. It is suggested that sea lettuce extract substrate conditioner can be used as substitute for chemical fertilizers to improve the sustainable mushroom growth and yield. Furthermore, this study emphasizes the application of sea lettuce extract substrate conditioner that can be effectively used as eco-friendly approach to do mushroom cultivation industry.

***Indexed Terms*— oyster mushroom (Pleurotus ostreatus), sea lettuce (Ulva lactuca) extratct, substrate conditioner, mushroom cultivation.**

INTRODUCTION

Food insecurity emerges as one of the world's biggest dilemma. This is present in poor countries that usually have inadequate food production systems and suffer from vital malnutrition [1]. To lessen hunger and malnutrition, mushroom cultivation is a very reliable and profitable option [2]. Moreover, mushroom cultivation helps reduce vulnerability to poverty and strengthens livelihoods through the generation of a fast yielding and nutritious source of food and a reliable source of income [3]. However, this mushroom cultivation and consumption culture is only more developed in China, Japan, Thailand, America and Korea [4]. In the Philippines, one of the difficulties encountered by innumerable mushrooms farmers is the limited cultivation due to the lack of local knowledge about its culture [5].

Usage of different inorganic fertilizers, pesticides, insecticides has damaged the soil ecosystem extensively. This kind of practice makes the soil environment unsuitable for crop growth in future [6]. As the disadvantages of chemical fertilizers are becoming more apparent, farmers are slowly but surely turning towards organic fertilizer [7]. Due to this

hazards, consumer`s preferences are shifting towards organic farming, organic manure and fertilizers [8].

Large amounts of sea lettuce are deposited along the shores of Cawa-Cawa, R.T. Lim Boulevard, Zamboanga City. These produce unpleasant odour along the shores which may create anoxic condition to the marine organisms and to people [9]. Hence, the proponent of this research study envisioned the potential of sea lettuce *(Ulva lactuca)* extract as substrate conditioner in cultivating oyster mushroom *(Pleurotus ostreatus)*.

- Statement of the Problem

This research study aimed to determine the potential of sea lettuce *(Ulva lactuca)* extract as substrate conditioner in oyster *(Pleurotus ostreatus)* mushroom cultivation.

Specifically, it sought to answer the following questions:

1. Which among the treatments [T_1 (1:1), T_2 (2:1), T_3 (1:2) of sea lettuce extract is to water and T_4 (pure sea lettuce extract)] is a good substrate conditioner in oyster mushroom cultivation in terms of number and weight of oyster mushroom fruiting bodies?
2. Is there any significant difference among the treatments as substrate conditioner in oyster mushroom cultivation in terms of number and weight of oyster mushroom fruiting bodies?
3. Is there any significant difference among the treatments comparable to T_0 (water) as substrate conditioner in oyster mushroom cultivation in terms of number and weight of oyster mushroom fruiting bodies?

This research study is significant in determining the potential of sea lettuce extract as substrate conditioner in cultivating oyster mushroom. This would be beneficial to mushroom farmers having abundant sea lettuce in its area and urge others in cultivating oyster mushroom as an additional source of income. This study attempted to find other uses of sea lettuce instead of becoming a factor of pollution. With this study, the number of sea lettuce that produces unpleasant odor along the shores would be diminished.

Moreover, the findings of this study could serve as a substitute model in cultivating oyster mushroom that might act as a useful springboard towards the discovery of new natural components of fertilizers from plants which could aid the yield of mushroom industry.

Mushroom is medicinal and good health food. Mushroom growing could be an ideal income generating activity for men and women and out of school youth [3]. Finally, the outcome of this study would provide baseline information that could help the future researchers to generate new findings in terms of empirical contributions to enrich the existing literature. Ultimately, the results of this study would serve as a potential solution to the focal problems the world is facing today.

This research study was delimited to determine the potential of sea lettuce extract as substrate conditioner in oyster mushroom cultivation in terms of number and weight of oyster mushroom fruiting bodies within twenty-one (21) days. Moreover, this study was limited to (5) five treatments [T_0 (water), T_1 (1:1), T_2 (2:1), T_3 (1:2) of sea lettuce extract is to water and T_4 (pure sea lettuce extract)] and also limited on using five (5) replicates for every treatment using 10 mL of the five (5) treatments as substrate conditioner in cultivating oyster mushroom scientifically known as *Pleurotus ostreatus*.

II. METHODS AND MATERIALS

- Research Locale

The experimentation of the study was conducted at the Department of Agriculture, Bureau of Plant Industry, La Paz Experiment Station located at Upper Ayala, La Paz, Zamboanga City with the supervision of the LPES-personnel.

- Gathering of Materials

The collection of sea lettuce was gathered from the shorelines of Cawa-Cawa, R.T Lim Boulevard, Zamboanga City using gloves and safety mask. The oyster mushroom fruiting bags were procured from Orlando O. Telmo, La Paz Experiment Station. Laboratory apparatuses such as weighing scale, beakers, stirring rods and graduated cylinders were

borrowed at Zamboanga National High School- West Science Laboratory.

- Proper Protocol in Identification of the Substrate Conditioner

A letter was given to Mr. Malli L. Usman, Department of Agriculture, Bureau of Fisheries and Aquatic Resources Regional Office No. IX at R.T. Lim Boulevard, Zamboanga City regarding the identification of the substrate conditioner called sea lettuce with proper certification.

- Proper Protocol in Securing the Oyster Mushroom Fruiting Bags

A letter was given to Mr. Orlando O. Telmo, Department of Agriculture, La Paz Experiment Station regarding proper protocol in securing the oyster mushroom fruiting bags. The oyster mushroom fruiting bags were prepared and secured with the assistance of the LPES-Personnel. The bags were arranged; the cotton plugs were removed then tied vertically using a rope and locked using a metallic hook. A total of twenty- five (25) oyster mushroom fruiting bags were prepared for this study.

For the mushroom culture, sorghum seeds were washed and boiled for 20-25 minutes. It was then transferred to a clean bottle plugged with cotton and covered with clean paper. The grain containing bottle was sterilized at 15 psi. When it was cooled, a pure culture stub of a young oyster mushroom was inoculated inside the bottle for 14 days.

The matured oyster mushroom fruiting bags were fermented with the general mixture of 70 % sawdust and 30 % rice hulls that were composted for about a week then filled inside the polypropylene bags of 6 x 12 inches bag size, placed with a PVC pipe neck (1 inch diameter), plugged with cotton and covered with a clean paper. The bags were then steamed in a pasteurizer drum for 6-8 hours and were cooled.

The inoculation of the bags with the grain spawn was conducted in an aseptic laboratory. Each bag was planted with 5 spawns of oyster mushroom. The grain spawn bottle was shaken to loosen the grains, the plug was removed then the mouth was flamed. Using the inoculating needle that was sterilized in alcohol and heated by the flame, the spawns were planted and transferred to the polypropylene bags. The transferring and planting of oyster mushroom spawns was near the flame of the alcohol lamp to avoid any contaminations.

The plastic bags were tightly closed so that no air would get inside the substrates. The spawned bags were placed in a ventilated dry room for about 3 weeks. Inoculated bags was piled and incubated in the La Paz laboratory with the assistance of the LPES-Personnel until the bags are fully covered with mycelia [10].

- Sterilization of Materials

The apparatuses were sterilized by using hot water about 20 minutes. Thereafter, the apparatuses were cooled for 10 minutes, cleaned thoroughly.

- Preparation of Extracts

The sea lettuce was washed thoroughly to remove inert matter then chopped into smaller pieces and pounded using mortar and pestle. The pounded sea lettuce was squeezed using clean cheesecloth to recover extract. Manual squeezing was done to recover most of the extract and was placed in a clean beaker and was then boiled.

- Treatments of the Study

There were five (5) treatments in this study; in T_0 (water) which is the negative control, 100 mL of water; T_1 (1:1), 50 mL of sea lettuce extract: 50 mL of water; T_2 (2:1), 100 mL of sea lettuce extract: 50 mL of water; T_3 (1:2), 50 mL of sea lettuce extract: 100 mL of water; and T_4 (pure sea lettuce extract), 100 mL of pure sea lettuce extract using 10 mL of the five (5) treatments as substrate conditioner in cultivating oyster mushroom.

- Spraying of the Different Treatments

Five treatments were prepared to determine the potential of sea lettuce as substrate conditioner in cultivating oyster mushroom. In each treatment, there were five (5) oyster mushroom fruiting bags weighing 750 grams planted with five (5) spawns of oyster mushroom. In each bag, 10mL of each treatment was sprayed twice a day (a total of 20mL) by the LPES-Personnel at the opening of the PVC pipe (1 inch diameter) to provide enough moisture at the mushroom house that has a temperature not less than

28 degree Celsius. The same procedure was done to the other treatments.

- Gathering of Data

The data were gathered during the 21st day harvest time of oyster mushroom in terms the number and weight of oyster mushroom fruiting bodies[10]-[13].The mushrooms were then harvested by the LPES-personnel after emerging by grasping the stalk of the fruit, gently pulling it out. The numbers of mushroom of each replicate were counted after the 21st day. The LPES-personnel counted the number of oyster mushroom that had grown in each treatment per replicate. Thereafter, the mushrooms were weighed using a weighing scale.

- Proper Waste Disposal

The wastes were properly disposed after the experimentation. The excess sea lettuce extract was diluted with water and disposed in the sink. The used materials such as the test gloves, plastic container, polypropylene bags and masks were disposed properly in a plastic bag and thrown in a non-biodegradable trash bin. The excess fermented substrates were used as fertilizer for plants in the La Paz Experiment Station.

- Statistical Tool

To facilitate the analysis of data, the following statistical measuring tools using the Statistical Package for Social Sciences (SPSS) version 16.0 was employed:

Mean was used to determine the average number and weight of oyster mushroom fruiting bodies, in the different treatments. The One-way Analysis of Variance (ANOVA) was used to determine the significant difference among the different treatments. If there is a significant difference, Post Hoc Test (Tukey) was used to determine the significant difference of the treatments compared to each other. Independent T-test on the other side of the spectrum was used to determine the significant difference of the most significant variant of the number and weight of oyster mushroom fruiting bodies produced compared to T_0 (100% water) as substrate conditioner in oyster mushroom cultivation.

III. DISCUSSION

In recent years, the use of different species of seaweeds as fertilizer, has allowed for partial substitution of conventional synthetic fertilizer [7]. A number of seaweed species are used as liquid fertilizer by applying them as foliar spray, soil drench or in granular form as soil conditioners and manure. These extracts are marketed because the chemical analysis in their extracts revealed the wide variety of plant-promoting substances such as auxins, cytokinis and betaines [14].

Among the different treatments tested, the Statistic results revealed that T_3 (1:2) of sea lettuce: water gave the most promising mean results which can be gleaned from table 1 and 5 with mean number of 2.2 and mean weight of 22.2. Since T_3 obtained the highest mean results, therefore it is better than the other treatments (T_1, T_2, and T_4).

Furthermore, it is the most recommended treatment with 50 mL of sea lettuce extract: 100 mL water as substrate conditioner in oyster mushroom cultivation in terms of the number and weight of oyster mushroom fruiting bodies produced after 21 days. This indicated that concentration of the sea lettuce must lessen for it to give a promising effect in oyster mushroom cultivation.

This finding can be supported by the evidence that lower concentration of seaweed species were found to show promoting effect on the growth and productivity of plants that is currently used today in combination with the commercial fertilizer [15]. The fertilizing efficiency of liquid extracts of the algae is due to the presence of micro and macro nutrients, growth hormones and vitamins at preferential levels [16]. This may be attributed to nitrogen, phosphorus and potassium (NPK) content present in the sea lettuce but lower concentrations are found to be efficient for the reason that high concentration of the algae contains salt that builds up and kills the plant. This can be used as fertilizers or compost but needs to be mixed with other materials [17]. The more intensely green coloured sea lettuce, the higher the nitrogen content [18].

Based on the One-way Analysis of Variance (ANOVA) from table 2 and 6, there is a significant difference among the four (4) treatments as substrate conditioner in terms of the number and weight of oyster mushroom fruiting bodies. This further implies that the four (4) treatments have varying effects as substrate conditioner in oyster mushroom cultivation.

Post Hoc Test (Tukey) from table 3 and 7 revealed that T_3 vs. T_1, T_3 vs.T_2 and T_3 vs. T_4 had a significant difference which led to the implication that the different treatments have varying effects as substrate conditioner in terms of number and weight of oyster mushroom fruiting bodies. Hence, T_3 as substrate conditioner in oyster mushroom is the most significant variant in terms of number and weight of oyster mushroom fruiting bodies.

Whereas, T_1 vs. T_2, T_1 vs. T_4, and T_2 vs. T_4 accepted the null hypothesis, no significant difference among the treatments and implies that the treatments have the same effect as substrate conditioner in oyster mushroom cultivation in terms of the number and the weight of oyster mushroom fruiting bodies produced.

The Independent Samples T-test from table 4 and 8 revealed that T_3 (1:2) of sea lettuce extract is to water compared to T_0 (water) had a significant difference as substrate conditioner in oyster mushroom cultivation in terms of both the number and weight of oyster mushroom fruiting bodies, rejecting the null hypothesis meaning there is a significant difference among the two as substrate conditioner.

Furthermore, it is revealed that T_3 is better than T_0 (water) as substrate conditioner in oyster mushroom cultivation in terms of number and weight of oyster mushroom fruiting bodies. It is suggested that sea lettuce extract substrate conditioner can be used as substitute for chemical fertilizers to improve the sustainable mushroom growth and yield.

CONCLUSION

Food insecurity is alarming. This indicates the need to upgrade the government's programs to eradicate this dilemma. Mushroom cultivation enhances an individual`s and a community`s capacity to act upon other economic opportunities [3]. Mushrooms have good flavour, texture, nutritional value and high productivity that are classified as an excellent food source to diminish malnutrition in countries [19]. This research study utilized and minimized the large amounts of sea lettuce deposited along the shores of Cawa-Cawa, R.T. Lim Boulevard, Zamboanga City that nowadays become a factor of pollution into an efficient substrate conditioner in oyster mushroom cultivation.

In accordance to the Statistics, T_3 gave the most promising mean results. One-Way Analysis of Variance (ANOVA) implied that the treatments have varying effects as substrate conditioner. Post Hoc Test (Tukey) identified T_3 as substrate conditioner in oyster mushroom is the most significant variant. Independent Samples T-test revealed that T_3 is better than T_0 (water) as substrate conditioner. The study revealed that the application of low concentration of sea lettuce combined with water significantly increased the number and weight of oyster mushroom fruiting bodies while higher concentration gave unpromising results [15].

This study concluded that all the proportions of the treatments has a greater probability that it can serve as substrate conditioner in oyster mushroom cultivation that could be a very reliable and profitable option in alleviating malnutrition [2]. Furthermore, this study emphasizes the application of sea lettuce extract substrate conditioner that can be effectively used as eco-friendly approach to the mushroom cultivation industry.

In view of the aforementioned conclusion, the researcher recommends to the future researchers some missing items that seems likely to be further studied at some points; characterize the sea lettuce components in order to determine which is responsible for the characteristics of sea lettuce odour; conduct an analysis on the neutraceutica value of the harvested mushrooms; extend the harvest time for 2 months; and conduct a preliminary qualitative phytochemical analysis of sea lettuce.

REFERENCES

[1] Diriba, Muleta, Beje, Gume and Dawit, Abante. Evaluation of locally Available Substrates for Cultivation of Oyster Mushroom (*Pleurotus ostreatus)*. African journal of Microbiology Research. Jimma, Ethiopia. (2013)

[2] Imtiaj, Ahmed and Rahman, Syed Ajijur. Economic Viability of Mushroom Cultivation to Poverty Reduction in Bangladesh. Trop. Subtrop. Agroecosyst. (2008)

[3] Food Agriculture Organization (FAO). Make money by growing mushroom. Rural Infrastructure and Agro- Industries. www.fao.org. Division Diversification booklet No 7. United Nations. (2009)

[4] Feeney, Mary Jo and Beelman, Robert. Mushrooms in a Class of Their Own. Sindh Agriculture University, Tandojam printing press. (2004)

[5] Edep, John. The promising business of growing mushrooms. Retrieved July 15, 2018 from

http://www.jmpmushroom.com/2013/01/the-mushroom-business-in-philippines.html.(2013)

[6] Povero Giovanni, Mejia John, Di Tommaso A systematic approach to discover and characterize natural plant biostimulants. Front. Plant Sci. 7:435. (2016)

[7] Zodape, Sudhakar, Mukhopadhyay Soumita, Eswaran Karuppan, Reddy Muppala and Chikara Jitendra. Enhanced yield and nutritional quality in green gram (Phaseolus radiate L.) treated with seaweed (*Kappaphycus alvarezi)* extract. J Sci Ind Res 69:468-471. (2010)

[8] Bhattacharjee, Ritika and Dey, Utpal. Biofertilizer, a way towards organic agriculture: A review. Journal of Scientific & Industrial Research. African journal of Microbiology Research. (2014)

[9] Mackenzie Jr.,Clyde. Removal of Sea Lettuce, *Ulva spp*., in Estuaries to Improve the environments for invertebrates, fish, wading birds, and eelgrass. Marine Fisheries Review. (2005)

[10] Regional Agriculture and Fisheries Information Division. Mushroom Production. Department of Agriculture. (n.d)

[11] Pasanting, Riza Aibie, Adres Miriam Ruth and Cabarles, Jr. Noel. *Musa parasidiaca* (Banana sap) extract : an alternative for water in oyster mushroom cultivation.(2003)

[12] Mahmud, Zainab, Rivas, Hazel Reign, Nur, Ninna Aleeza, Pesidas, Christine and Bakil, Hamsarah. Banana *(Musa parasidiaca)* Pseudo-Stem Extract as Growth Enhance on Oyster Mushroom Cultivation. (2016)

[13] Rodriguez, Devine Grace. Comparative Study of Water Hyacinth and Banana Leaves as Substrate in Straw Mushroom Cultivation. (2013)

[14] Khan Wajahatullah, Rayirath Ushah, Sowmyalakshimi Subramanian, Jitesh Mundaya, Rayorath Prasanth, Hodges Mark, Critchley Allan, Craigie James, Norrie Jeffrey and Prithiviraj Balakrishan. Seaweed extracts as biostimulants of plant growth and development. J Plant Growth Reg. 28:386-399. (2009)

[15] Chatterji, Aaron. Anti-influenza activity with Indian seaweeds. A preliminary investigation. In the proceeding of the 2004 National Seminar on New Frontiers in Marine Bioscience Research. (2015)

[16] Sivasangari Ramya. Foliar application of liquid biofertilizer of brown alga *(Stoechospermum marginatum)* on growth, biochemical and yield of *Solanum melongena*. Int J Recycl Org Waste Agriculture. 4:167-173. (2015)

[17] Mussings, Tony. Sea lettuce as fertilizer: some considerations. Retrieved August 2, 2018 from http://www.tonymussings.blogspot.com/2016/08/sea-lettuce-as-fertilizer-some considerations. html. (2016)

[18] Robert- Anderson DV, Wilson DT, Bolton JJ, Anderson RJ, Maneveldt GW. Rapid assessment of tissue nitrogen in cultivated Gracilaria gracilis and Ulva lactuca. African Journal of Aquatic Science 34(2):169-172. (2009)

[19] Eswaran, Ramabadran. Studies on physiological, cultural and post-harvest aspects of oyster mushroom. Tropical Agricultural Research. 12:360-374. (2000)