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Mycelial growth, yield and decomposition capability of white oyster mushroom (*Pleurotus florida*) grown in low-density polyethylene (LDPE) plastic and lignocellulosic wastes

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Abstract

White Oyster Mushroom (*Pleurotus florida*) is one of the domesticated mushrooms in the Philippines and known to grow in various biodegradable wastes. Low Density Polyethelene (LDPE) plastic is one of the most common non-biodegradable wastes in the environment which causes pollution. At present, biodegradation process is one of the ways to degrade plastics because of its non-polluting mechanism and cost-effectiveness. Thus, this study aimed to determine if *P. florida* could grow in LDPE plastic and facilitate its decomposition. Fourteen treatments were used in the study, laid out in Complete Randomized Design, and replicated thrice. The first four (4) treatments were composed of substrates with no LDPE plastics (rice straw, sawdust and cocopeat) while the rests were substrates with certain percentage of LDPE plastics. Observation was done for eight (8) weeks in the PUP Lopez Mushroom Labouratory and the data obtained was analyzed using ANOVA. Result shows that slight decomposition happened in LDPE plastic mixed with lingo-cellulosic wastes. Small spots or dark holes appear in the surface of LDPE plastics observed under microscope which is a sign of decomposition. In terms of mycelial ramification, fruiting bags with LDPE plastics were first to occupy the fruiting bag. There was also a decreased weight of the fruiting bags after 8 weeks. In terms of yield, substrates without LDPE plastics grew mushrooms with the highest weight. However, yield in sawdust and cocopeat substrates were not significantly different with the yield of mushrooms grown in substrates with LDPE plastics.

Key words: Decomposition, ligno-cellulosic waste, low-density polyethylene (LDPE) plastic, mycelial ramification, *Pleurotus florida*

Introduction

In today's world, solid waste management has become a top priority. The Philippines produces 2.7 million tons of plastic garbage annually, making it one of the largest contributors to global plastic waste production. This leads to significant levels of pollution in urban areas. It is anticipated that garbage generation in the Philippines will continue to rise in the coming years. The agricultural industry is also one of the biggest producers of agricultural solid waste, which can either be exploited as a raw material for the bioeconomy or left to build indiscriminately and pose a threat to world health and food security. Knowing the different things that can be done to assist lessen the wastes in our country is one of the ways that can be done to help the environment and the community.

The plastics sector in the Philippines is crucial to the national economy, contributing US$2.3 billion in 2018, but it also provides low-cost consumer items to low- and middle-income people, according to the World Bank Organization (2021). Nevertheless, the Philippines has developed a "sachet economy" due to its heavy reliance on single-use plastics like multilayer sachets and pouches, which has contributed to the worrisome levels of marine plastic pollution in the area. An astounding 163 million pieces of sachets are reportedly consumed daily in the Philippines. The Philippines struggles with unsustainable plastic manufacturing and use as well as a lack of solid waste disposal infrastructure, similar to many other rapidly rising nations. The Philippines produces a remarkable 2.7 million tons of plastic garbage annually, of which 20% is thought to wind up in the ocean. The Philippines, which is made up of more than 7,500 islands, is particularly vulnerable to the effects of marine debris due to its coastal population as well as its fishing, shipping, and tourism industries. And the majority of the plastic wastes being evaluated are made of plastic resins including polyethylene terephthalate (PET), low density polyethylene (LDPE), high density polyethylene (HDPE), and polypropylene (PP), which are thought to account for 1.1 million tons of daily resin use.

The thermoplastic known as low density polythene (LDPE) is created from the ethylene monomer. It has good mechanical capabilities, water-repellent qualities, light, inexpensive, and very energy-efficient, making it the most popular packing material (Kyaw *et al.*, 2012). One form of plastic that is generally safe for use with food is polyethylene, sometimes known by its generic name thermoplastic (Lubis *et al.*, 2020). Low-Density Polyethylene (LDPE), which is used to make lightweight plastic bags, food containers, and beverage containers the size of glasses, is one type of polyethylene. LDPE is highly resistant to water, rigid, transparent, flexible, and resistant to chemical compounds below 60°C. LDPE is recyclable and works well as a food container because food packed in it does not readily undergo chemical reactions.

However, just like any other plastics, LDPE does not readily decompose and because of serious and alarming problem of plastic pollution, researchers around the globe have begun to look for methods by which plastic can be degraded. As a result, "plastic-eating mushrooms" were found. Numerous fungi were put to the test in 2011 by Yale University researchers to see if they could break down the synthetic polymer polyester polyurethane (PUR), a form of plastic (Russell *et al.*, 2011). They discovered that a number of fungus belonging to the Pestalotiopsis genus were capable of converting plastics into organic materials in both solid and liquid solutions. Additionally, a number of species of mushrooms, including some that are edible, like oyster mushrooms, are capable of degrading plastic, according to numerous experts throughout the world.

One of the most popular varieties of cultivated mushrooms worldwide is the white oyster mushroom (*Pleurotus florida*) which may be eaten, and it also has a number of biological effects due to the presence of significant bioactive compounds (Yang *et al.*, 2013). According to certain studies, *Pleurotos* species develop more quickly than other mushrooms. Its fruiting body is rarely affected by diseases and pests, and it may be produced in a straightforward, inexpensive manner with a high yield in a controlled environment. Pink, yellow, white, grey, and black oyster mushrooms are also available, with white and grey varieties being the most common. The most popular substrates for oyster mushrooms include sawdust, cocopeat, rice straw, and other agricultural leftovers. Scientists have demonstrated that it can decompose plastic and yet produce an edible mushroom. Pieces of plastic were placed in capsules containing oyster mushrooms in the study of Unger, who developed a prototype for an at-home oyster mushroom recycling device dubbed the "Fungi Mutarium". Once it had grown on the capsule, the fungi could be picked and eaten (Greene, 2022). Furthermore, it was effectively demonstrated in the study by Da Luz *et al.* (2015) that oyster mushrooms can break down oxo-biodegradable plastics and grow mushrooms on them.

In connection with the alarming issues in the environment due to Low-density polyethylene (LDPE) plastics and with the potential of oyster mushroom in degrading plastic components, the idea of using LDPE mixed with ligno-cellulosic wastes as substrates was conceived. This generally aimed to make use of plastics and reduce the growing wastes problem in our locality and in our country and making it part of the substrates to produce edible and delicious mushrooms.

Materials and methods

Source of strain: *P. florida* pure culture was obtained from the collection of mushroom culture media (Pf_01) of PUP Lopez Tissue Culture Labouratory. It was subsequently inoculated in sorghum spawn and allowed to ramify for substrate inoculation.

Research design and locale: The researchers used an experimental design laid out in Complete Randomized Design. The experiment was done in the PUP Lopez Tissue Culture Labouratory and mushroom growing was done in the PUP Lopez Mushroom house.

Treatment and layout: Fourteen (14) treatments with 10 replications were observed in the study. Different ligno-cellulosic wastes such as sawdust, coco peat, rice straw and rice bran served as its growing medium and mixed with Low-Density Polyethylene (LDPE) plastic. The control treatment is based on the protocol used by Philippine Rice Research Institute on Mushroom Production using rice straw as the main substrate. The following were the treatments (Table 1).

Table 1. Treatments used in the study

Treatment	Percentage	Rice Straw (g)	Saw dust (g)	Coco peat (g)	Rice bran (g)	LDPE plastic (g)
1	70%, 30%, 10%	572.67	245.43		81.81	
2	100%, 10%	818.1			81.81	
3	100%, 10%		818.1		81.81	
4	100%, 10%			818.1	81.81	
5	100%, 10%				81.81	818.1
6	75%, 25%, 10%	613.58			81.81	204.53
7	50%, 50%, 10%	409.05			81.81	409.05
8	25%, 75%, 10%	204.53			81.81	613.58
9	75%, 25%, 10%		613.58		81.81	204.53
11	50%, 50%, 10%		409.05		81.81	409.05
12	25%, 75%, 10%		204.53		81.81	613.58
13	75%, 25%, 10%			613.58	81.81	204.53
14	50%, 50%, 10%			409.05	81.81	409.05
15	25%, 75%, 10%			204.53	81.81	613.58

Materials used: Low-Density Polyethylene (LDPE) plastics, fruiting bags, mushroom spawns, rubber bands, paper, cotton and agricultural wastes such as sawdust, coco peat, rice straw and rice bran. Spawns were obtained from PUP Tissue Culture Labouratory. While, the agricultural wastes were obtained from the agricultural lands in Lopez, Quezon. For the purpose of the study, the LDPE plastics used were new and never been used garbage bags bought in Lopez, Quezon Public Market.

Fruiting bag preparation: Polypropylene (PP) bags were used in the preparation of oyster mushroom fruiting bags. Following the proper preparation of substrates in the protocol used by Jacob *et al.* (2015), treatment ratio were prepared, where each fruiting bag hold a total weight of 900 g. The LDPE plastic sheets on the other hand were prepared and cut into smaller pieces, then mixed with the other substrates. To kill pathogens which might cause contamination, fruiting bags were pasteurized for 8 hours.

Inoculation and incubation of mushroom fruiting bags: The pasteurized fruiting bags were brought to Mushroom Labouratory Room where they were left for 8 hours to facilitate cooling as preparation for inoculation. Subsequently, inoculation of 5 g spawn to each of the bag was performed. They were immediately transferred in the incubation room for mycelial growth and ramification.

Percentage of decomposed LDPE: The appearance of LDPE plastics was observed using a compound light microscope (V21) and measured (Table 2).

Mycelial growth: Mycelial ramification was measured weekly (cm) from the neck of the bag to the bottom of the bag using a ruler and was done until the bag is fully ramified.

Weight of fresh mushroom: weight of fresh mushroom was measured using digital weighing scale in grams and yield measurement was observed for a month of fructification.

Table 2. Criteria used in measuring decomposed LDPE plastic

Percentage	Interpretation
76-100%	Fully decomposed
51-75%	Decomposed
26-50%	Moderately decomposed
1-25%	Slightly decomposed
0%	No decomposition

Statistical analysis: The gathered data was analyzed using Analysis of variance (ANOVA) of the Statistics for Agricultural Research (STAR).

Results and discussion

Decomposition activity of oyster mushroom grown in different substrates with LDPE plastic: *Pleurotus* is a species of mushrooms, a lignocellulytic fungi that has been gaining popularity due to its role in bioremediation processes for agro-industrial residues, pollutants and unmanageable compounds. Their highly effective enzymatic system contributes to their capacity to vitiate a wide variety of substances (Sanchez, 2009). Table 3 shows the percentage of decomposed LDPE plastics. It shows the different treatments containing 25, 50, 75 and 100% of Low-density Polyethylene (LDPE) plastic mixed with lignocellulosic wastes and rice bran. It reflects that based on the criteria formulated for the observation of decomposition of LDPE plastics, no visible decomposition was evident but when observed under the compound light microscope (V21), showed holes which indicates a sign of 1-25% decomposition. The lack of variation on the percentage of decomposition among the treatments with LDPE plastics may indicate that regardless of the amount of other lignocellulosic wastes combined with LDPE, if there are mushroom mycelia present, with proper lighting it will act on the plastics. It is evident that changes occurred in the plastic after eight weeks of exposure to mycelia. According to Eich *et al.* (2015), those small spots or holes are a sign of decomposition and based on the characteristics observed in the plastic samples, the capability of oyster mushrooms to decompose LDPE plastics is possible. Generally, LDPE plastics have been predicted to degrade when exposed to ultraviolet radiation in as short as 500 years, with a conservative average period of 1000 years. The plastic may stay an eternity if it is not exposed to light, like at the bottom of a landfill (Veethahavya *et al.*, 2016). As stated by Carter (2023), fungi are very adaptable and could degrade almost every substrate. Their production of potent enzymes, which are expelled and used to break down substrates into simpler molecules that the fungal cells can then ingest, is what gives them their ability. These fungi have frequently evolved to decompose woody materials, but they can also target other substrates using same capacity. This explains why fungi may grow on a variety of man-made objects, including carpets, painted furniture, grout between tiles, shower curtains, upholstery, and even automobile headlights. Recent research also suggest that some fungi may even be able to break down some of the so-called "forever chemicals," including Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS), but the process is sluggish and not fully understood. Additionally, there is proof that the ocean's accumulation of plastic is lower than what might be predicted based

Table 3. Percentage of decomposition and appearance of LDPE in different substrates

Treatment	Percentage	Interpretation
100% LDPE plastic+10% ricebran	1-25%	Slightly decomposed
75% rice straw+25% LDPE plastic+10% rice bran	1-25%	Slightly decomposed
50% rice straw+5-% LDPE plastic+10% rice bran	1-25%	Slightly decomposed
25% rice straw+75% LDPE plastic+10% rice bran	1-25%	Slightly decomposed
75% sawdust+25% LDPE plastic:10% rice bran	1-25%	Slightly decomposed
50% sawdust+50% LDPE plastic+10% rice bran	1-25%	Slightly decomposed
25% sawdust+75% LDPE plastic+10% rice bran	1-25%	Slightly decomposed
75% coco peat+25% LDPE plastic+10% rice bran	1-25%	Slightly decomposed
50% coco peat+50% LDPE plastic+10% rice bran	1-25%	Slightly decomposed
25% coco peat+75% LDPE plastic+10% rice bran	1-25%	Slightly decomposed

Table 4. Mycelial growth (mm) and yield of oyster mushroom in 4 weeks of incubation and 4 weeks of harvest

Treatment	Mycelial growth (mm)	Yield (g)
T1 (70% rice straw+30% Saw dust+10% rice bran)	74.49 fg	142.00 a
T2 (100% rice straw+10% rice bran)	73.62 gh	94.00 b
T3 (100 sawdust and 10% rice bran)	62.20 h	56.88 d
T4 (100% coco peat and 10% rice bran)	93.63 b	69.34 c
T5 (100% LDPE plastic+10% rice bran)	85.05 c	18.00 h
T6 (75% rice straw+25% LDPE plastic+10% rice bran)	79.44 de	43.00 f
T7 (50% rice straw+50% LDPE plastic+10% rice bran)	77.41 ef	40.66 f
T8 (25% rice straw+75% LDPE plastic+10% rice bran)	81.66 d	39.34 f
T9 (75% sawdust+25% LDPE plastic+10% rice bran)	60.48 h	52.00 e
T10 (50% sawdust+50% LDPE plastic+10% rice bran)	79.57 de	42.00 f
T11 (25% sawdust+75% LDPE plastic+10% rice bran)	81.41 d	32 g
T12 (75% coco peat+25% LDPE plastic+10% rice bran)	98.91 a	41.34 f
T13 (50% coco peat+50% LDPE plastic+10% rice bran)	100.00 a	35.34 g
T14 (25% coco peat+75% LDPE plastic+10% rice bran)	99.78 a	21.34 h

*Means with the same letters are not significantly different at $P < 0.05$ using Tukey's Honest Significant Different Test

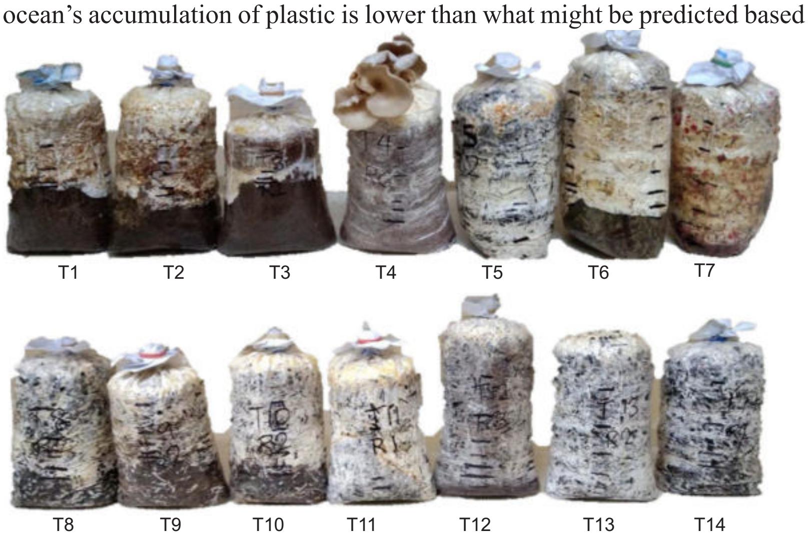

Fig. 1. LDPE plastics before and after inoculation of mushroom spawn showing 1-25% decomposition

on production and disposal rates, and it has been hypothesized that part of this "missing" plastic may have been broken down by marine fungi. Furthermore, fungi have a special aptitude for removing or binding heavy metals, radiation, and other chemical contaminants, such as oil and pesticides and even plastics (Akpasi *et al.*, 2023).

The result of the study coincides with the study of da Luz *et al.* (2015) who stated that *Pleurotus ostreatus* PLO6 could break down green polyethylene regardless of sunshine exposure. However, to speed-up the decomposition process, other alternative methods might be considered to shorten the half-life of plastic garbage in landfills or dumps which include exposing the plastic to sunshine and allowing it to subsequentlyincubate in the presence of fungus. Gupta and Prakash (2019) also mentioned that there are numerous varieties of mushrooms, including the ubiquitous edible oyster mushroom which have the ability to use plastic as their primary supply of carbon. *Pestalotiopsis microspore*, a unique kind of mushroom that can only survive on plastic, has been found in the Amazon rainforest. The primary component of plastic items, polyurethane, is ingested by it and converted to biological stuff. This fungus can also survive without oxygen, demonstrating its enormous potential for feeding on and so cleaning even the most extreme landfills.

Several scientists recently created a mechanism known as the Mutarium of fungi. This was a prototype created by the scientist in Utrecht University Labouratory. This is mostly a little garden that allows for the growth of *Schizophyllum commune* (split gill mushrooms) and *Pleurotus ostreatus* (oyster mushrooms). They were put in an agar media with sugar and plastics on it. They said that both mushrooms can 'consume' large amounts of plastics and are edible (http://www.livinstudio.com/fungi-mutarium). Furthermore, Munir *et al.* (2018) also revealed that the fungi *Trichoderma viride* and *Aspergillus nomius* were able to degrade plastics in landfill and reduced the weight of LDPE plastics after 45 days of cultivation. Groves and rough were also found on the surface of LDPE film which were not found on plastics without fungi.

Mycelial growth and fruit yield of white oyster mushroom (*Pleurotos florida*) in LDPE plastic and ligno-cellulosic wastes: Mycelium is the primary structure of mushrooms which enables them to get nutrient from the food source and eventually lead to fructification. Pleurotus can fruit as soon as two weeks after grain spawn is inoculated into pasteurized bulk substrates (Mkhize *et al.*, 2016). As shown in Table 3 and Fig. 1, among the substrates without LDPE plastics, mycelia in coco peat was the fastest to ramify, followed by the control treatment (combination of rice straw, sawdust and rice bran), rice straw and sawdust. While, among the substrates with LDPE substrates, mycelial ramification in various percentage of coco peat combined with LDPE plastics were the fastest. It was then followed with pure LDPE plastic with rice bran and subsequently different percentage of rice straw and sawdust with LDPE plastics respectively. In terms of yield, oyster mushrooms inoculated in substrates without plastics had higher yield as compared to those fruiting bags with LDPE plastics. The control substrate (7 rice straw; 3 sawdust: 1 rice bran) gained the highest yield and was statistically different with the rest of the treatments. It was followed by the combination of rice straw and rice bran (94 g), coco peat and rice bran (69.34 g) and sawdust and rice branwith 56.88 g of harvested mushrooms. While, in those substrates with LDPE plastics, that with 75% sawdust had the highest yield and statistically different with the rest of the treatments.

The fast growth of mycelia in cocopeat could be attributed to its low nutrient content. When the substrate is depleted of nutrient, the tendency of the mycelia is to grow faster and longer to look for nutrient. In the case of coco peat where fastest mycelial run was observed, there is a lower lignin (10-25%) and cellulose percentage (20%), as compared to rice straw and sawdust which both contains 30% lignin and 50% and 40% cellulose (Chen *et al.*, 2020; Muley *et al.*, 2016). Moreover, as stated by Rahma and Purnomo (2018), the growth of mycelium is also caused by the decomposition on fruiting bag that is not too dense making the growth of mycelium fast. Thus, could also be the reason why those substrates with sawdust had the slowest mycelial growth.

It could be noticed that the higher the amount of ligno-cellulosic waste present, the higher the yield and the lower the amount of ligno-cellulosic wastes, the lower the yield. Thus, a very evident low yield on substrates with only LDPE and rice bran was observed. The higher yield obtained from the control treatment could be attributed to the fact that various mushrooms have different nutrient requirements and oyster mushroom grows best in the combination of rice straw, sawdust and rice bran in a 7:3:1 ratio as reported and recommended by the Philippine Rice Research Institute and the Center for Tropical Mushroom and Development at CLSU, Muñoz, Nueva Ecija, Philippines. The lowest yield from LDPE plastics with rice bran can be attributed to the nutrient profile of rice bran, which contains carbohydrates, amino acids, and essential minerals (Moonmoon *et al.*, 2011). Rice bran is also a source of phosphorus, iron, magnesium, and provides 11.5% dietary fiber and 11%–13% crude protein (Ijaz et al., 2021). Moreover, there is not enough nutrient on LDPE which could easily be used by the mushroom mycelia. Though decomposition might have started and took place, it will take time to fully grow mushrooms and totally decomposed LDPE.

In a similar study done by Rambey *et al.* (2020) and Soriano and Mangune (2022), oyster mushroom grows best in a combination of rice straw and sawdust substrate with highest yield and biological efficiency. While, in the case of oyster mushrooms growing in plastics, a "Fungi Mutarium" was created by Utrecht University scientists and Austrian designer Katharina Unger. It makes use of agar gelatin pods that feed the fungus sugars and starches until UV-treated plastic is inserted into the center. The fungus needs a few months to completely digest the plastic, after which it produces a puffy, mushroom-like cup with a sweet taste and licorice-like odor (Greene, 2022)

Signs of decomposition evident in low density polyethylene plastic when viewed using a compound light microscope since small spots or holes appeared in the surface of LDPE plastics signifying a start of decomposition. Mycelia spread more quickly in the fruiting bags with LDPE plastics since insufficient nutrients were obtained, thus, also leads to lower yield. While, slow mycelial growth was observed in fruiting bags without LDPE plastics for the reason that it has enough source of nutrients, thus higher yield was obtained. However, additional study is recommended to further establish the ability of oyster mushroom to decompose LDPE plastics.

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