# Comparative Study of *Pleurotus Djamor* Cultivation on Sustainable Waste Substrates

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## Abstract

*Fungi are diverse and essential to the health of many ecosystems. They break down organic material from waste and in turn are able to provide other organisms with essential nutrients. Mushrooms are critical to the health of Earth because they recycle tremendous amounts of waste. This experiment tested the productivity of 4 different recycled substrates and their ability to produce the largest final mass of Pleurotus Djamor. Oak mulch, palm mulch, coffee grounds, and a mix of all three were tested. The levels of mycelium growth were measured rather than mushroom mass due to time constraints. The mixed substrate was predicted to have the best results; however, coffee surpassed it and had the overall fastest development times. The mixed substrate had the second quickest colonization, the palm mulch came in third, and oak mulch mycelium growth was the least, never surpassing 20%. Although coffee had the quickest colonization rates, the experimenters determined the mixed substrate to be the most sustainable because of coffee's vulnerability to environmental factors. Even though this is a small-scale experiment, certain outcomes may lead to future large-scale changes, thus reducing the amount of waste in public spaces by a noticeable amount.*

## Introduction

For hundreds of years, scholars have been debating the carrying capacity of earth in relations to the logistic growth model. Carrying capacity is the maximum population size that an environment can support (Campbell et al. 2011). With over seven billion people on Earth, when will Earth reach its maximum carrying capacity and what will cause the human population decrease? Could it be that human's leading predator is waste itself?

According to a recent BBC publication on global waste production, the average American produces over 700 kg of waste per year (BBC, 2013). That means each person in the United States produces 1,543 pounds of trash each year. An average college student (21 years old) has already produced 16.2 tons of trash over their lifetime. At this rate, if an American lives for 80 years, they produce roughly 62 tons of trash. Putting it in a different perspective, an 80-year-old person produces a quantity of waste that is equivalent in mass to about approximately 31 elephants. If we compare this trend to the entire planet, Earth's 7 billion inhabitants produce 5 billion tons of trash each year.

This waste comes from the Linear Model of Production, a very common model used in industrialized countries. There is a long history of linear models of economic activity, tracing back to Soviet economists material balance tables (Blume). These models fluctuate between general and partial equilibrium and have no recycling pattern to balance the system (Blume). More production means more disposal and in this linear model, there is no use for the waste. As seen in Figure 1, businessmen go home happy with their paycheck, but will they be happy when the Earth's resources are barren?

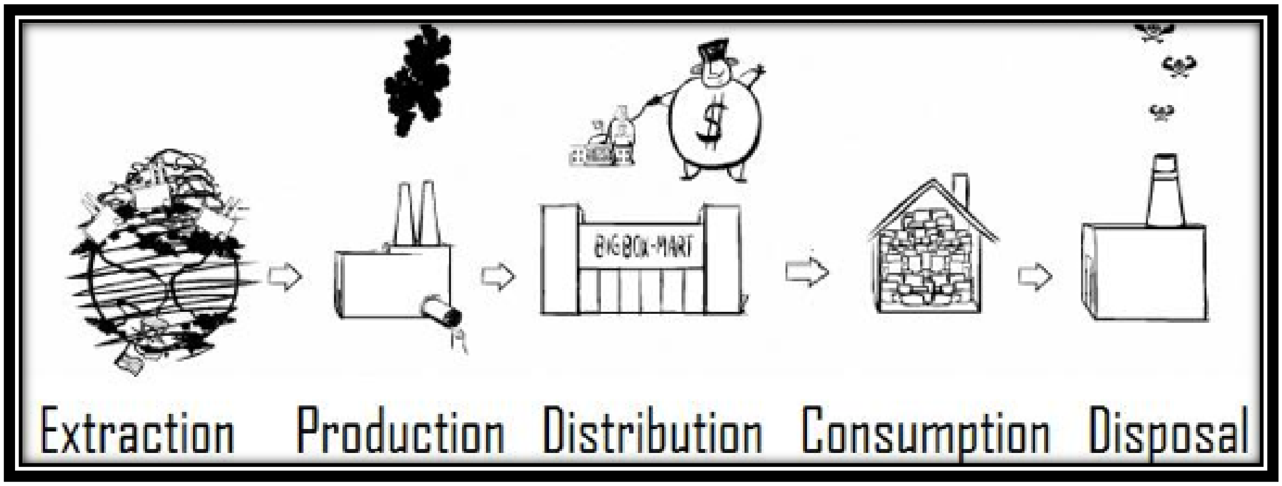



**Figure 1: Diagram From Story of Stuff by Annie Leonard (Leonard, 2007).**

Where does all this waste go? If there were no decomposers, this waste would suffocate the planet and massively pollute living conditions. It is easy to say that without decomposers, trash really would be a human 'predator' and the carrying capacity of Earth would have been reached hundreds of years ago. However, decomposers are essential organisms that degrade organic materials into a form that can be easily utilized by other organisms. Decomposers in nature often form a sustainable cycle of death, growth, and birth. When biotic organisms die, decomposers grow from the remnants and break down the larger molecules into a form more readily for plant uptake (Stamets, 2000).

Sustainability is the key focus of this experiment. The three pillars of sustainability, people, planet, and profit, are of utmost importance in order for a system to maintain itself (Bernard, 2013). This experiment was designed to maximize "profit" through using free, recycled materials as growing mediums for mushrooms that can be sold. Through using a variety of natural, readily available resources, this experiment explores the most effective substrate for mushroom cultivation. This experiment also focuses on "planet", as the mushrooms will potentially degrade their substrates sufficiently enough to be used as compost, which in turn can be used to grow other plants. "People" were also focused on through the use of purely waste products as the substrates. Even though this is a small-scale experiment, certain outcomes may lead to future large-scale changes, thus reducing the amount of waste in public spaces by a noticeable amount. These three pillars of sustainability: people, planet, and profit are all benefitted by the closed loop system created by decomposers (Figure 2, pg. 3).

Edible mushrooms, particularly fast growing ones, such as *Pleurotus spp.* can successfully form a closed loop system. The first part of the system is to provide an edible product (mushrooms) by using waste byproducts (i.e., coffee grounds, tree trimmings, palm fronds, etc.) as the growing medium**.** Once the mushrooms have been sold, the spent substrate can in turn be composted and eventually converted into fertilizer to add nutrients back into the soil. The fertilizer encourages other plant growth and eventually cycles around to produce more mushrooms (Rinker, 2002). If not used for compost, the spent substrates can be utilized to produce animal feed or other crops and purify water and soil (Rinker, 2002).

**Figure 2: Sustainable Cycle of *Pleurotus Djamor***

According to an article published in Conservation Biology, "Ecological Sustainability as a Conservation Concept", sustainability is achieved by meeting human needs without compromising the health of an ecosystem (Callicott, 1997). Mushrooms exceed these criteria for sustainability by benefitting the ecosystem with a wide array of ecosystem services. "Humans benefit from fungi's services to agriculture and forestry as well as their essential role in making products ranging from bread to antibiotics" (Campbell, 2008). Also, human nutritional needs are met by the fruiting body of the mushroom. Commercial mushroom producers could benefit greatly and increase their profit by creating their "product" from free, recycled substrates. While humans have already found a wide range of uses for the products and services produced by these organisms, there is still room for further incorporation into societies' everyday means of operation.

Decomposers can be separated into the 5 phyla of fungi, or Eumycota (Campbell et al. 2011). Fungi are diverse and essential to the health of many ecosystems. They break down organic material from useless waste, and provide other organisms with essential nutrients. The 5 phyla of fungi include: chytrids, zygomycetes, glomeromycetes, ascomycetes (sac), and basidiomycetes (Campbell et al. 2011). Basidiomycetes, or mushrooms, are the most commonly known phyla of fungi and will be the main focus of this experiment.

Similar to plants, basidiomycetes disperse their genetic material via aerial or water dispersal in seed-like capsules known as spores. The visible part of a mushroom is actually an entire fruiting body, woven with reproductive hyphae in thread-like structures (Campbell, 2008). The fungi produce a fruiting body above ground in order to maximize the surface area in which the spores will be exposed too. After the spores are released, they spread out in search of nutrients and a stable place for survival (Campbell, 2008). Substrate is focused on in this experiment because ideal substrate and climatic conditions are essential for maximal mycelia growth and mushroom production.

Substrates chosen for this study were recycled materials that could all be found within the UCF campus. Coffee was chosen because it has high nitrogen content and has been used many times as additives to traditional substrates. Oak mulch was chosen due to the observation that mushrooms often typically grow on trees, such as oak, in nature. Lastly, palm mulch was chosen because palm trees are an abundant natural resource in Florida. As well, many previous studies have been found to test palm fronds as a substrate with success (Kalita & Mazumder, 2001). A blend of all three substrates will also tested be as a fourth trial. Hypothetically, *Pleurotus Djamor* mushrooms should produce at the highest yield on the blend of coffee grounds, oak mulch, and palm fronds because each substrate will contribute to maximize the nutrient content.

The UCF Arboretum supplied the oak mulch and palm mulch. Campus coffee shops supplied the coffee. All three substrates in the experiment are readily available, inexpensive, and have few alternative uses, which make them ideal substrates for this experiment.

Additional components that are typically used to increase mycelia growth and mushroom production such as rice bran, calcium carbonate, and cottonseed oil will not be used in this experiment (Stamets, 2000). This is to allow direct observation of each individual substrate's ability to provide adequate conditions for mycelia growth and mushroom production. For example, coffee already has high nitrogen content, and adding an additional source of nitrogen is unnecessary. Also, adding supplements to the substrate increases production costs and creates a need for stricter sanitation (Stamets, 2000).

The species, *Pleurotus Djamor*, was chosen for this experiment for many reasons. *Pleurotus* cultivation popularity has recently increased due to its desired taste as well as numerous nutritional and medicinal benefits. In 2008, oyster mushrooms rose to the third most commercially produced mushroom in the world (Dundar, Yildiz, 2008). *P. Djamor* prefers tropical and subtropical regions and is known for its "speed to fruiting, adaptive ability to flourish on a wide variety of base materials, and high temperature tolerance" (Stamets, 2000). Also, *P. Djamor* grows on a wider range of forest and agricultural waste products than any other species within the basidiomycota phyla. In past research, *P. Djamor* has been cultivate on most all hardwoods, on wood by-products (sawdust, paper, pulp sludge), all the cereal straws, corn and corn cobs, sugarcane bagasse, coffee residues (coffee grounds, hulls, stalks and leaves) banana fronds, cottonseed hulls, agave waste, soy pulp, and numerous other materials containing lignin and cellulose (Stamets, 2000).

Further research within this genus has been stressed due to the species' adaptability. The potential to utilize otherwise considered waste products has been studied as a means of reducing hunger in developing nations (Anoliefo et al., 1999). Other research has been performed about the bioremediation abilities of *P. Djamor*. When grown on materials containing significant levels of mercury they have been found to accumulate these heavy metals from the ground. Growing mushrooms in such conditions may not be useful for human consumption but can play valuable role in the bioremediation of contaminated ecosystems (Bressa et. al, 1988). In a study done by Paul Stamets, oyster mushrooms were used to breakdown residual oil in soil in a lot near a high traffic area resulting in significantly less oil in the soil and mushrooms free from petroleum residues (Stamets, 1999). *Pleurotus spp.* have also been shown to stun and digest nematodes for access to necessary nitrogen, which can be a nuisance in agriculture and water quality (Thorn and Barron, 1984).

## Methods

| | Coffee | Oak Mulch | Palm Mulch | Blend |
|---|---|---|---|---|
| Trial 1 | x3 | x3 | x3 | x3 |
| Trial 2 | x3 | x3 | x3 | x3 |

**Table 1: Testing the substrates consisted of 3 replicates of each substrate, for a total of 24 jars. Overall, there were 6 jars of coffee ground substrate, 6 jars of oak mulch substrate, 6 jars of palm debris substrate, and 6 jars with a proportional mixture of all three substrates.**

This experiment was designed to test the productivity of 4 different recycled substrates and their ability to produce the largest final mass of *Pleurotus Djamor*. A total of six trials were performed for each substrate, creating an overall test of 24 substrates (Table 1).

A 25 lb. bag of *Pleurotus Djamor* Sawdust Spawn was ordered from an online distributor (www.everythingmushrooms.com). The recycled substrates were collected directly from the University of Central Florida campus to portray the accessibility and sustainability of the chosen substrates. The UCF Arboretum provided the oak mulch and palm mulch. A local campus coffee shop provided the coffee grounds.

**Figure 3: Above- Substrates were submerged in water in order to reach 100% moisture content. Below- To reach 55-65% moisture percentage, substrates with excess moisture were baked at 350°F for 15 min intervals.**

The growing mediums (coffee, oak mulch, and palm mulch) were collected in separate five-gallon buckets and submerged in water. The substrates were soaked for about three hours to ensure 100% moisture content. The wet substrates were then laid out on tarps to sundry for 3 hours.

Initial moisture content is of utmost important for proper mycelium growth. Many hobbyists use a method called the palm test to determine proper moisture. The palm test is determined by squeezing a handful of substrate. If the substrate is squeezed and it drips, the moisture content is too high. Optimal moisture content is achieved when wet substrate is squeezed and no water drips out (Stamets, 2000). There is another method called the oven method (described below). This method is typically preferred by scientists. In this experiment, the oven method was used to get more consistent moisture content in the substrates.

| Initial Moisture Content Per Substrate | | | |
|---|---|---|---|
| | Coffee | Palm | Oak |
| Trial 1 | 62.70% | 58.70% | 62.43% |
| Trial 2 | 61.60% | 57.67% | 61.79% |

**Table 2: The initial moisture percentage for both trials. Moisture percentage was determined by subtracting the dry mass from the wet mass, dividing the total quantity by the dry mass, and multiplying by 100.**

The mass of one cup of each substrate was found using a gram scale. The dry substrate mass was subtracted alongside the experiment by placing a cup of each substrate in the oven at 350°F for 1 hour. The dry and wet masses were used to find the percent moisture content, ideally aiming for 55% to 65%. Proper moisture is essential for mycelium colonization, and 55% to 65% moisture content is the optimal condition (Stamets, 2000). If the percentage was too low, water was added, and if the percentage was too high, the substrate was baked at 350 degrees for15 minute intervals.

The substrates were then placed in baking trays, covered, and heated to 160° F for one hour by oven. Each substrate was then placed into an individual autoclaved wide mouth glass quart jars. *Pleurotus Djamor* spawn was layered within the substrate to ensure thorough colonization In order to minimize risk of contamination; this step was completed under a laminar flow hood. The jars were then capped, labeled accordingly, and placed in a dark incubation room at ideally 75-85° F.

**Figure 4: Capped jars were placed in a dark incubation room at 75-85° F.**

Over the colonization period, the amount of mycelium growth in each jar was observed every two days. The data was recorded based on visual observation of the quickness and quality of mycelium growth per substrate. Due to time constraints of this class, the data cut off point was collected before full mycelium colonization. Therefore, results were recorded in mycelium growth. However, the experiment continued and just a few days after the data cut off point, the jars were fully colonized and ready to fruit.

At around 100% colonization (about 24 days), the jars were uncapped and placed in a terrarium containing 100% humidity for fruiting. The jars were wrapped in aluminum foil to encourage vertical growth.

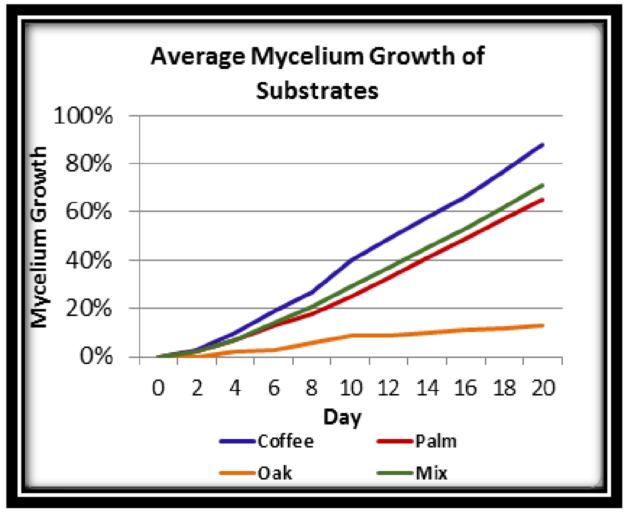
**Figure 5: Jars covered in aluminum foil in terrarium.**

## Results

The results of this experiment were measured as a percentage of visible mycelium growth rather than mass of fruiting bodies due to time constraints. As visible in Figure 6, coffee had the overall fastest colonization time within this experiment. Coffee was followed by the mixed substrate, which had the second fastest colonization time, and palm mulch finished third. Oak mulch had barely any colonization remaining less than 20% throughout the experiment.

Additionally, the second run of the experiment had faster development times than the previous run. This held true for all four substrates being tested. The mixed substrate's second trial results developed at the same pace as the first trial of coffee, only being surpassed at the end. The second trial of palm mulch surpassed the growth the first mixed trial at the end of collection as well (Figure 6).

The findings that coffee produces the highest yield, contradicts the original prediction that the mixed substrate would have the greatest success in growing mushrooms.



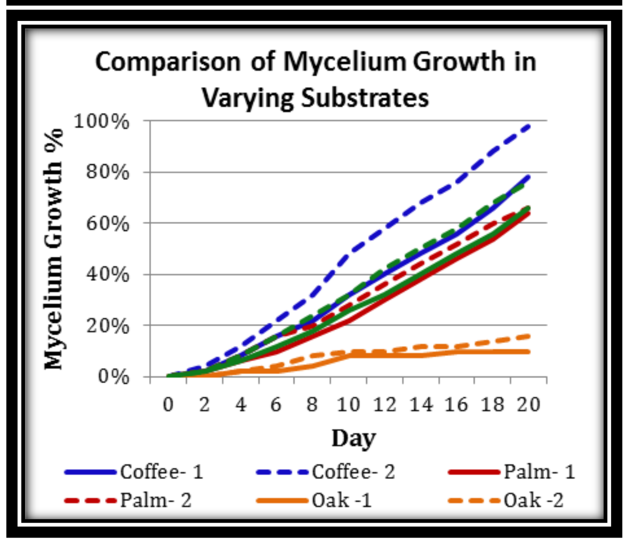




**Figure 6. Above-The overall growth rates averaged among the two trials. Below- Trial 1 is represented solid lines and Trial 2 with dashed lines.**

## Discussion

Contrary to the original hypothesis that the mixture of palm mulch, oak mulch, and coffee grounds would colonize at the fastest rate, the coffee grounds used as a substrate gave the quickest colonization times. The mixed substrate had the second quickest colonization, the palm mulch came in a close third, and oak mulch mycelium growth never surpassed 20% growth. As a result of this, it is believed that by oak mulch being in the mixture, it may have decreased the nutritional value of it enough to put it at a disadvantage when compared to coffee alone, which is extremely nutrient rich. If not the nutritional value, then the high density of oak mulch pieces may have been the culprit for the slow rate of mycelium growth. While mushrooms are one of the few things able to break down lignin, a very complex polymer found in wood, it can cause growth to be a much slower process as the nutrients are not as easily accessible as in other substrates, ie. coffee (Buswell & Odier, 1987).

Although coffee had the quickest colonization rates, it was later determined that the mixed substrate is the most sustainable because of coffee's vulnerability to environmental factors. Despite proper pasteurization techniques, the nutrient richness of coffee attracted other types of fungi and some jars were contaminated. This was visible through a green mold growing throughout 3 of the jars. Contamination such as this would be detrimental to mushroom growers. In Pennsylvania in the 1990s, the mushroom industry experienced losses of between 30-100% as a result of this mold (Tisdale, 2004). Coffee was also determined to be less stable than the other substrates. At one point late in the experiment, the humidifier stopped for a brief period of time (approximately 5 hours), which caused the coffee substrate pull away from the sides of the jar and mushrooms to slightly shrivel, whereas the other substrates remained stable. Coffee's inability to maintain moisture content would be unfavorable in larger outdoor experiments that could potentially be faced with periods of drought and flood.

Due to time constraints of the project, the experimental data was collected before fruiting bodies emerged. Therefore, instead of determining the efficiency substrates by comparing mushroom mass, the mycelium growth was analyzed. As a result of this, there may be some bias in the mycelium growth results reported as they were based on outward visual appearance, and inner growth in the jars could not be properly accounted for. This was discovered after the appearance of mushrooms in all of the jars. These results were unexpected, especially in the case of oak mulch, where barely any mycelium growth had been observed. It is believed that even though the mycelium growth may not have been visible, it was still occurring in the middle of the substrate, where it was not visible. It is possible this is due to the density differences among the substrates, something like coffee grounds having a very low density and being easy to maneuver through, compared to chunks of oak mulch being hard to break apart.

**Figure 7. Mushrooms growing on the coffee substrate.**

Mushrooms started to appear only a few days after the experimental cut off point, and shocking observations were reported. Even the oak mulch, which visually had the least mycelium growth produced mushrooms. A future experiment analyzing the mass of fruiting bodies will provide more information about the effectiveness of growing on each substrate.

As far as measuring the percentage of substrate converted to compost, mushrooms grow as they decompose more and absorb more from their substrate. As a result of this, it is reasonable that the larger the mushrooms are, the more the soil has been broken down into compost. Based off of these results, both coffee and the mixed substrates were composted the most, as they produced the largest mushrooms, presumably from degrading the soil at a higher rate. If removal of waste is the main focus of future growers, then mixed substrate would be the best option as it allows for conversion of three types of waste into compost rather than just one.

This can cycle back into the concept of sustainability. By using the spent substrates, they can then be composted and eventually converted into compost to add nutrients back into the soil. Coffee grounds are especially a readily available source of "waste", which is highly beneficial to the process and will allow for a continuous supply of substrate to produce at high yields for the community.

Some may see placing all jars in the same terrarium as another possible bias in this experiment; however, even if spores transfer substrates during the cultivation period, the spores will not have enough time to develop into fruiting bodies and will therefore not affect the final mass, creating no experimental bias. Also, there is no need to worry about cross contamination of species since all of the spawn used across the various substrates will be one consistent species, *Pleurotus Djamor*.

## Future Work

The findings of this experiment have led to further questions being asked. After discovering that coffee rather than mixed grew the fastest, possibly being due to high lignin content/high density or low nutritional value of oak mulch in the mix, the experiment was rethought out. The experiment should be repeated in the future with a mix of coffee and palm alone. Predicted results from this experiment are shown in Figure 8.

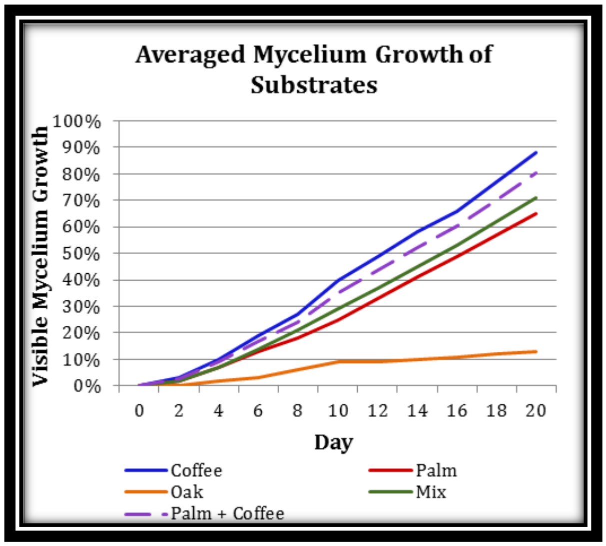



**Figure 8. Previous experiment results along with predicted results for a palm and coffee mixture (dashed line).**

In theory, the combination of palm and coffee without oak will be the best combination of the three. This is because the palm much should decrease the risk of contamination as it will just slightly lower the richness of the coffee. Additionally, the oak mulch will not be an obstacle for mycelia growth. As well, by using a combination of two of the three rather than one alone, more waste will be able to be composted.

An additional issue in this experiment was finding the exact moisture content of the substrates in the beginning. Eventually, the oven method was decided on, but even though this was the most precise method found, it was still not exact. While pasteurizing the substrates in the oven, some moisture tended to escape through the lids of the containers, so the final exact moisture content was most likely 1-5% off of what it had prior to this process.

As a result, a further experiment is planned in which autoclavable bags will be filled with dried substrates (0% moisture content) to start. The amount will be weighed and 65% water will be added to the bags. The bags will then be sealed shut and autoclaved. This process should theoretically cause the water within the bags to steam the substrate throughout, thus creating a completely uniform moisture content within the bags. The substrates should then give more consistent results.

## Acknowledgements

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