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Development and Characterisation of Mycelium Bio-Composites Produced from *Pleurotus ostreatus* by Using Paddy Straw and Cotton Wastes

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Mycelium biocomposites are a combination of mycelium and agricultural waste or organic matter from industrial waste. Agricultural productivity has increased due to the growing need to feed the world's population. The lack of studies on the treatment of this waste can lead to significant financial losses and pose a significant risk to human health due to environmental pollution, as most of it has been burnt in open areas. Fungi can solve this problem by utilising agricultural waste as a source of sustainable building materials through the growth of mycelium. Therefore, the study focussed on the local production of mycelium. The surface area, weight, moisture, density, morphological analysis and compressive strength were measured to determine the physico-chemical and mechanical properties of mycelium in different substrates. 100 g of rice straw and cotton soaked in hydrated lime were mixed with 3 g of *Pleurotus ostreatus* mushrooms in a transparent plastic bag, and the mycelium grew for about two months. The results show that paddy straws have a larger surface area (179.33cm2) than cotton (129.67cm2), which is due to the different surface dimensions (size, shape and weight). However, the compression resistance at 20% deformation test shows that cotton has a higher result, 0.09130 MPa and 0.01619 MPa for rice straw. This study shows that the development of mycelium in cotton and rice straw shows positive results. Their mechanical and physical properties also vary depending on how well they absorb water and how different their densities are. It is advisable to conduct further research in the future to determine which substrate is most suitable for a particular type of mushroom to be used and to standardise size, shape and weight to obtain reliable results.

Keywords: *Pleurotus ostreatus*; mycelium; substrate; physico-chemical properties; mechanical properties

I. INTRODUCTION

Pleurotus ostreatus is a species of fungus that has a growth characteristic that allows it to thrive in very humid conditions. For example, Pleurotus ostreatus has easy-to-develop hyphae and can grow fruiting bodies at room temperature (Hoa & Wang, 2015). It can also grow by using substrate, a mixture of natural ingredients specifically made for mushroom cultivation, such as grain spawn. Agricultural waste can also serve as a substrate and provide the necessary amounts of carbon and nitrogen for fungal metabolism (Suwannarach *et al.*, 2022). Examples of agricultural wastes include sugarcane bagasse, maize straw, wheat straw, rice

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husk and rice straw, which are usually burnt or left behind after harvest (Oluseun *et al.*, 2021). As all these wastes are rich in fibres and nutrients, the mycelium spreads over the substrate, bonding the lignocellulosic substrate particles together through the adhesive properties of the fungal mycelia (Sun *et al.*, 2022). It can then develop into mushroom-like fruiting bodies.

In addition, fungal mycelium is used as a natural adhesive material to create a complex of mycelium and organic substrates, which is called mycelial biocomposites (Alemu *et al.*, 2022). Both the type of fungus and the substrate influence the quality of the composite. They are in high demand as alternative materials in the construction industry due to their low carbon footprint, low energy and processing costs, biodegradability and a variety of appealing properties (Alaneme *et al.*, 2023). Mycelium can be grown into precise shapes and used after drying as an extremely durable, mould and fire-resistant building material (Dessi-Olive, 2022), minimising the effort required for processing.

The mechanical and physico-chemical properties of the mycelial biocomposite are improved by the addition of suitable fungi and agricultural waste (Peng *et al.*, 2023). The mycelium must have high compressive strength and be tough to prevent shearing (Gou *et al.*, 2021). Temperature is also an important factor to increase the strength of the mycelium during the compression process. Peng *et al.* (2023) reported that the sawdust substrate had the highest initial density and the hardest texture, resulting in the highest compressive strength. The results of Lingam *et al.* (2023) showed that bagasse and Junicao grass- mycelium composites had higher density of mycelium growth and higher compressive and flexural strength (63.4 and 399.39 kPa, and 13.81 and 78.34 kPa, respectively). According to some studies, the mycelium of the fungus *Pleurotus ostreatus* also grows differently depending on the temperature (Hu *et al.*, 2023). Based on these results, the hardness of mycelial biocomposites depends on the type of fungus and suitable agricultural waste.

II. MATERIALS AND METHOD

Pleurotus ostreatus is the fungus used in this study. The fungus was grown to the mycelium stage. Other materials used were coconut palms, rice straw and cotton, which serve as substrate for the mycelium, hydrated lime and water. The equipment used was a 2-litre beaker, a balance, a ruler, a digital microscope, a pH paper indicator, a container for soaking, 'tempeh' plastic and an INSTRON universal testing machine to test the compressive strength of the substrate.

A. Preparation of Fungi

The *Pleurotus ostreatus* spawn was purchased from a local source. The spawn then stored at room temperature and in the dark area.

B. Preparation of Substrate

The substrates used were cocopeat, paddy straw and cotton. Prior use, a total of 500 g of cocopeat, paddy straw and cotton that were soaked in 3 L of water with 7 g of hydrated lime for eight hours in different containers. During this step, the substrate, water and hydrated lime were thoroughly mixed for the first two minutes to guarantee their homogeneity (Muswati *et al.*, 2021). These agriculture waste will be use as a substrate for the development of hyphae that adhere to the surface of the substrate (Gou *et al.*, 2021). After eight hours, the water was drained off before the substrate were packed into a clear plastic bag. The substrate in every plastic bag will be weighed to obtain an equal weight around 100 g.

C. Growing the Mycelium in Agriculture Waste

About 5 g of spawn was added into the substrate pack to form the mycelium bio-composites in this experiment. The transparent plastic was used to enable researchers to observe the mycelium with an unaided eye. The plastic was tightly sealed and placed in the dark at room temperature. The days of mycelium growth were counted together with the moisture, pH and environment temperature data to observe the mycelium growth (Zhao *et al.*, 2022).

D. Measurement Growth of Mycelium

1. Surface area measurement

The surface area was measured every 5 days until 60 days or until it had full coverage of mycelium in the substrate. It was measured by drawing the mycelium development on transparent paper and redrawing it on graph paper. The measurement was taken in square unit (cm^2).

2. Weight measurement

The weight of grain and substrate before and after mycelium growth was recorded. It is important to know how much water evaporates from the sample.

3. Morphological analysis

Using a digital microscope, morphological characterisation was performed in order to assess the microstructural arrangement of the mycelium. The images from the microscope were processed to discuss the morphological characterisation and to reveal the present of mycelium, substrate coverage, and failure part before or after compression. It was connected to the computer, and the picture was taken at resolution 640×480.

E. Determination of Physico-chemical and Mechanical Properties

1. Moisture

The moisture was calculated with the weight of before and after drying the sample. The formula used was stated as below:

$$Moisture\ \% = \frac{weight\ of\ wet\ sample - weight\ of\ dry\ sample}{weight\ of\ wet\ sample} \times 100 \qquad (1)$$

2. Density

The density of mycelium is crucial for mechanical testing, especially in compressive resistance. The formula used is stated as below:

$$Density\ \left(\frac{g}{cm^3}\right) = \frac{weight\ of\ dry\ sample}{length \times width \times height} \qquad \text{qq} \qquad (2)$$

3. Compressive strength test

Compression tests were conducted using an INSTRON universal testing machine under displacement control and all the tests were performed in ambient conditions (25 °C and ~50% relative humidity). This test was conducted at Universiti Putra Malaysia (UPM), Serdang.

4. Statistical analysis

In this research, the data results were expressed as mean±standard deviation of triplicate results. The results were analysed by using a T-test to compare the results from two different substrates on the growth rate of mycelium, area measurement, and mechanical testing.

II. RESULT AND DISCUSSION

A. Growth of Mycelium in Substrate

The prepared mixture of substrate undergone for two months of incubation until the mycelium fully covered the surface as shown in Figure 1 and Figure 2. Based on the figures, a positive result of mycelium development. is shown According to Girmay *et al.* (2016), early mycelial colonisation in the paddy straw substrate commonly took 11.5 days. In this experiment, early mycelial growth was on day 2. The day for mycelium growth may differ because the quantity of substrate used was different.

Figure 1. Mycelium coverage in paddy straw for two months

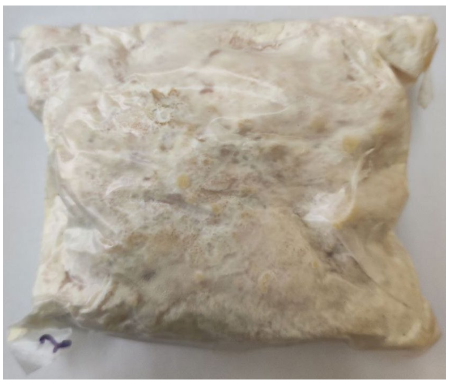

Figure 2. Mycelium coverage in cotton for two months

B. Physical Characteristics of Substrates

Pleurotus ostreatus was cultivated until the mycelium stage in paddy straw, and cotton. Before mycelium growth, each substrate was weighed 100 g and 3 g for grain spawn of

Pleurotus ostreatus. After mycelium growth, it shows that there was a reduction in weight for both samples. The reduction of weight after mycelium growth for paddy straw was 72.83 ± 2.20 g and it was higher than cotton which is 91.23 ± 1.64 g. The result for the physical characteristics of the substrate is summarised in Table 1. From the data, it can be seen that cotton holds more moisture than paddy straw. Cotton can raise absorbency means that it can hold onto water for a longer period before evaporating (Candido, 2021). Besides, the reduction in bio-composite weight resulted from fungal deactivation (Saini *et al*., 2023). The organic material gradually degrades while fungal biomass grows on and within substrate parts. In the end, hyphae emerge from the substrate into the air, covering the substrate in a fluffy or compact layer. This mycelium produces enzymes that break down polymers in the substrate into substances that can be used up to serve as nutrients (Appels *et al*., 2019). The mass loss also may be attributed to water evaporation from samples and internal moisture loss of mycelium.

Table 1. Weight and pH of sample during growth

Substrate	pH	Initial weight	Final weight
Paddy straw	11	103	72.83±2.2
Cotton	11	103	91.23±1.64

C. Area Measurements of Mycelium on the Surface

Hyphae are long, filamentous cells that the fungi use to colonise their substrate (Manan *et al*., 2021) and they grow both on and inside the substrate (Vidal-Diez de Ulzurrun *et al*., 2017). In this study, area measurements of mycelium on the surface that penetrated the sample were recorded starting from day 5 into day 60. The results are summarised in Table 2. From the table, the highest mycelium area on the surface was paddy straw which is 179.30 ± 11.00 cm². Paddy straw is rich in nutrients and simple to decompose, making it an excellent substrate for growing mycelium (Sayner, 2022). It also has a porous structure which makes mycelium penetrate faster than cotton. In addition, from day 5 to 15, cotton shows a faster development of mycelium compared to the paddy straw. Since the cotton structure was packed more than paddy straw that has a porous structure, mycelium of cotton was spread on the surface first before entering the middle part of the substrate. According to Schoder *et al.* (2024), Each type of substrate has a significant impact on the pace of mycelium development since the hyphae contact with it directly.

Table 2. Mycelium area coverage in the sample in cm ²

Day/ Sample	Paddy straw (cm²)	Cotton (cm²)
5	2.00±1.00	3.67±0.58
10	8.00±1.73	14.33±4.51
15	25.00±5.00	44.33±2.31
20	56.33±3.21	54.33±2.89
30	89.30±10.10	68.00±2.00
40	112.30±10.8	93.00±4.36
50	132.00±8.19	111.67±6.43
60	179.3±11.00	129.67±6.51

D. Morphological Analysis

Mycelium characteristics in paddy straw and cotton are shown in Figure 3 and 4. In Figure 3 and 4, there was a formation of hyphae and mycelium observed clearly. Hyphae are filamentous structures that resemble threads. Almost all hyphae extend at the tips, expanding outward from the site of formation. According to Samson (2016), hyphae have a stable diameter, and freely mycelium expands to create a circular colony on solid substrates that allowing the fungal growth. The abundance of hyphae provide strength to the compost mixture.

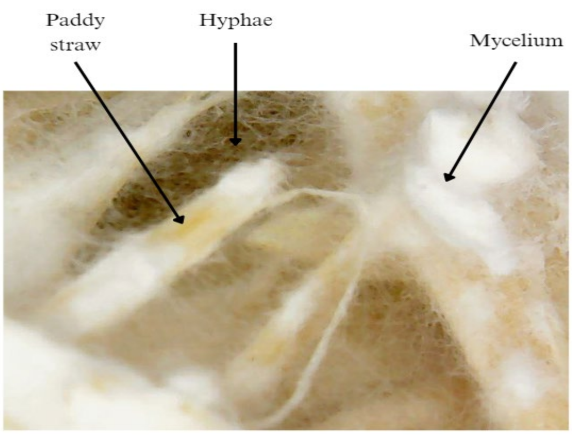

Figure 3. Mycelium in paddy straw

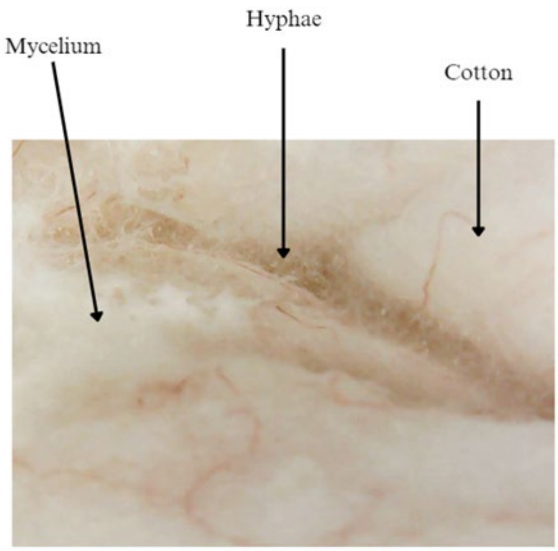

Figure 4. Mycelium in cotton

E. Mechanical Properties of Mycelium Composites

There are various mechanical properties based on the substances that can be tested including plasticity, durability, fragility, strength, toughness, stress resistance, rigidity, elasticity and ductility are a few examples of mechanical properties (Saleh, 2022). In this study the compressive strength is chosen based on the suitability of the sample used in this experiment. Prior to the compressive strength test, the full-grown compost of paddy straw and cotton underwent a drying method. According to Appels *et al.* (2019), the material can be dried or heated to prevent fungus development. By drying, the fungus is kept in a state known as "hibernation," which enables it to begin growing again when favourable moisture conditions return. However, the fungus will be killed by heating. Figure 5 and 6 show the sample that already undergoes the drying method.

Figure 5. A dried sample of paddy straw

Figure 6. A dried sample of cotton

Based on Figures 5 and 6, all of the samples' surfaces were regionally yellow. According to Peng *et al.* (2023), the Maillard reaction between the fungal cell wall, sugars, and proteins in the plant substrate may be the reason for this change in appearance. It also resulted from the material's polysaccharide caramelisation and organic matter's thermal deterioration. The Maillard reaction will occur when introduced to heat and amino acids will interact chemically with reducing sugars (Tamanna & Mahmood, 2015).

Table 3 presents the weight reduction following the drying process, where the mean weight loss for paddy straw was 36.20 g, whereas cotton exhibited a significantly higher mean weight loss of 64.06 g. The moisture content was calculated based on the initial sample weight of 103 g and its post-drying weight, revealing that cotton retained a greater percentage of moisture compared to paddy straw. This higher moisture retention is statistically significant and suggests that cotton can hold more water, which may enhance mycelium growth. Prior studies support this finding, as Zapach (2023) reported that insufficient humidity negatively affects mycelium proliferation. Additionally, Elsacker *et al.* (2019) observed that mycelium naturally contains over 60% water content, confirming the necessity of moisture for its development. However, as Yang *et al.* (2017) noted, to optimise mechanical performance and halt further growth, excess moisture must be removed.

Table 3. Weight and moisture profile of mycelium composites

Substrate	Weight (g)		Moisture (%)
	Initial	After drying	
Paddy straw	72.83±2.2	36.20±3.97	64.85
Cotton	91.23±1.64	27.17±6.09	73.62

The compressive strength data are presented in Table 4. Both cotton and paddy substrates in this study produced a cracking sound during the compressive strength test, likely due to the mycelium breaking within the substrate, behaving similarly to an open-cell foam under compression and exhibiting a hysteresis effect under cyclic loading (Islam *et al.*, 2017). Cotton has a higher density which is 0.753 g/cm³ than paddy straw which is 0.151 g/cm³. The compressive resistance for cotton, 0.09130 ± 0.02404 MPa was higher than paddy straw, 0.01619 ± 0.00579 MPa.

Paddy straw exhibited a lower compressive strength than the Ecovative grow kit (0.055 MPa), despite both materials having comparable densities (0.151 g/cm³ for paddy straw and 0.055 g/cm³ for the Ecovative grow kit). This variation in compressive strength may be statistically significant and is likely influenced by the fungal species present in the substrate. Specifically, Pleurotus fungi, used in paddy straw-based mycelium block fabrication, are known to decompose cellulose at a higher rate, potentially weakening structural integrity (Aiduang *et al.*, 2022). In contrast, the Ecovative grow kit likely incorporates a different fungal strain with enhanced mechanical properties, resulting in greater compressive strength.

Table 4. Compressive strength data of mycelium composites

Substrate	Density (g/cm³)	Comprehensive resistance at 20% deformation (MPa)
Paddy straw	0.151	0.01619±0.00579
Cotton	0.753	0.09130±0.02404

In addition, the pH, type of fungi, type of substrates, and physical characteristics play an important role in getting a stronger compressive resistance. Besides, in this study, the mycelium bio-composite did not get any additional water during incubation and was sealed in transparent plastic, so the substrate needs to have characteristics that can hold moisture for a longer time. Consequently, there are various studies have been conducted to observe which types of mushrooms are most suitable for specific substrates (Chang & Miles, 2004; Phan & Chang, 2000; Rogerson, 2004). These studies collectively highlight the importance of matching specific mushroom species to their ideal substrates for optimal growth, and they represent key sources in advancing mushroom cultivation techniques.

III. CONCLUSION

In conclusion, mycelium bio-composites using paddy straw and cotton demonstrated full mycelium development within approximately two months. Additionally, it has been established that substrates with higher moisture content accelerate mycelium growth. Consequently, this enhancement also improves mechanical properties by increasing the sample's compressive strength. Based on the findings, cotton outperforms paddy straw in terms of moisture retention and compressive strength.

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