Fabrication and characterization of mycelium-based composites from *Lentinus squarrosulus* and *Pleurotus ostreatus* with improved physico-mechanical properties for versatile applications

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***Abstract*— This study explores the potential of fungal mycelium-based plate-like composites as sustainable and cost-effective alternatives for synthetic materials. Locally available lignocellulosic waste and fungi isolated locally were used to fabricate mycelium composites. The medium primarily consists of Albizia sawdust and the fungi *Lentinus squarrosulus* and *Pleurotus ostreatus* were used. All the composites identified here were subjected to analyses based on scanning electron microscopy, moisture content, dry density, water absorption, flammability, thermal stability, and compression strength following ASTM and ISO standards. The mycelium plates produced with *L. squarrosulus* mycelium (ASL) exhibited lower dry density and less water absorption. The ASL mycelium plates exhibited a soft and foamy appearance, whereas the *P. ostreatus* material (ASP) appeared dense mycelium growth only in the top and bottom parts of the plate. The UL-94 rating demonstrated that both samples exhibited superior flame retardancy properties (rated as V1) compared to commercially used expanded polystyrene (EPS). These variations were due to the difference between mycelium density. Therefore, these new bio-composites have the potential to replace conventional packaging or interior construction materials.**

Keywords- biodegradable, biocomposites, fungi, mycelium, synthetic materials

I. Introduction

The rapid growth of global population has led to environmental pollution and the depletion of natural resources. The utilization of foam-based packaging materials like Styrofoam for protective packaging has contributed to environmental pollution in terrestrial and aquatic ecosystems. These materials are associated with high costs, energy consumption, and environmental unfriendliness [1].

There is potential in developing sustainable materials through a combination of fungal mycelium and organic substrates. Mycelium, which is the vegetative part of fungi consisting of long, branching, filamentous structures known as hyphae, serves as a natural adhesive. When the growing process of mycelium on organic substrates is halted through drying, it yields mycelium-based materials, often referred to as biocomposites [2]. One example of such a product is mycelium based composite plate or Mycelium plates, which is composed of organic substrates and employs mycelium as a natural adhesive.

Existing research has demonstrated the versatility of mycelium-based materials in various applications, including insulation, partition walls, packaging, utensils, and furniture, as well as different design and architectural purposes. They are entirely biodegradable and possess a lower carbon footprint when compared to conventionally manufactured packaging materials. The quality of these products can be improved through methodological variations, such as altering the types of substrates used, selecting specific strains, adjusting cultivation durations, and modifying molding techniques and temperatures.

This study primarily focuses on utilizing organic waste materials to produce alternative and cost-effective solutions by using fungal mycelium from two species of fungi as a natural adhesive.

II. Materials and Methods

A. Strain Cultivation

The fungal strain *P. ostreatus* was obtained from the Mushrooms Development and Training Centre, Rathmalana, Sri Lanka. *L. squarrosulus* (GenBank accession number; PP

001103) mushroom species was isolated from Kottawa, Sri Lanka. Potato Dextrose Agar (PDA (39 g/L) was used for the growth of the strain after autoclaving at 121°C for 15 min.

B. *Spawn Preparation*

Rice grain purchased from the local market was cleaned and soaked. Then the grain was spread on a water-permeable cloth to remove the excess water. Glass bottles filled with 100 g (on a wet weight basis) grain were autoclaved at 121°C for 20 min and allowed to cool overnight in an aseptic condition. After that fungal cultures from the petri dish were inoculated and incubated at 27°C until the substrate was fully colonized.

C. *Production Phase*

Albizia sawdust samples were collected from Morawaka Sri Lanka. The substrate was selected due to its abundance and based on our preliminary studies. The average size of the substrate was obtained by sieving it manually.

Fig. 1. Image showing Mycelium plates made from different fungal strains: ASL- *L. squarrosulus* with Albizia sawdust, ASP- *P. ostreatus* with Albizia sawdust

Sawdust was soaked in tap water overnight and drained off the excess water until moisture content became 60% to 70%. Sawdust samples were sterilized in an autoclave at 121°C for 20 min and allowed to cool overnight. The substrate was inoculated with 10% spawn and mixed manually. Mycelium was filled to PVC moulds and samples were kept in a dark room at (27 ± 1°C) until mycelium fully colonized the substrate. After 10 days in the moulds, the samples were removed and dried in a convection oven at 80˚C for 10 to 12 hours until their weight stabilized (Fig. 1).

III. Physical and Mechanical Characterization Techniques

A. *Moisture content*

The wet basis moisture content of the samples was calculated following the ISO 16979:2003 [3], with the formula:

$$M = \frac{(w_w - w_d)}{w_w} \times 100 \quad (1)$$

Where:

M= moisture content [%], Ww = initial mass [g], wd = mass after drying [g] (Table 1).

B. *Dry density*

The dry density of the composite materials was calculated following ISO 9427:2003, with the formula:

$$V = \frac{Weight\ of\ Dry\ Material\ (kg)}{Volume\ of\ Material\ (m3)} \quad (2)$$

C. *Capillary water absorption*

The capillary water absorption of the composite materials was calculated following ISO 15148:2002(E), with the formula:

$$C_w = \frac{(m_i - m_d)}{(A \cdot \Delta\sqrt{t_i})} \quad (3)$$

where C_w = capillary water absorption coefficient [kg/(m2.h0.5)], mi = weight of the specimen at any time ti [kg], md = dry weight of the specimen [kg]; A = surface area of the specimen in contact with water [m2], ti = time [h].

D. *Scanning Electron Microscopy (SEM)*

The surface morphology of the composites was analyzed using SEM (ZEISS EVO 18, Germany).

E. *Thermogravimetric analysis*

Thermogravimetric analysis was conducted on a TGA5500 instrument made in Germany. The temperature ranged from 30 ^{0}C to 700 ^{0}C with a heating rate of 10 ^{0}C/min under nitrogen (N_2) atmosphere conditions.

F. *Compression test*

Samples were tested for compression strength following ASTM D638 on a testometric DBBMTCL-500kg–1.5-000 machine.

G. *Flame retardancy test*

Five specimens for each composite sample and EPS sample were tested for fire resistance by vertical burning (UL-94 V) and the horizontal burning (UL-94 HB).

IV. Results and Discussion

A. *Foam appearance and structure*

The two materials that were produced from Albizia sawdust and two different fungi strain (Fig. 1) showed different visual characteristics. Both materials showed a white colour surface. The composite produced from Albizia sawdust with *L. squarrosulus* (ASL) showed rough texture than the composite produced from Albizia sawdust with *P. ostreatus* (ASP). However, the ASP appeared tougher at visual inspection. The ASL looked foamy and appeared flexible than the ASP. The cross-section of ASP showed that fungal colonization was dense close to air-exposed sides of the material when compared to the material center.

B. *Physical characteristics of mycelium plates*

The physical properties of the current mycelium plates were affected by fungi strain. The moisture content of the composites was not statically significance. The ASP composite had increased density and increased water absorption, 257.0 kg/m3 and 66.25% respectively (Table 1). The ASL composite had decreased density and decreased water absorption, 170.6 kg/m3 and 63.46% respectively (Table 1). However, fungal colonization is positively affects to the density of the material. On the contrary, an extensive colonization leads to complete degradation of the feeding substrate, which causes a decrease in density. In this case, ASL composites were more colonized than ASP composites. The extensive fungi colonization may be the reason for decreased density and decreased water absorption.

Compressive strength is one of the most important material properties, particularly for packaging applications that are intended to protect the inside contents from mechanical damage. The studied mycelium composites differed in their compressive strengths (Fig. 2). The ASP composites showed higher compressive strength (0.15 MPa). The ASL composites had 0.13 MPa compression strength due to their higher fungal colonization.

TABLE I. THE MEAN AND STANDARD DEVIATION OF MYCELIUM PLATESS AT DIFFERENT PROPERTIES AND FUNGAL SPECIES

Properties	**Statistics**	**ASL**	**ASP**
Moisture Content (%)	Mean	87.00^{a}	85.80^{a}
	Std. deviation	0.70	1.78
Density (kg/m3)	Mean	170.6^{b}	257.0^{a}
	Std. deviation	8.29	21.39
Water absorption (%)	Mean	63.46^{a}	66.25^{a}
	Std. deviation	1.62	2.51
Compression strength (MPa)	Mean	2.79^{a}	2.99^{a}
	Std. deviation	0.20	0.65

Letters (a,b,c,d) indicate significant differences based on Tukey's family error rate at $p<0.05$ for sample-specific ANOVA

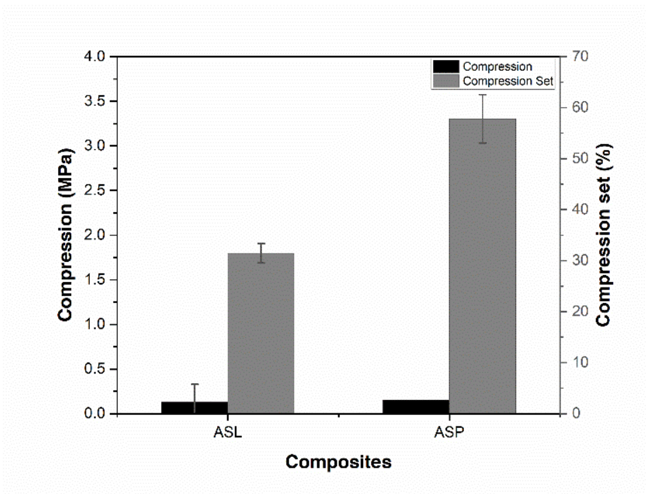

Fig. 2. Compression strength and compression set of the mycelium plates

The present findings support the previous knowledge that the compressive strength of mycelium composites is highly dependent on the substrates and mycelia strains, which also affect the morphology of the mycelium composites [4]. Other than the substrate, the presence of chitin in the fungal cell wall has been suggested to provide mechanical strength to mycelium composites, as chitin is aggregated into fibrils that decrease crack formation during compression and support the material structure. Proteins and lipids may function as plasticizers, while polysaccharides give stiffness to mycelium.

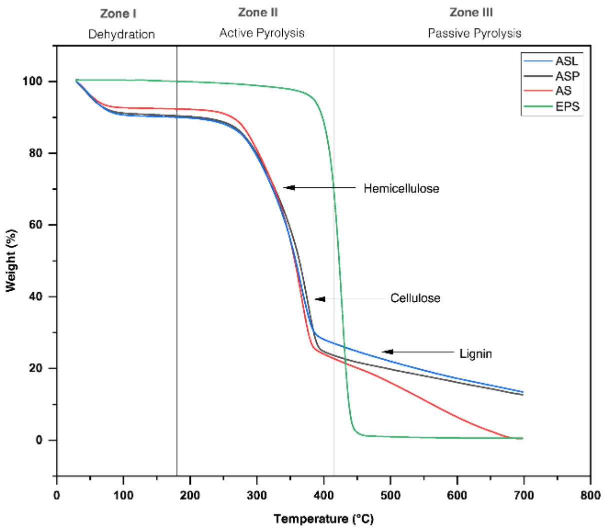

Fig. 3. TGA profile of mycelium plates

The analysis of thermal degradation patterns of produced mycelium plates indicates that the majority of degradation took place within the temperature range of 280 °C to 400 °C, as shown in Fig. 3. There was no significant difference in TGA profile up to 400 °C. The pure Albizia sawdust showed rapid degradation after 400 °C. The ASL and ASP composites were more thermal stable than albizia sawdust. This is due to the incorporation of fungi mycelium. Additionally, the composites produced from *L. squarrosulus* (ASL) showed stable thermal profile than composites produced from *P. ostreatus* (ASP). The SEM images of the composites revealed that the ASL composite has denser fungal colonization than ASP composites (Fig. 4).

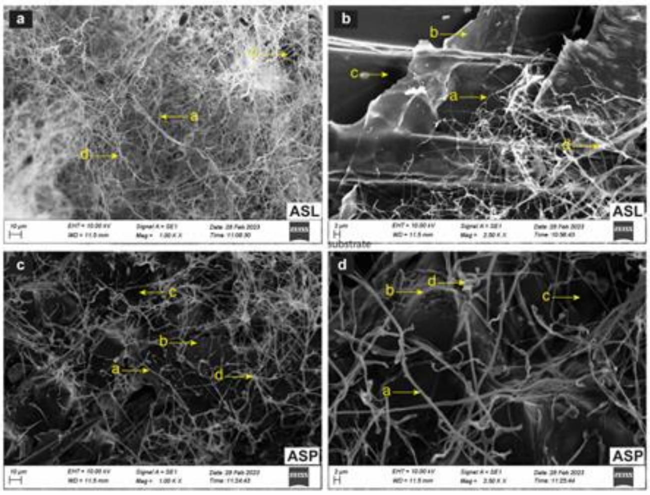

Fig. 4. SEM images of mycelium plates. (a) mycelium, (b) substrate, (c) air void, (d) hyphae anastomosis

Table 2 reports the results for the horizontal burning (UL-94 HB) and vertical burning (UL-94 V). The ASL and ASP Mycelium platess presented better results than EPS. The burning rate of the tested EPS sample was 400 mm/min. The samples with lower burning rates have flame-retardant additives. According to the UL-94 classification, V1 samples have a lower level of flame resistance compared to V0 (which is the maximum classification). V1- Samples must not burn with flaming combustion for more than 30 seconds after either test flame application and the total flaming combustion time must not exceed 250 seconds for each set of 5 samples. No flaming drips are allowed.

The EPS sample, which had a higher flammability, burned before taking the reading. The mycelium plate produced in this study fulfills the mechanical standards for applications in partition, architectural design, and insulation. They could potentially replace synthetic polymer materials such as expanded polystyrene, which is commonly used in construction. Notably, mycelium-based composites are significantly more cost-effective, with mycelium-based composites costing only $18.92 per m3 compared to $936.87 per m^3 for cement-based blocks [5]. While Mycelium plates may not match the strength, density, and water absorption standards of cement-based materials, their environmentally friendly synthesis process, local availability, and non-toxicity are major advantages. However, it is important to note that Mycelium plates technology faces challenges related to its sensitivity, as it relies on biological growth influenced by environmental factors.

TABLE II. UL-94 RESULTS OF MYCELIUM PLATES

Sample	**UL-94 Rating**	
	UL-94 HB (Burning rate mm/min)	***UL-94 V***
ASL	33.34^b	V1
ASP	51.34^b	V1
EPS	51.34^b	Not achieved

Letters (a,b,c,d) indicate significant differences based on Tukey's family error rate at $p<0.05$ for sample-specific ANOVA

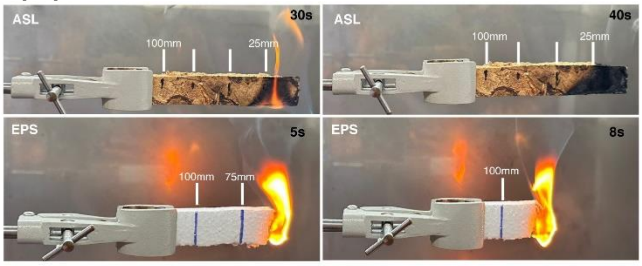

Fig. 5. Images of UL-94 HB test

Currently, mycelium-based composites are used in various applications, including packaging, insulation, partition walls, and architectural design.

V. CONCLUSION

In this study, Albizia sawdust was used to produce Mycelium plate using fungal strains *P. ostreatus* and *L. squarrosulus*. Important parameters including SEM, TGA, density, compressive strength, fire retardant, and water absorption were analyzed to confirm the standard of the Mycelium plates. The composites produced by inoculating *L. squarrosulus* mycelium demonstrated better mechanical and physical properties compared to that used by *P. ostreatus*. These findings offer valuable insights for the development of Mycelium plates with improved mechanical performance in future research.

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