

Article

Characterization of Uncoated and Coated Fungal Mycelium-Based Composites from Water Hyacinth

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Abstract: Mycelium-based composites are a promising avenue for innovating sustainable materials from the hyphae of fungi. This study focuses on the use of fibers from four local fungal species, namely, *Pleurotus ostreatus*, *Pleurotus sajor-caju* (Fr. Singer), *Auricularia auricula-judae*, and *Schizophyllum commune* Fr., to produce mycelium-based composites from water hyacinth. An inoculum of each of the mushroom species was cultivated on PDA medium at 25 and 30 °C to determine the optimal temperature based on the growth rate. The obtained optimal condition was used to grow the fungi on water hyacinth (WH) mixed with rice bran in different proportions (100% WH, 70% WH, and 50% WH) with various numbers of fungal inocula (10, 20, and 30 plugs). The obtained composites were coated with a solution of either starch, chitosan, or epoxy resin. *Schizophyllum commune* Fr. exhibited the highest growth rate and fiber density, with a growth rate of 1.45 ± 1.92 mm/day at 30 °C. Ten inocula of *Schizophyllum commune* Fr. incubated at 30 °C for seven days on a mixture of 50% WH and 50% rice bran gave the optimal composite. Coating the obtained composite with chitosan improved its mechanical properties, but coating it with epoxy resin improved its water absorbency. Buried in soil, the composite coated with a chitosan solution decomposed within 30 days. The results indicate that *Schizophyllum commune* Fr. can be used as a binder to produce mycelial composites on a substrate of WH mixed with rice bran. The implications of these results will enable the further development and tuning of mushroom-based materials, especially for the production of sustainable bio-construction materials derived from local mushrooms and bio-waste.

Keywords: coatings; mushroom; mycelium-based composite; rice bran; water hyacinth



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1. Introduction

Mycelium-based composites (MBCs), also known as mycelium biocomposites, are emerging as sustainable alternatives to conventional materials [1]. The manufacturing process of MBCs starts with the inoculation of a substrate with fungal spores under suitable conditions for mycelial growth. The mycelium binds the substrate particles together to form a cohesive composite. MBCs are most commonly produced by growing fungi on substrates of lignocellulosic residues to obtain an easily sourced and cheap, sustainable material [2,3]. Different fungi and substrates can directly influence the functional properties of an MBC and ultimately alter its potential in different applications [4]. MBCs represent a promising biomaterial that is environmentally friendly and biodegradable and is consistent with the broader application of fungal biotechnology [5]. The applications of MBCs include the production of packaging materials, household items [4], and films. These products can

replace materials made of plastics, synthetic foams, and certain wood composites [6–10]. The practical difficulties of producing MBCs are currently related to the identification of suitable fungal species, substrates, and production technologies.

The constituent structure of fungi is a network of hyphae, which are spiral branches that form the mycelium. Hyphae grow on many substrates and in a variety of environmental conditions [11]. The fungal species considered for the production of an MBC should be a species that produces leathery or woody fruiting bodies and must simultaneously cause white rot on the substrate [1]. *Ganoderma*, *Pleurotus*, *Pycnoporus*, and *Trametes* are the most commonly used fungal species, as they produce longer, thinner, and more elastic hyphae than those growing on a complex cellulosic substrate [4,6,7,12]. However, among the mushroom species widely distributed in the southern region of Thailand, four species that grow well stand out. These species are *Pleurotus ostreatus* (the Hungarian oyster mushroom), *Pleurotus sajor-caju* (Fr. Singer) (the Sarjou-caju mushroom), *Auricularia auricula-judae* (the wood ear mushroom), and *Schizophyllum commune* Fr. (the split mushroom). These fungi produce white rot and grow well on lignocellulosic material. Kohphaisansombat et al. [13] produced MBCs from *Pleurotus ostreatus* by using natural pineapple fibers (NPFs) at a level of 10% to 30% in spent coffee grounds. It was found that MBCs with 10% NPFs had the highest density and compressive strength, while those with 30% NPFs showed higher flexural strength, water absorption, swelling, and sound absorption. Oyster mushroom-based MBCs with NPFs offer promising environmentally friendly alternatives to certain manufactured products due to their practical advantages and recyclability. Ismail and Abdullah [14] used *Pleurotus sajor-caju* to produce MBCs from various substrates, including paper, rice husk ash, and rubber sawdust, supplementing each substrate with rice bran and calcium carbonate. Rubber sawdust was the most suitable substrate. Ly and Jitjak [15] produced mycelial composites using four fungi (*Pleurotus ostreatus*, *Auricularia auricula-judae*, *Lentinus squarrosulus*, and *Lentinus polychrous*) on three agricultural waste substrates: coconut husks, rice husks, and rice straw. The composites made from rice straw showed the highest compressive strength and the highest biodegradability, as measured by weight loss, while the *Lentinus squarrosulus*-based MBC from rice straw showed the highest moisture uptake. Aiduang et al. [4] found that the type of lignocellulosic residue used as the substrate (sawdust, corn husk, and rice straw) and the fungal species that produced the mycelium (*Ganoderma fornicatum*, *Ganoderma williamsianum*, *Lentinus sajor-caju*, and *Schizophyllum commune*) affected the properties of the resulting MBC.

Water hyacinth (WH) is a highly invasive aquatic plant that has spread worldwide. Its rapid growth has led to widespread problems, such as the destruction of ecosystems, irrigation problems, and an increase in mosquito populations, as it creates breeding sites in its dense coverage of watercourses [16]. WH consists of about 20% cellulose, 48% hemicelluloses, and 3.5% lignin [17–20]. Despite being one of the most productive plants on Earth, it poses a serious threat to biodiversity and has been declared a threat by many countries [21]. Efforts to control WH have proved costly and labor-intensive. Various methods have been tried, including biological, physical, and chemical approaches, but none has provided a lasting solution. However, the problem presented by WH can be turned into an opportunity through innovative approaches. Entrepreneurs have seized this opportunity by producing handicrafts, briquettes, and industrial cellulase from the plant [21,22]. Limpiteprakan et al. [23] reported that cushioning materials made from WH and bagasse fibers passed drop tests without breaking glass ceramics. In Thailand and Zimbabwe, WH has been used as a substrate for mushroom cultivation [21]. Mukhopadhyay [24] showed that WH biomass can be safely used with rice straw (1:1) as an alternative substrate for the cultivation of *Pleurotus* species. Nguyen [25] reported that WH can be used to reinforce green composites and that composites of WH and rice straw showed significant improvements in flexural and compressive strength. However, the production of MBCs from WH using the fungi *Pleurotus ostreatus*, *Pleurotus sajor-caju* (Fr. Singer), *Auricularia auricula-judae*, and *Schizophyllum commune* Fr. has not yet been investigated.

The present study deals with the characterization of MBCs produced from WH and rice bran using these fungi as binders. WH was mixed with rice bran in different proportions, and the number of mycelial plugs used to inoculate the different substrates was varied. Well-formed composites were coated with a solution of either wet starch, chitosan, or epoxy resin before physical and mechanical properties and biodegradability were investigated.

2. Materials and Methods

2.1. Materials

2.1.1. Mushroom Species and Inoculum Preparation

Pure cultures of four indigenous mushroom species, namely, *Pleurotus ostreatus*, *Pleurotus sajor-caju* (Fr. Singer), *Auricularia auricula-judae*, and *Schizophyllum commune* Fr., were purchased from the DuangDao mushroom farm, Songkhla, Thailand. Inocula of all four species were grown on sorghum seeds in potato dextrose agar (PDA, HIMEDIA, Mumbai, India) at 30 °C for seven days. Plugs of the fungal mycelium (0.5 cm × 0.5 cm) were cut from the edge of an actively growing colony and used to inoculate substrates.

2.1.2. Water Hyacinth and Rice Bran Preparation

WH was collected from water sources in Hat Yai, Songkhla, Thailand. It was thoroughly washed with water to remove dirt and soil particles before being dried at 60 °C to a constant weight and then ground into small particles (1–2 mm) using a blender (Tefal, Sarcelles, France, BL91H). Fine rice bran was purchased from Chokchai Rice Mill, Phatthalung, Thailand. WH and rice bran were mixed in a blender in the following ratios: 100% WH, 70% WH mixed with 30% rice bran, and 50% WH mixed with 50% rice bran. Water was added to the mixed materials to achieve a moisture content of 60%. Then, 60 g of each mixed material was weighed out, placed in a Petri dish, and sterilized in an autoclave at a temperature of 121 °C at 15 psi for 15 min.

2.2. Methods

2.2.1. Culture Conditions to Produce Mycelium-Based Composites

Mycelial plugs (0.5 cm × 0.5 cm) were used to inoculate the substrates according to the method of Daengrot et al. [26]. The plugs were grown in the substrates at 25 °C and 30 °C for seven days. The diameters of the fungal colonies were measured daily. The growth rate was calculated as the diameter of the mycelium divided by the incubation time [27]. The temperature that resulted in the higher growth rate was selected for the optimization study.

The culture conditions of MBC production were varied. The number of mycelial plugs used was varied at 10, 20, and 30 plugs. The mixed substrate material included 100% WH, 70% WH, or 50%WH. All three substrate mixtures were inoculated with 10, 20, and 30 mycelial plugs from each of the four fungi. The plugs were incubated at the optimal temperature for seven days. The resulting MBCs were dried in a hot air oven at 90 °C until a constant weight was achieved.

The MBCs were coated with three different solutions: wet starch, chitosan, and epoxy resin.

2.2.2. Preparation of Coating Solution and Composite Surface Coating

To prevent the absorption of moisture, MBCs were coated with three different solutions: starch, chitosan (from shrimp shell 75%, deacetylated, Loba, Mumbai, India), and epoxy resin (SKC, Bangkok, Thailand). The starch solution was prepared with 20 g of rice flour (16% amylose and 84% amylopectin), 10 g of tapioca starch (17% amylose and 83% amylopectin), and 10 g of preservative (C_6H_5COONa , Kemaus, Cherrybrook, Australia). The three materials were dissolved in 1000 mL of water and heated until the starch was completely dissolved, and a sticky solution was formed. The chitosan solution was prepared with 3 g of chitosan dissolved in 100 mL of dilute acetic acid (CH_3COOH , RCI Labscan, Bangkok, Thailand) (1% v/v) under stirring [28]. The epoxy resin solution consisted of an epoxy resin (A) and a hardener (B, $[(CH_3)(C_2H_5)C(O_2H)]_2O_2$). The coating

was prepared by mixing 2 parts of resin A and 1 part of hardener (B). MBC samples were coated with each of the three solutions to a wet-film thickness of 250 μm . A wire rod coater was used at a constant forward speed. The finished coatings were dried in an oven at 65 $^{\circ}\text{C}$ for 24 h [28].

2.2.3. Physical and Mechanical Characterization

Physical Properties

The density of the MBC samples was determined according to ASTM D 3574-95 (ASTM, 2001) [29]. The rectangular test sample was weighed using a 4-position precision balance. The volume of the test piece was determined by measuring its width, length, and height with a Vernier caliper. The density was calculated as follows:

$$D = M/V \quad (1)$$

where D is the density of the test piece (g/cm^3), M is the weight of the test piece (g), and V is the volume of the test piece (cm^3).

The water absorption of the MBC samples was determined according to the ASTM International D 570-98 protocol (ASTM, 2008) [30]. The rectangular test sample was weighed using a 4-position precision balance and then soaked in water for 24 h. The sample was then dried with blotting paper and weighed. Water absorption was calculated as follows:

$$\text{Water absorption (\%)} = ((W_1 - W_0)/W_1) \times 100 \quad (2)$$

where W_0 is the weight before soaking in water (g), and W_1 is the weight after soaking in water (g).

Mechanical Properties

Before testing, the MBC samples were dried at 90 $^{\circ}\text{C}$ for 12 h [31]. At least eight specimens per test were tested at room temperature. The compressive and bending forces of the samples were determined using the INSTRON3365 load platform (Instron, Norwood, MA, USA). Compression specimens had a diameter of 9 cm and a thickness of 1 cm. Bending specimens had dimensions of $2 \times 5 \times 1.8$ cm. To test compressive strength, specimens were compressed up to 50% at a speed of 5 mm/min with a force of 10,000 N. To test flexural strength, specimens were placed on the lower beam and compressed at a rate of 5 mm/min until they were completely broken.

2.2.4. Morphologies of Uncoated and Coated Mycelial Composites

The morphologies of uncoated and coated MBC samples were examined by scanning electron microscopy (SEM, JSM-5800 LV, JEOL; Tokyo, Japan). The surface of the sample was coated with carbon and then gold-plated for electrical conductivity. The SEM acceleration voltage was 20 kV.

2.2.5. Determination of Biodegradation

Uncoated and coated MBC samples were tested for biodegradability using burial tests. The samples were weighed, packed in mesh bags, and buried in soil. Samples were recovered every 15 days after burial. Each piece was cleaned of all adhering debris and dried overnight at room temperature. The dried sample was placed in a desiccator until a constant weight was reached. The weight of each sample was recorded. The deterioration of the sample was expressed as percent weight loss (Ong and Sudesh, 2016 [32]).

$$\text{Weight loss (\%)} = ((W_i - W_o)/W_i) \times 100 \quad (3)$$

where W_i is the weight of the initial sample (g), and W_o is the weight of the sample after decomposition (g).

3. Results and Discussion

3.1. Mycelial Growth Rates and Optimal Culture Temperature

The growth rates of *Pleurotus ostreatus*, *Pleurotus sajor-caju* (Fr. Singer), *Auricularia auricula-judae*, and *Schizophyllum commune* Fr. were higher when incubated at 30 °C than at 25 °C. At 30 °C, *Schizophyllum commune* Fr. achieved the highest growth rate of 1.45 ± 1.92 mm/day, followed by *Pleurotus ostreatus* at 1.19 ± 1.46 mm/day, *Pleurotus sajor-caju* (Fr. Singer) at 1.06 ± 1.28 mm/day, and *Auricularia auricula-judae* at 0.94 ± 1.27 mm/day (Table 1). Therefore, incubation at 30 °C was chosen as the optimal incubation temperature.

Table 1. Growth rates of fungal mycelia cultivated on potato dextrose agar at 25 °C and 30 °C.

Mushroom Species	Growth Rate (mm/day)	
	At 25 °C	At 30 °C
<i>Pleurotus ostreatus</i>	0.61 ± 0.09	1.19 ± 0.06
<i>Pleurotus sajor-caju</i> (Fr. Singer)	0.79 ± 0.05	1.06 ± 0.08
<i>Auricularia auricula-judae</i>	0.41 ± 0.05	0.94 ± 0.07
<i>Schizophyllum commune</i> Fr.	1.17 ± 0.03	1.45 ± 0.92

3.2. Optimization of Composite Production Conditions

None of the mycelia could grow on 100% WH. However, all four fungal mycelia could grow on 50% WH mixed with rice bran. The fungal mycelium of *Schizophyllum commune* Fr. grew well and covered the surface of the substrate better than other species (Figure 1). The addition of rice bran provided a pool of nutrients that accelerated the growth of the mycelium and promoted a more homogeneous shape [33]. When considering the optimal number of mycelial plugs used to inoculate the substrate, it was found that the fungal hyphae could migrate through the substrate equally well when 10, 20, or 30 plugs were used. Thus, the optimal condition for the production of MBCs was a substrate of 50% WH and 50% rice bran and 10 plugs of *Schizophyllum commune* Fr, incubated at 30 °C. This condition was chosen for the production of an MBC for further studies.

The morphologies of the four fungal mycelia were also examined using a fluorescence microscope (Olympus, BX51 DP72, Tokyo, Japan). The observations showed that *Schizophyllum commune* Fr. had the highest fiber density (Figure 2). This finding was consistent with the observed growth rates of the mycelia. Since the network structure of the mycelial binder affects the mechanical properties of MBCs [34], this result indicated that the mycelium of *Schizophyllum commune* Fr. was potentially a good binder for the production of MBCs.

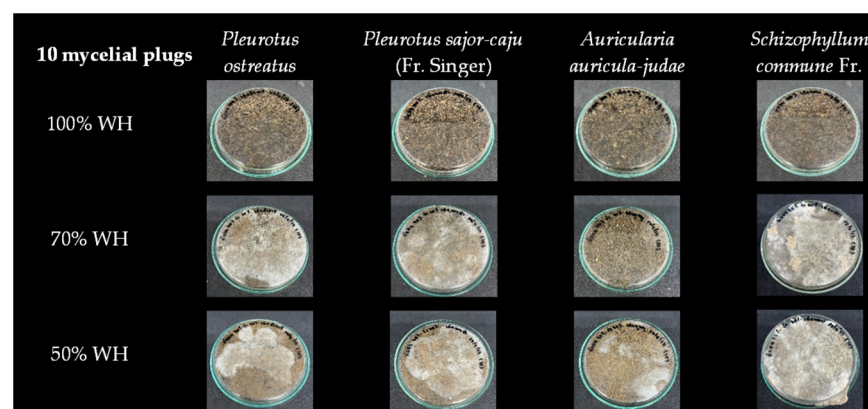


Figure 1. Cont.

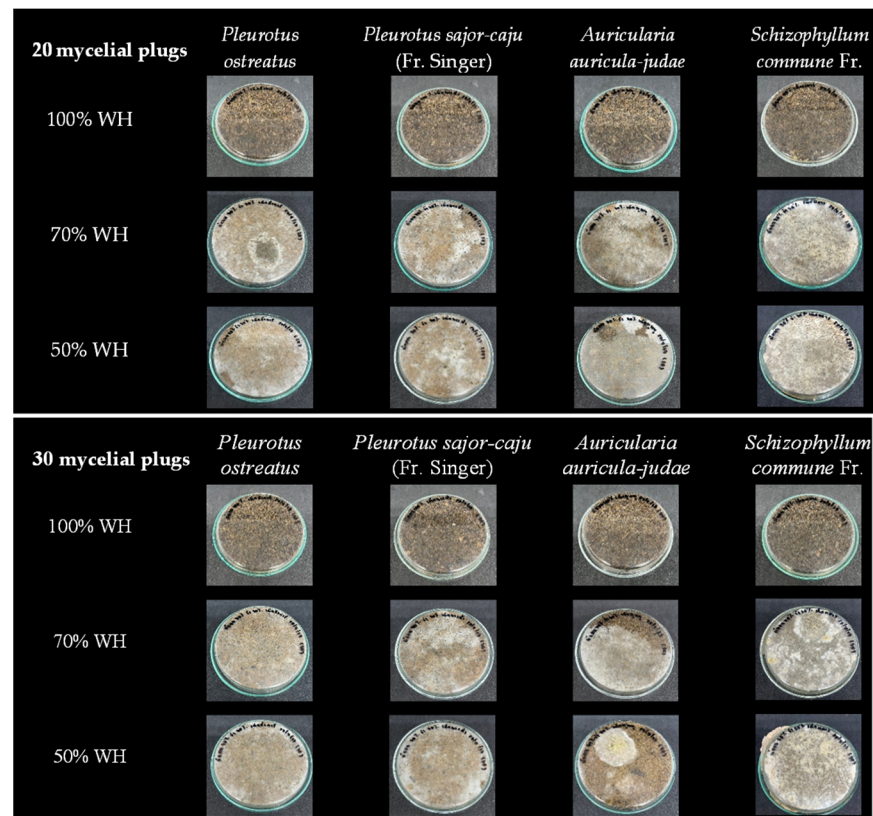


Figure 1. Growth characteristics of the fungal mycelia of *Pleurotus ostreatus*, *Pleurotus sajor-caju* (Fr. Singer), *Auricularia auricula-judae*, and *Schizophyllum commune* Fr. on 100% WH, 70% WH with 25% rice bran, and 50% WH with 50% rice bran, incubated at 30 °C for seven days.

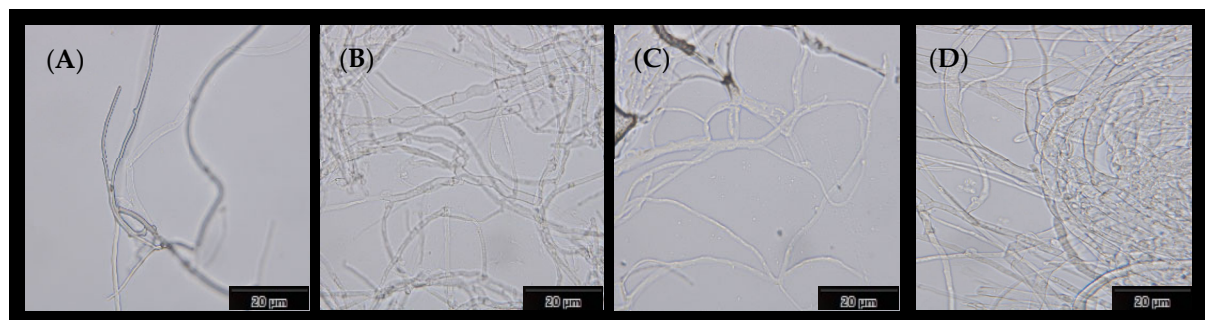


Figure 2. Morphologies of the fungal mycelia of (A) *Pleurotus ostreatus*, (B) *Schizophyllum commune* Fr. (C) *Auricularia auricula-judae*, and (D) *Pleurotus sajor-caju*.

3.3. Physical and Mechanical Properties of Coated Mycelial Composites

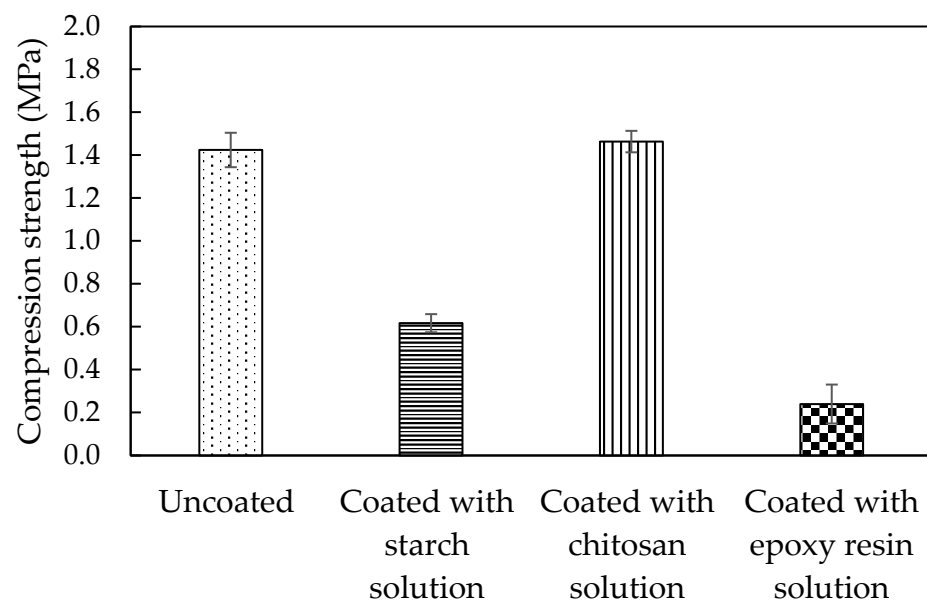
The density of the MBC produced in optimal conditions was determined after applying the three different coating solutions, and the obtained values were compared with the density of the uncoated MBC, which was $0.0389 \pm 0.0016 \text{ g/cm}^3$. The values for the optimal MBC coated with the starch solution, chitosan solution, and epoxy resin were $0.0399 \pm 0.0037 \text{ g/cm}^3$, $0.0475 \pm 0.0029 \text{ g/cm}^3$, and $0.0942 \pm 0.0021 \text{ g/cm}^3$, respectively (Table 2). The coatings therefore increased the density of the MBC. Coating with epoxy resin gave the highest density because the viscous resin adhered strongly and cured fastest.

Table 2. The density and water absorption of the uncoated and coated optimal mycelium-based composite.

Type of Coating	Density (g/cm ³)	Water Absorption (%)
Uncoated composite	0.0389 ± 0.0016	50.05 ± 0.66
Composite coated with starch solution	0.0399 ± 0.0037	51.37 ± 2.09
Composite coated with chitosan solution	0.0475 ± 0.0029	53.32 ± 1.00
Composites coated with epoxy resin	0.0942 ± 0.0021	43.60 ± 0.73

The water absorption values of the MBC without a coating and coated with the starch solution, chitosan solution, and epoxy resin were $50.05 \pm 0.66\%$, $51.37 \pm 2.09\%$, $53.32 \pm 1.00\%$, and $43.60 \pm 0.73\%$, respectively (Table 2). Coating with epoxy resin inhibited water absorption because the resin is hydrophobic [35]. Epoxy resin is often used to coat various materials for protection and as an adhesive with high chemical resistance and good processing properties [36].

The compressive strength of the optimal MBC was determined without a coating and with coatings of starch and chitosan solutions and epoxy resin. The respective values were 1.30 ± 0.08 MPa, 0.62 ± 0.04 MPa, 1.46 ± 0.05 MPa, and 0.24 ± 0.09 MPa (Figure 3). The coated MBC with the chitosan coating performed the best in terms of compressive strength. It is difficult to compare the compressive strength of MBCs produced by different types of fungi grown on different lignocellulosic substrates, and it is not easy to determine which conditions are best, but the available data can be used as a guide. In the present study, the compressive strength of the optimal MBC coated with the chitosan solution was 1.46 ± 0.05 MPa. This value is higher than those of MBCs produced from *G. resinaceum* grown on rose petal debris (1.03 MPa) and on lavender straw (0.72 MPa) [37]. The flexural strength of the optimal MBC without a coating was 0.74 ± 0.139 MPa. When coated with solutions of starch, chitosan, and epoxy resin, the flexural strength of the MBC was 0.66 ± 0.008 MPa, 0.28 ± 0.003 MPa, and 1.18 ± 0.138 MPa, respectively (Figure 4). The MBC coated with epoxy resin exhibited the highest flexural strength, perhaps because fibers from the water hyacinth plant reinforced the resin solution [38].

**Figure 3.** The compressive strength of an uncoated and coated mycelium-based composite of *Schizophyllum commune* Fr. grown on 50% WH and 50% rice bran incubated at 30 °C for seven days.

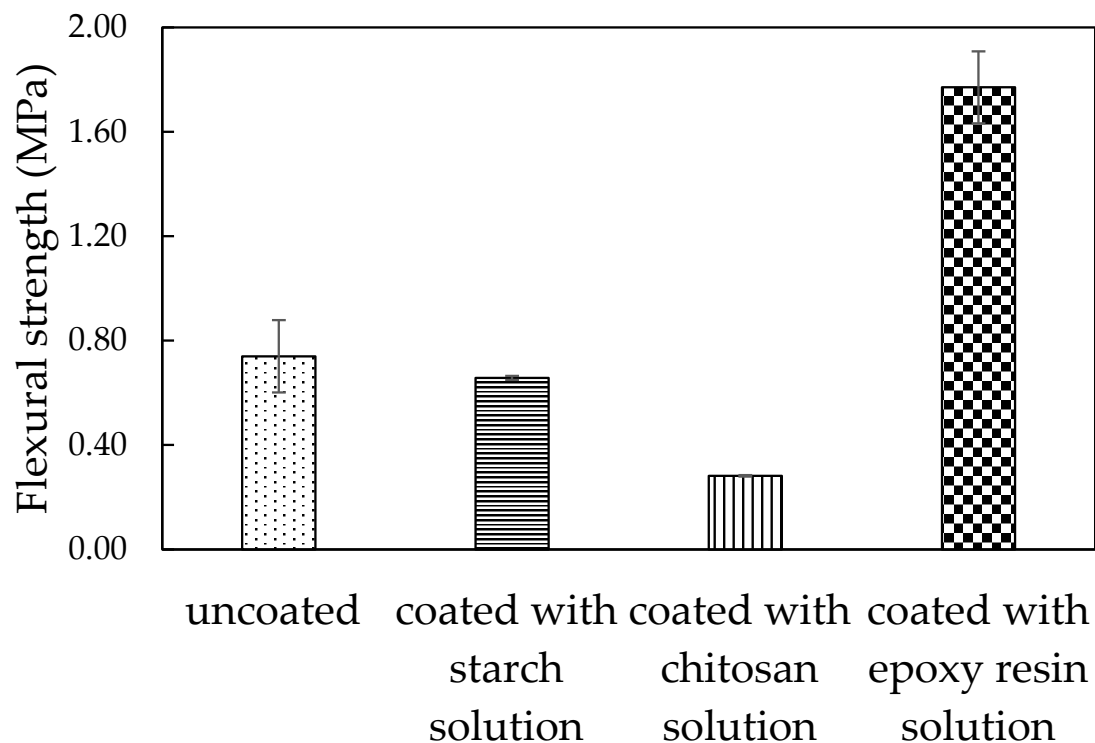


Figure 4. The flexural strength of an uncoated and coated mycelium-based composite of *Schizophyllum commune* Fr. grown on 50% WH with 50% rice bran incubated at 30 °C for seven days.

3.4. Morphologies of Coated Mycelial Composites

The morphologies of the uncoated and coated optimal MBC were examined by SEM. The mycelial hyphae were visible in the uncoated MBC but not in the coated MBC samples (Figure 5). This result indicated that the coatings filled the pores between the cellulose fibers and the mycelium, contributing to compressive strength, which is consistent with [28]. Coating with the wet starch solution gave the best results, followed by the epoxy resin and chitosan coatings. However, when comparing the hydrophobic properties of the composites, it was found that the resin produced the best result. The resin coating therefore helped prevent the absorption of water.

3.5. Biodegradation of Uncoated Mycelial Composites and Chitosan-Solution-Coated Mycelial Composites

To produce sustainable bio-building materials with high compressive strength, an uncoated MBC sample and a sample coated with the chitosan solution were buried in the soil, and the biodegradation of the MBC samples was investigated for 45 days. The uncoated MBC decomposed completely within 45 days, whereas the MBC coated with the chitosan solution decomposed completely within 30 days. Chitosan is a naturally biodegradable substance. It absorbed and retained water in the soil, promoting decomposition [39,40]. The percentage weight loss and physical changes in the uncoated and chitosan-solution-coated MBCs are shown in Figures 6 and 7, respectively.

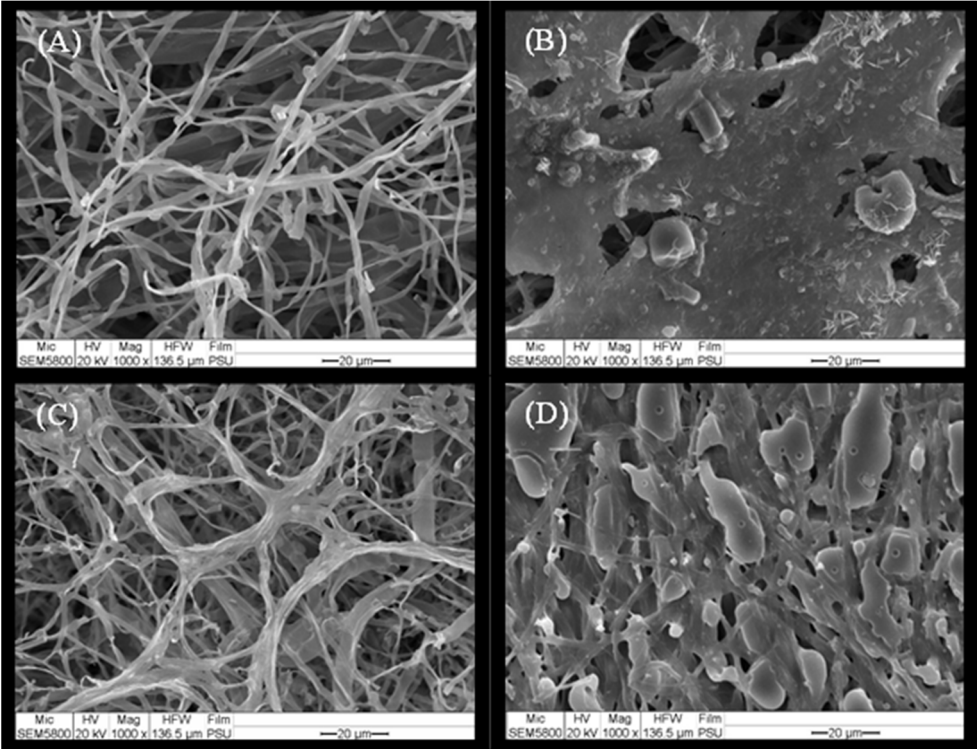


Figure 5. Samples of the mycelial-based composite were observed under a scanning electron microscope. The images show (A) the uncoated mycelium-based composite and the mycelium-based composite (B) coated with a starch solution, (C) coated with a chitosan solution, and (D) coated with epoxy resin.

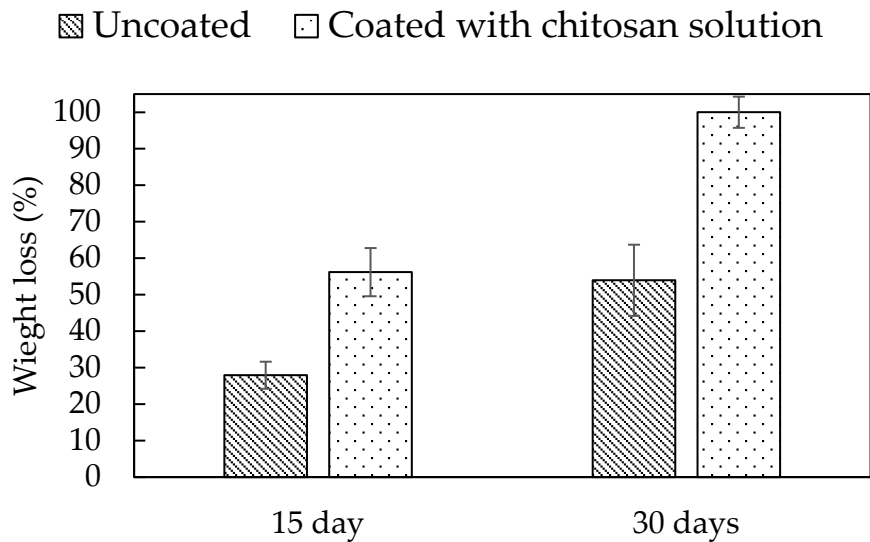


Figure 6. Percentage weight loss of the proposed mycelial composite during 30 days buried in soil. A sample coated with the chitosan solution and an uncoated sample are compared.

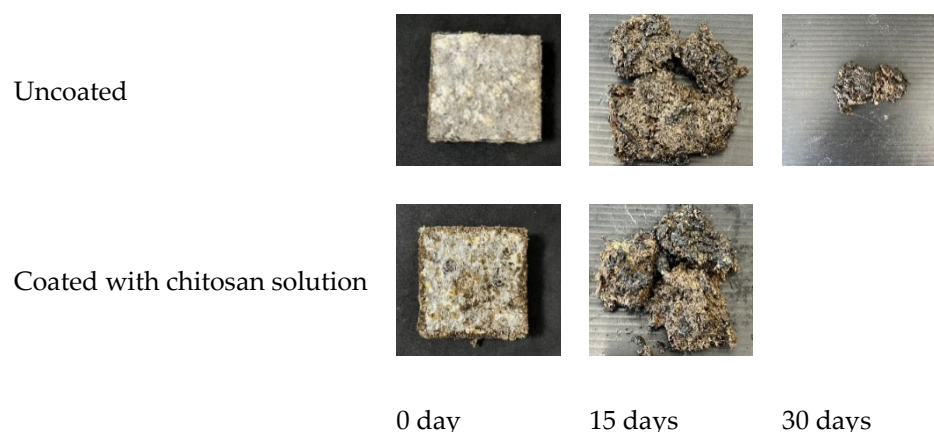


Figure 7. Biodegraded samples of the optimal mycelium-based composite are shown during a burial-in-soil study lasting 30 days. A sample coated with the chitosan solution and an uncoated sample are compared.

4. Conclusions

Mycelium-based composite materials were produced by inoculating a water-hyacinth-and-rice-bran substrate with the hyphae of four mushroom species, namely, *Pleurotus ostreatus*, *Pleurotus sajor-caju* (Fr. Singer), *Auricularia auricula-judae*, and *Schizophyllum commune* Fr. The hyphae bound the substrate particles in suitable conditions. The hyphae of *Schizophyllum commune* Fr. exhibited the fastest growth rate and the highest density of fibers. Its growth rate was 1.45 ± 1.92 mm/day. The optimal conditions for the formation of fungal mycelial composites were a mixture of 50% water hyacinth with 50% rice bran inoculated with 10 mycelial plugs of *Schizophyllum commune* Fr. incubated on the substrate at 30 °C for seven days. Coating the mycelium-based composite with a chitosan solution improved the mechanical properties, but coating with a resin improved the water absorbency of the material. The mycelial composite coated with the chitosan solution biodegraded fastest, degrading within 30 days. The results show that *Schizophyllum commune* Fr. can be used to bind water hyacinth mixed with rice bran to produce mycelium-based composites. The findings of this work also provide useful information that not only increases the value of water hyacinth but also can be used as information on a new indigenous fungus for the production of fungal mycelium-based composites.

Author Contributions: P.S. and J.I. wrote the manuscript and analyzed the data; P.S. carried out the experiments; W.T., J.C., S.L., and J.I. supervised and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: Author Siwapong Luenram was employed by the company BIOAXEL Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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