



Research article

Valorization of agricultural waste to produce myco-composite materials from mushroom mycelia and their physical properties

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Abstract

Importance of the work: Mushroom mycelia can be utilized in novel and low-cost bio-fabrication for recycling agricultural wastes into eco-friendly biomaterials known as myco-composites.

Objectives: To investigate mushroom isolates and agricultural waste that could be used to make myco-composite materials.

Materials & Methods: The mycelial growth rate was investigated of each mushroom isolate along with the physical properties of myco-composites produced from five different mushroom isolates (*Earliella* sp. CMU-NK0461, *Hexagonia* sp. CMU-KA018, *Lentinus* sp. CMU-0712, *Pleurotus* sp. CMU-NK1032 and *Pycnoporus* sp. CMU-0189) and two different agricultural wastes (sawdust and rice straw).

Results: *Lentinus* sp. had the fastest growth rate and produced the highest mycelial biomass on potato dextrose agar. *Lentinus* sp. had the fastest growth rate on sawdust and rice straw. The various tested mushroom isolates and agricultural substrates produced myco-composites with different physical properties. Sawdust-based myco-composites had higher densities than rice straw-based myco-composites. However, the myco-composites produced from rice straw had greater water absorption and average shrinkage capabilities than sawdust. The myco-composites produced from sawdust and *Lentinus* sp. had the highest density (302.36 kg/m³) with the lowest average shrinkage (6.81%). The myco-composites produced from rice straw and *Lentinus* sp. had the greatest water absorption (284.41%).

Main finding: Notably, these myco-composite materials have a density that is similar to foams; however, these materials are more advantageous in the natural decomposition process. Additionally, the high water absorption ability of these myco-composites could be beneficial in future agricultural applications.

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Introduction

Currently, the rapidly increasing global population has caused an increase in annual agricultural product consumption, which has resulted in the production of greater amounts of agricultural waste at an average rate of 5–10% per year (Wang et al., 2016; Xue et al., 2016). Agricultural waste management has always been a major concern for farmers. Normally, burning is believed to be the most effective and cost-effective approach to managing agricultural waste. However, this action has the potential to directly pollute the soil, air and water (Adejumo and Adebiyi, 2020; Duque-Acevedo et al., 2020). In response to these problems, many studies have focused on the development of different strategies and modern management techniques that use agricultural waste in the manufacturing of high-value-added products generated through the recycling process (Sadh et al., 2018). Agricultural waste is a well-known raw material in the production of bioproducts and is one of the richest renewable resources (Usmani et al., 2021). Nowadays, these forms of agricultural-waste can be used to produce bioenergy, biofuels, organic acid and various high-value biomaterials (Gutierrez-Macias et al., 2017; Sadh et al., 2018). In the development of sustainable practices, alternative agricultural materials have become one of the most beneficial raw materials that could be used in the production of biomaterials.

Agricultural waste is lignocellulosic in nature and is composed of cellulose, hemicellulose, lignin and minerals (Saini et al., 2015). These forms of waste can be used in the production of myco-composite materials. Myco-composites are a form of biomaterial that is mainly produced from mushroom mycelia grown on agricultural waste. In this process, various forms of agricultural wastes, such as sawdust, husks, hulls, straw, cotton and hemp, are used as substrates (Teixeira et al., 2018; Appels et al., 2019; Ghazvinian et al., 2019; Elsacker et al., 2019; Tacer-Caba et al., 2020; Aiduang et al., 2022). Currently, the production of myco-composites has focused on the use of these forms of agricultural waste and the mycelia of several mushroom genera that include *Ganoderma*, *Lentinula*, *Pleurotus*, *Pycnoporus* and *Trametes* (Appels et al., 2019; Butu et al., 2020). To date, myco-composites have been utilized in a range of potentially beneficial applications in different fields of study, including the biomedical, paper, packaging, textile, furniture, agriculture and civil engineering fields (Attias et al., 2019; Jones et al., 2020; Manan et al., 2021; Yang et al., 2021). However, the structural properties of myco-composite materials vary depending on a variety of parameters,

such as the fungal strain, the substrate type, growth conditions and post-synthesis processing (Manan et al., 2021; Yang et al., 2021). Therefore, the current study aimed to evaluate the mycelial growth of different mushroom isolates on culture media and the agricultural substrates used in the production of myco-composites. In addition, the physical properties (density, average shrinkage and water absorption) of the obtained myco-composites were evaluated.

Materials and Methods

Source of mushroom mycelia

Pure cultures of five different mushroom isolates (*Earliella* sp. CMU-NK0461, *Hexagonia* sp. CMU-KA018, *Lentinus* sp. CMU-NK0712, *Pleurotus* sp. CMU-NK1032 and *Pycnoporus* sp. CMU-NK0189) were obtained from the culture collections of the Research Center of Microbial Diversity and Sustainable Utilization, Faculty of Science, Chiang Mai University, Thailand. All selected isolates had a high mycelium density and rapid growth rate. Each mushroom isolate was grown on a potato dextrose agar (PDA; Conda; Madrid, Spain) plate and incubated at 30 °C for 7 d.

Source of substrate and preparation

Two different forms of agricultural waste, namely the sawdust of rubber tree and rice straw, were used as the main substrates in the experiments. All substrate types were obtained from a rubber wood sawmill and a rice field located in Chiang Mai province, Thailand. Each substrate was ground in a woodchipper and sieved. Chips with a particle size of 5–10 mm were collected and used in this study.

Evaluation of mycelial growth rate on culture medium

The surface of the PDA plates was overlaid surface with a sterilized cellophane disc. A mycelial disc (5 mm in diameter) of each isolate was placed in the center of surface of separate PDA plates. The inoculated plates were incubated at 30 °C for 7 d in darkness. Colony diameters were measured daily and growth rates were calculated from the colony diameter divide by the incubation time (Tudses, 2016). Then, the cellophane discs were removed and dried at 80 °C for 24 h. The mycelial dry weight was measured (Ang et al., 2013), with 10 replications performed for each mushroom isolate.

Evaluation of mycelial growth on agricultural waste and myco-composite production

Each type of agricultural waste was mixed with 5.0% rice bran, 1.0% calcium carbonate, 2.0% calcium sulfate and 0.2% sodium sulfate (Kupradi et al., 2017). Then, the mixtures were adjusted to a relative humidity of 60% by adding water. A sample (30 g) of each substrate mixture was placed onto a glass plate (9 cm in diameter). The plates were autoclaved at 121 °C for 60 min. After cooling, a mycelial disc (10 mm in diameter) of each mushroom was placed onto the center of the tested plates. The plates were incubated at 30 °C for 14 d in darkness. The mycelial growth rate of each mushroom isolate was measured daily (Nashiruddin et al., 2021), with 10 replications for each treatment.

After 14 d, the mushroom mycelia had completely colonized the substrate. Then, myco-composite samples were removed from the glass plates, placed in separate plastic boxes and incubated at 30 °C. After 3 d incubation, the sides that were in contact with the glass plate were covered in mycelia. Then, all myco-composite samples were dried at 70 °C for 24 h or until their weight had stabilized (Elsacker et al., 2019). The myco-composite samples were evaluated in terms of their physical properties in future experiments.

Evaluation of physical properties

Two physical properties (the density and average shrinkage) were determined for the myco-composite samples. The density of each sample was calculated from its mass and volume. To measure average shrinkage, the pre-drying and post-drying volume of myco-composite samples were calculated based on the formula: average shrinkage (%) = $(V_1 - V_2 / V_1) \times 100$, where V_1 = original volume of the sample and V_2 = volume of the sample after being oven-dried (Elsacker et al., 2019). Ten replications were performed on each sample for each treatment.

Water absorption

Water absorption was determined according to the standard method (American Society for Testing and Materials D570; Nava et al., 2016). After being dried, the samples were allowed to cool in a desiccator for 24 h. Then, the initial weight of each sample was measured before being tested in deionized water. Ten repetitions of each sample were submerged in water for 24 h. Samples were weighed after 2, 4, 6, 12, 18, and 24 h and compared with the densities of two different types (8 kg/m³ and 20 kg/m³) of expanded polystyrene foam (EPS). The increase in mass was calculated based on the formula: increase in weight (%) = $(W - D) / D \times 100$, where W = wet weight and D = dry weight.

Statistical analysis

The data from each experiment using the 10 replications were analyzed using one-way analysis of variance in the SPSS program for Windows (version 17.0; SPSS Inc.; Chicago, IL, USA), while Duncan's multiple range test was used to indicate significant ($p < 0.05$) differences.

Results

Evaluation of mycelial growth rate on culture medium

The mycelial growth of the five different mushroom isolates presented in Table 1 indicated that the mycelial growth rate on PDA medium and dry mycelial biomass differed depending upon the mushroom species. The highest levels of growth rate and dry biomass were found in *Lentinus* sp. (19.20 mm/d and 74.73 mg), followed by *Pycnoporus* sp. and *Earliella* sp., respectively. The lowest level of mycelial growth rate and dry biomass were found in *Hexagonia* sp. (6.77 mm/d and 13.77 mg).

Table 1 Mycelial growth rate and dry biomass (mean ± SD) of mushroom isolate on potato dextrose agar

Mushroom isolate	Growth rate (mm/d)	Mycelial biomass (mg)
<i>Earliella</i> sp. CMU-NK0461	11.17±0.06 ^c	50.73±3.66 ^b
<i>Hexagonia</i> sp. CMU-KA018	6.77±0.15 ^e	13.77±2.41 ^d
<i>Lentinus</i> sp. CMU-NK0712	19.20±0.10 ^a	74.73±4.82 ^a
<i>Pleurotus</i> sp. CMU-NK1032	10.47±0.15 ^d	36.62±4.16 ^c
<i>Pycnoporus</i> sp. CMU-NK0189	16.27±0.21 ^b	51.90±3.55 ^b

Mean±SD in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Evaluation of mycelial growth on agricultural wastes

The mycelial growth rate observed on the sawdust and rice straw substrates varied with the mushroom isolate, with the highest growth rate observed in *Lentinus* sp. on both sawdust and rice straw substrates (17.65 mm/d and 19.50 mm/d, respectively), followed by *Pycnoporus* sp. and *Earliella* sp. The lowest mycelial growth rate on the agricultural waste was for *Hexagonia* sp. at 8.40 mm/d in sawdust and 11.85 mm/d in rice straw (Fig. 1 and Table 2).

Evaluation of physical properties

Density has been determined to be a good parameter of the physical and mechanical properties of the biomaterials

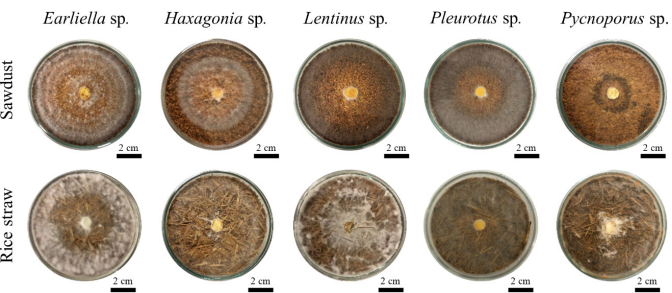


Fig. 1 Mycelial growth on agricultural wastes in this study

Table 2 Mycelial growth rates (mean±SD) of mushroom isolate on sawdust and rice straw

Substrate	Mushroom isolate	Growth rate (mm/d)
Sawdust	<i>Earliella</i> sp. CMU-NK0461	13.35±0.07 ^c
	<i>Hexagonia</i> sp. CMU-KA018	8.40±0.14 ^c
	<i>Lentinus</i> sp. CMU-NK0712	17.65±0.2 ^a
	<i>Pleurotus</i> sp. CMU-NK1032	12.75±0.07 ^d
	<i>Pycnoporus</i> sp. CMU-NK0189	14.35±0.21 ^b
Rice straw	<i>Earliella</i> sp. CMU-NK0461	18.35±0.21 ^b
	<i>Hexagonia</i> sp. CMU-KA018	11.85±0.49 ^d
	<i>Lentinus</i> sp. CMU-NK0712	19.50±0.28 ^a
	<i>Pleurotus</i> sp. CMU-NK1032	13.20±0.14 ^c
	<i>Pycnoporus</i> sp. CMU-NK0189	17.80±0.42 ^b

Mean±SD in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Table 3 Density of myco-composite samples (mean ± SD) in this study

Mushroom isolate	Density (kg/m ³)	
	Sawdust	Rice straw
<i>Earliella</i> sp. CMU-NK0461	275.43±5.99 ^b	172.30±3.45 ^b
<i>Hexagonia</i> sp. CMU-KA018	249.57±7.36 ^c	166.83±5.63 ^{bc}
<i>Lentinus</i> sp. CMU-NK0712	302.36±7.14 ^a	180.32±4.09 ^a
<i>Pleurotus</i> sp. CMU-NK1032	269.56±8.32 ^b	160.52±2.25 ^c
<i>Pycnoporus</i> sp. CMU-NK0189	291.10±5.41 ^a	183.57±2.86 ^a

Mean±SD in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

(Aiduang et al., 2022). In the current study, the densities of the myco-composites produced from sawdust and rice straw were in the ranges 249.57–302.36 kg/m³ and 160.52–183.57 kg/m³, respectively (Table 3). The sawdust-based myco-composites had higher densities than the rice straw-based myco-composites. Importantly, the highest density was in the myco-composites produced from sawdust and *Lentinus* sp. (302.36 kg/m³), while the lowest density was in the myco-composites produced from rice straw and *Pleurotus* sp. (160.52 kg/m³)

The sawdust-based myco-composites had a lower percentage shrinkage value than the rice straw-based myco-composites (Fig. 3). The average shrinkage levels of the myco-composites produced from sawdust and rice straw were in the ranges 6.81–9.78% and 11.89–14.56%, respectively.



Fig. 2 Myco-composite samples obtained in this study

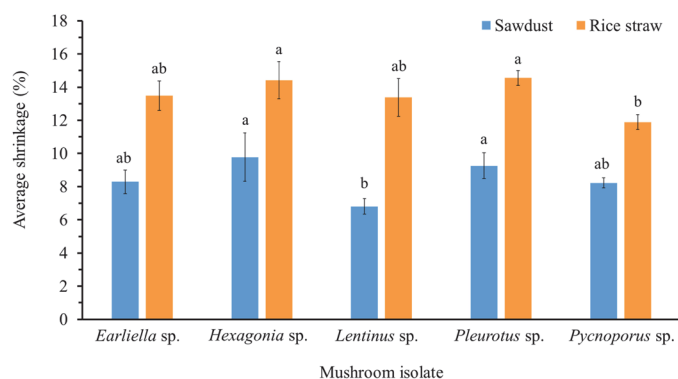


Fig. 3 Average shrinkage obtained from myco-composite samples in this study, where results are shown as mean $\pm$ SD of 10 replications and different lowercase letters above bars for the same parameter indicate significant ($p < 0.05$) differences

Water absorption

In terms of water absorption properties, the myco-composite samples produced from *Earliella* sp., *Hexagonia* sp. and *Pleurotus* sp. could not be maintained in water after 2 h because these were unstable materials. Therefore, the study focused on the water absorption properties of the myco-composites produced from *Lentinus* sp. and *Pycnoporus* sp. And compared them to the two EPS foams. The results indicated that the weight of the myco-composite samples increased after being placed into the water for 2 h and became slowly stable after 12 h. Furthermore, the sawdust-based myco-composites and rice straw-based myco-composites had higher levels of water absorption capability than the EPS foams. The myco-composites produced from *Lentinus* sp. and rice straw had the highest degree of water absorption (284.41% after 24 h). Furthermore, the findings indicated that the density had an

impact on material water absorption. Comparing the impact of material composition on water absorption and density, it was apparent that the water absorption of the materials dropped as the composite density increased. These results were observed when the two EPS foams with different densities had similar water absorption characteristics with the myco-composites. Notably, the high-density EPS foam had lower water absorption than the low-density EPS foam (Fig. 4).

Discussion

To date, myco-composites have been produced by growing the vegetative fungal hyphae on different forms of agricultural waste through the process of solid-state fermentation. The growth of mushroom mycelia and the substrate type are critical in myco-composite production. The current study, showed that different mushroom isolates had different growth rates on both PDA and agricultural substrates. These findings were consistent with those of other research studies that reported that different mushroom species, isolates and growth substrates had a significant impact on the mycelial growth rate (Xing et al., 2018; Ly and Jitjak, 2020; Suwannarach et al., 2022). The high degree of mycelial growth was related to high hyphal extension and resulted in a very dense mycelial biomass (Carlile et al., 2001; Nashiruddin et al., 2021). The chemical composition of each substrate had a significant impact on the mycelial growth of the mushrooms because the hyphae were in direct contact with the substrate and were involved in the extraction of essential nutrients from the substrate for use in mycelial growth (Belletini et al., 2019; Nashiruddin et al., 2021). The outcomes of the current research study revealed that the growth

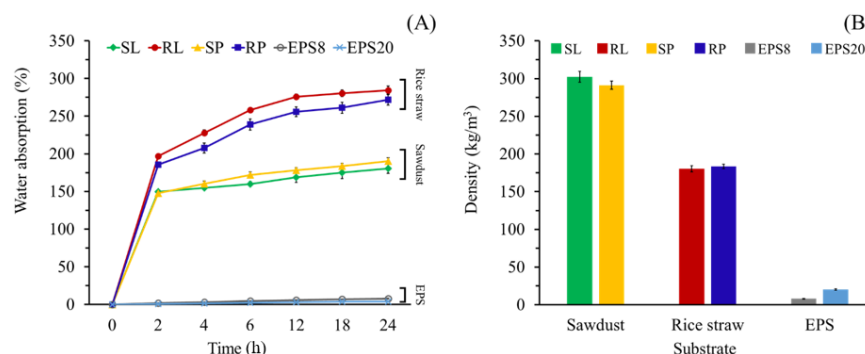


Fig. 4 Water absorption behavior and density of myco-composite samples in this study: (A) water absorption of myco-composites; (B) density of myco-composites, where results are mean $\pm$ SD of 10 replications, SL = myco-composite produced from sawdust and *Lentinus* sp.; RL = myco-composite produced from rice straw and *Lentinus* sp.; SP = myco-composite produced from sawdust and *Pycnoporus* sp.; RP = myco-composite produced from rice straw and *Pycnoporus* sp.; EPS8 = EPS foam with 8 kg/m³ density and EPS20 = EPS foam with 20 kg/m³ density

rates of all mushroom isolates in rice straw were higher than in sawdust because sawdust has a high lignin content. Generally, sawdust has a lignin content of 28–30% dry weight basis, while in rice straw it is 15–18% on a dry weight basis (Ali et al., 2013; Gou et al., 2018; Shakir et al., 2020). Similarly, other studies reported that when mushrooms were grown on substrates with a high lignin content, the mycelial growth rates were limited due to the poor rates of lignin breakdown and utilization of mushrooms (Pu et al., 2018; Kumla et al., 2020; Zhang et al., 2021).

The physical properties of myco-composites depend upon the fungal strain, the type of substrate, the growth conditions and post-synthesis processing (Pelletier et al., 2013; Islam et al., 2018; Manan et al., 2021; Yang et al., 2021). Notably, density is a key factor as an indicator of good material qualities. The density values of sawdust-based (249.57–302.36 kg/m³) and rice straw-based myco-composites (160.52–183.57 kg/m³) were within the ranges described in other reports (25–954 kg/m³) (Tacer-Caba et al., 2020; Chan et al., 2021; Aiduang et al., 2022). Furthermore, other studies reported that the myco-composite material had a density that was similar to that of synthetic foams (11–400 kg/m³) and wood-based products (160–1030 kg/m³) (Iejavs and Spulle, 2016; Yang, 2018; Jones et al., 2020). It is expected that these myco-composite materials could replace synthetic foams (polystyrene, polyurethane, phenolic formaldehyde resin and polyimide foams) and wood-based products (plywood and softwood) in the packaging, construction and furniture industries (Iejavs and Spulle, 2016; Yang, 2018; Jones et al., 2020; Aiduang et al., 2022). The average shrinkage of the myco-composites resulted from the dehydration of the samples via the drying process. Normally, the average shrinkage of myco-composite materials is in the range 5.10–20.00% (Holt et al., 2012; Nava et al., 2016; Elsacker et al., 2019). Notably, myco-composites produced from sawdust had a low average of shrinkage (6.81–9.78%) compared with the myco-composites produced from *Trametes versicolor* that were grown on hemp, flax and wheat straw (9.00–20.00%), according to Elsacker et al. (2019). Furthermore, a low average shrinkage value resulted in strong material stability (Nava et al., 2016). Therefore, in the current study, the sawdust-based myco-composites were more stable than the rice straw-based myco-composites. Furthermore, other studies indicated that the range of shrinkage involving myco-composites was comparable to that reported for wood-based materials (1–25%). Therefore, they could be used to successfully replace these wood-based materials (Jones et al., 2020; Aiduang et al., 2022).

Additionally, rice straw-based myco-composites had higher water absorption ability than sawdust-based myco-composites in the current study. These results were achieved by determining that the density of a material, which decreases composite density as a result of increasing the numbers of voids and pores in the material, also increases the water absorption ability (Attias et al., 2019). In addition, the results indicated that the myco-composites had many times greater water absorption properties than the synthetic foams because myco-composites are generally hygroscopic (Jones et al., 2020). The high-water absorption affinity of the myco-composite was related to their typically cellulosic filler ingredients, which include hydroxyl groups that are easily accessible, a hydrophilic porous mycelium binder and biologically derived filler phases, which all contribute to the water absorption capability of the material (Jones et al., 2017). However, the essential advantage of the myco-composite material was associated with its higher and faster water absorption capabilities compared to the synthetic foams. Notably, these characteristics can be beneficially utilized in future agricultural application as a biodegradable pot.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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