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Research Article

Biodegradable Sheets from Dried Mycelia of Edible Mushrooms

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ABSTRACT
Due to its quick growth and biodegradability, mushroom mycelium has been used to create alternative materials. This study aimed to produce mycelium sheets from market-purchased edible mushrooms (*Lentinus* sp. and *Pleurotus* sp.) and to assess the mycelium sheet properties. They were isolated and cultured in various liquid media. The production of four mycelium sheets was successful. After drying, the mycelium sheets of *Pleurotus* sp. using potato dextrose broth had the largest water contact angle. With a tensile strength, the mycelium sheet of *Lentinus* sp. using malt extract broth obtained the highest value. The dried mycelium sheet from *Pleurotus* sp. cultured on yeast extract broth had the greatest hardness value in the microhardness testing. After 7 days, the residual dry weight of the mycelium sheets in different conditions—soil burying, soil surface exposure, and water immersion—was less than 50 % of the initial weight. This work has demonstrated the biodegradability of mycelium sheets.

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INTRODUCTION

Synthetic materials e.g., plastics are known to have a long period of degradation which is problematic to the environment including marine life, soil pollution, ecosystems, and natural resources. One of the causes of death to marine mammals, sea birds, and sea turtles is the ingestion of plastic debris. Thus there is a strong need for renewable and biodegradable alternatives to reduce environmental impact. One of them is mushrooms Click or tap here to enter text. (Bond et al. 2013; Zhu et al. 2019; Silverman et al. 2020). Ecologically, the mushroom is a decomposer that can grow rapidly in large quantities when environmental conditions are favorable. The mushroom species are different in their textures ranging from soft to hard, shapes, and properties such as waterproof ability, germicidal properties, and flexibility (Haneef et al. 2017). Interestingly, mushrooms, agricultural waste, and other biomasses containing fibers can be combined to create alternatives for biodegradable materials (Bayer et al. 2014; Mostafa et al. 2018).

Materials derived from mushrooms in combination with natural plant fibers have been increasingly studied such as bricks, soundproof walls, and green packaging, e.g., boxes including fibers for textile industries (Haneef et al. 2017; Islam et al. 2017; Girometta et al. 2019; Jones et al. 2018; Whabi et al. 2024). This involves the production of mycelium-based composites with plant fibers to enhance the physical properties e.g. tensile and compressive strength (Ziegler et al. 2016). Moreover, mycelium-based composites can be applied to various objects with different purposes – decoration, furniture and insulation panels (Appels et al. 2019). Environmentally, these materials are also biodegradable which can reduce the carbon footprint which is one of global sustainability goals (Elsacker et al. 2021). There are various types of mushrooms in Thailand. The edible mushrooms are now widely cultivated and can be found in local markets in northeastern Thailand such as *Lentinus* spp. and *Pleurotus* spp. Silverman et al. (2020) prepared composites of *Pleurotus ostreatus* (oyster), *Pleurotus citrinopileatus* (yellow oyster), *Pleurotus eryngii* (king oyster), and *Ganoderma lucidum* (reishi) with fabric mat and sawdust to make environmentally friendly footwear. Also, Nawawi et al. (2019) made chitin paper from crab shells, *Agaricus bisporus*, and some polypores mushrooms. However, the mycelium sheets and the physical properties of *Lentinus* sp., or log white mushroom as the mushroom local to the northeastern region of Thailand, have not largely been studied like *Pleurotus* which was therefore the objectives of this research.

MATERIALS AND METHODS

Source of mushroom and pure culture isolation

Two edible local mushrooms, *Lentinus* sp. (log white mushroom, Lw) and *Pleurotus* sp. (oyster mushroom, Oy), were purchased from a local market in Muang district, Khon Kaen province. To prepare the pure mycelium of each mushroom, tiny pieces (0.2×0.2 cm^2) were isolated from the inside mushroom stalk and placed at the center of a Petri dish containing potato dextrose agar (PDA). After an incubation period of 2-3 days, mycelia obtained from pieces of fresh basidiocarp were placed on PDA. Then, the edge of the grown mycelium (0.2×0.2 cm^2) was taken and placed onto new PDA dishes to obtain the pure mushroom mycelia for the next experiments.

Mycelium sheet production

Four different broth media were used, namely potato malt peptone broth (PMPB), potato dextrose broth (PDB), malt extract broth (MEB), and yeast extract broth (YEB) in static condition. Preliminary experiments indicated that PMPB and MEB were the most suitable media for the growth of Lw, but PDB and YEB were found optimal for Oy growth which was determined

based on the average distance of the mycelia on the media. An equal volume (50 mL) of the liquid medium was added to each flask and autoclaved at 121 ° C for 15 minutes. After the broth medium was cooled down, the mushroom mycelium plugs from the pure culture (1 cm diameter) were inoculated. The mycelium sheets were grown on the surface of the liquid media in static conditions at laboratory room temperature (20–35 °C). After 30 days, the mycelium sheets were taken out of the flasks and dried in the hot air oven for 3 hours at 60 °C to remove moisture and inhibit further growth. The dried mycelium sheets were packed in plastic bags and stored in desiccators containing silica beads to protect them from contamination and moisture.

Determination of mycelium sheet properties

The intact, dried mycelium sheets underwent various tests to assess their morphology, water protection, flexibility, hardness, and degradability.

Morphology

The mycelium sheet morphology was visualized under a Scanning Electron Microscope (SEM, Hitachi S–3000 N) to observe the fibrous structure. The sample was coated with gold using Emitech sputter coater K500X before SEM analysis.

Water protection

The water resistance of the mycelium sheets was assessed using a water contact angle (WCA) goniometer (OCA 15EC, Dataphysics). It was to determine the waterproof ability of the mycelium sheet surface. The samples were flattened using 2 pieces of cover glass, and 5 µL water was dropped on the dried mycelium sheets. After 10 sec, the side view images were captured at room temperature (29-35 °C). The WCA was then automatically calculated using SCA20 software) (Massa-Angkul et al. 2020). This experiment was done with 5 replicates.

Flexibility test

The flexibility test was performed using a universal testing machine (Lloyd Instruments LR30K). Each sample was cut into a strip shape (0.4 cm × 0.25 cm) and was fixed with clamps on the machine. The deformation rate was set at 2 mm min^{-1} until failure. The ultimate tensile strength (UTS) and test-load value (max-min) dwell time were calculated from NEXYGEN™ PLUS Materials Testing Software. The mechanical analysis of the mycelium sheet was referred by Appels et al. (2020). This experiment was done with 5 replicates.

Hardness test

The hardness of the mycelium sheets was evaluated using microhardness tester Future-Tech FM-800 machine and FT-ARS software version 1.15.13 (Future-Tech Corporation, Japan) which automatically calculated the surface Hardness values of Vickers (HV). The test machine was the Vickers indenter that pressed into a surface under a static load. The indenter was a spherical diamond-tipped cone. The mycelium sheets were fixed on a wooden cube before testing. The compression force value for the Lw sheet was applied at 25 gf/15s and 50 gf/15s for the Oy sheet. The software visualized microscopic images of the pyramid shape after the materials were pressed. This experiment was done in 5 replicates.

Biodegradability

Mycelium sheet samples (0.10 g) were put inside a nylon teabag with 3 replicates and exposed to 3 environments; 1) on the soil surface (SS), 2)

soaked in water (SW), and buried in the soil (BS). For conditions SS and BS, soil for planting was purchased from an agriculture shop and was mixed with rough sand in a ratio of 1:1. For condition SS, the bags were placed on the soil in the transparency box with holes at the bottom to drain excess water and circulate the air. The bags were kept in position by rocks around the mycelium sheet and watered every 3 days. In condition BS, the samples were buried in the soil mixture then watering water to make the soil moist up to 85 -90 %. The samples exposed to SW condition were immersed in 150 mL water collected from a natural reservoir. The experiment was conducted at room temperature (20-29 °C). The soil temperature was 22-29 °C and the water temperature was 21-29 °C with pH of 7.11-8.23. After 1-week, the samples were carefully rinsed with water and dried in the hot air oven at 60 ° C for 3 hours and weighed again by sensitive electronics (Ibrahim et al. 2014; Ounkaew et al. 2018).

Statistical analysis

Analysis of variance (ANOVA) was used to compare the values derived from the tests to compare the performances of the mycelium sheets ($p<0.05$).

RESULTS AND DISCUSSION

Mycelium sheet production

According to Figures 1A-1D, grown mycelium sheets were shown successfully on the liquid media after 30 days. When comparing the two mushroom species, Lw was able to rapidly produce the fibrous structure and covered the surface of both MEB (Figure 1A) and PMPB (Figure 1B). Meanwhile, Oy could grow and stay on the surface of both PDB (Figure 1C) and YEB (Figure 1D). After drying, the Lw fibers were formed into a thick layer and the texture was leathery and tough (Figures 1E and 1F). The Oy fibers were thick and soft like a sponge and the dried mycelium sheet was thin, crispy, and fragile (Figures 1G and 1H).

Material analysis of mycelium sheets

Morphology

The dry mycelium sheets were stored in a desiccator with silica gel before being investigated under the scanning electron microscope. The micrographs of the morphological characteristics of the dry mycelium sheets are illustrated in Figure 2 below. All mycelium sheets showed a similar microstructural morphology. The mycelium fibers of the sheets were flat because of dehydration and were woven together without a specific pattern (arrowheads).

In this study, intact mycelium sheets of *Lentinus* sp. and *Pleurotus* sp. were successfully grown in 30 days at room temperature. The toughness presented a major weakness because the mycelium sheet was composed of the filamentous structure of the mycelia. The dried mycelia of the mushroom became very fragile. The electron microscope images revealed the mycelium sheets were porous because of the mycelial crosslink.

Water protection

The waterproofing ability of the mycelium sheets was tested by measuring the water contact angle (WCA). A higher water contact angle means a higher hydrophobicity and thus waterproofness. Here, the values of the water contact angle of the mycelium sheets were 103.3 ± 19.13° (LwMEB), 72.0 ± 32.9° (LwPMPB), 131.91 ± 9.70° (OyPDB), 113.14 ± 30.55° (OyYEB). The greatest WCA value was from the mycelium sheet of OyPDB, which was only significantly different from LwPMPB ($p < 0.05$) as shown in Table 1.

The hydrophobic performance of the mycelium sheets was determined from the water contact angle, i.e., a higher value implied a better waterproof

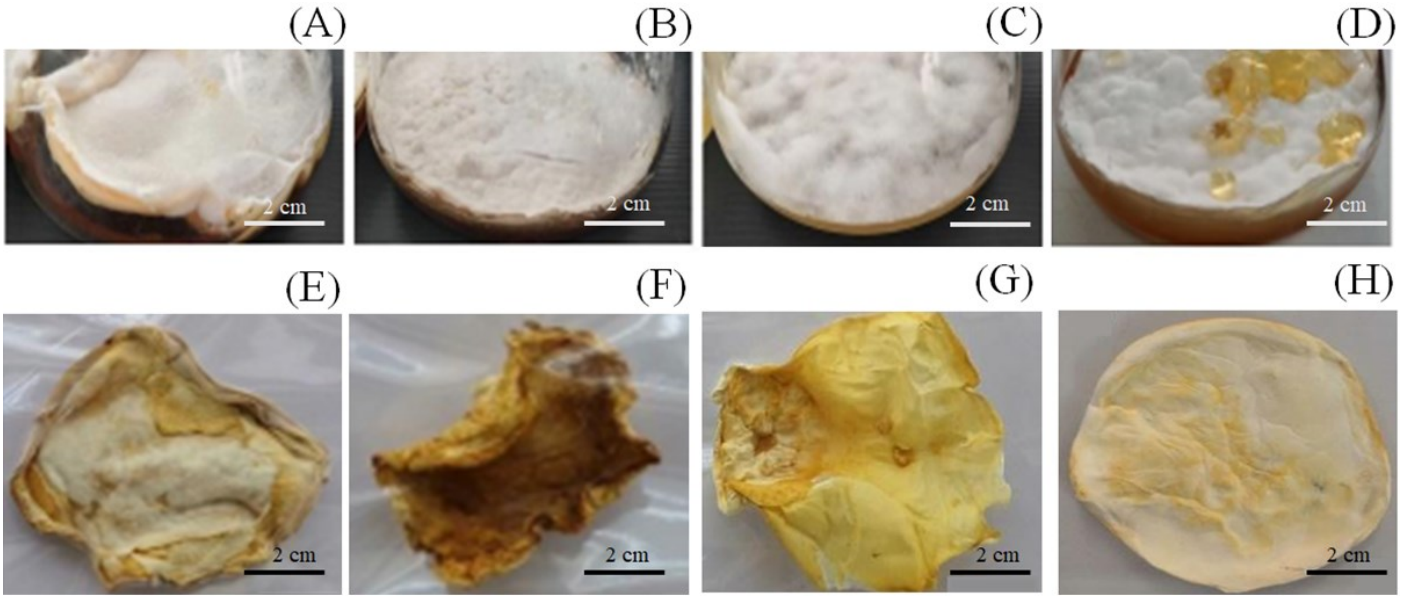

Figure 1. Freshly grown mushroom mycelia on the surface of the broth media after 30 days (A-D) and dried mycelium sheets (E-H). The following samples are displayed: LwMEB (A and E), LwPMPB (B and F), OyPDB (C and G), and OyYEB (D and H).

Table 1. Average values of water contact angles, ultimate tensile strength and average hardness values of the mycelium sheets. The different letters indicate significantly different ($p < 0.05$).

Mycelium Sheet	Water Contact Angle (° ± SD)	Ultimate Tensile Strength (MPa ± SD)	Hardness (HV ± SD)
LwMEB	103.3 ± 19.13a	15.88 ± 11.62a	5.81 ± 0.96b
LwPMPB	72.0 ± 32.9b	0.86 ± 0.54b	6.89 ± 1.76b
OyPDB	131.91 ± 9.70a	1.54 ± 1.71b	12.39 ± 1.72b
OyYEB	113.14 ± 30.55a	1.43 ± 1.14b	25.82 ± 6.05a

ability (Shen et al. 2024). The water contact angle was the highest on the OyPDB sheet at 131.9° and the lowest was found for the LwPMPB sheet (72.0°). The contact angle is the measurement to determine whether the material could lessen the moister absorption (Shen et al. 2024). This water protection property is derived from hydrophobins, proteins produced by fungi, leading to hydrophobicity on the mycelium surfaces. These proteins act as a protective layer that is resistant to water. Due to this property, the water absorption rate is lower on the mycelium sheet surface (Walter & Gürsoy 2022). Nawawi et al. (2019) produced paper from blue swimming crab shells, *A. bisporus*, and polypores mushrooms. The paper from these components gave the water contact angles at 65.6°, 24.2°, and 54.5° for the crab shell paper, *A. bisporus*, and polypores mushrooms respectively but the value of our mycelium sheets was relatively high at 131.9°. The WCA of *Schizophyllum commune* mycelium film from Appels et al. (2020) study was 129 ± 2° but when they added glycerol, the WCA was decreased depending on glycerol concentration. This implies the potential to prevent water on the material surface.

Flexibility test

The mycelium sheets were studied with a dual-column universal testing machine for their flexibilities, and the average tensile strengths were as follows. LwMEB (15.88 ± 11.62 MPa) had the highest value, which was significantly different from the others, OyPDB (1.54 ± 1.71), LwPMPB (0.86 ± 0.54 MPa), and OyYEB (1.43 ± 1.14 MPa) ($p < 0.05$) (Table 1). The highest tensile strength values were 15.88 ± 11.62 MPa, which was in the

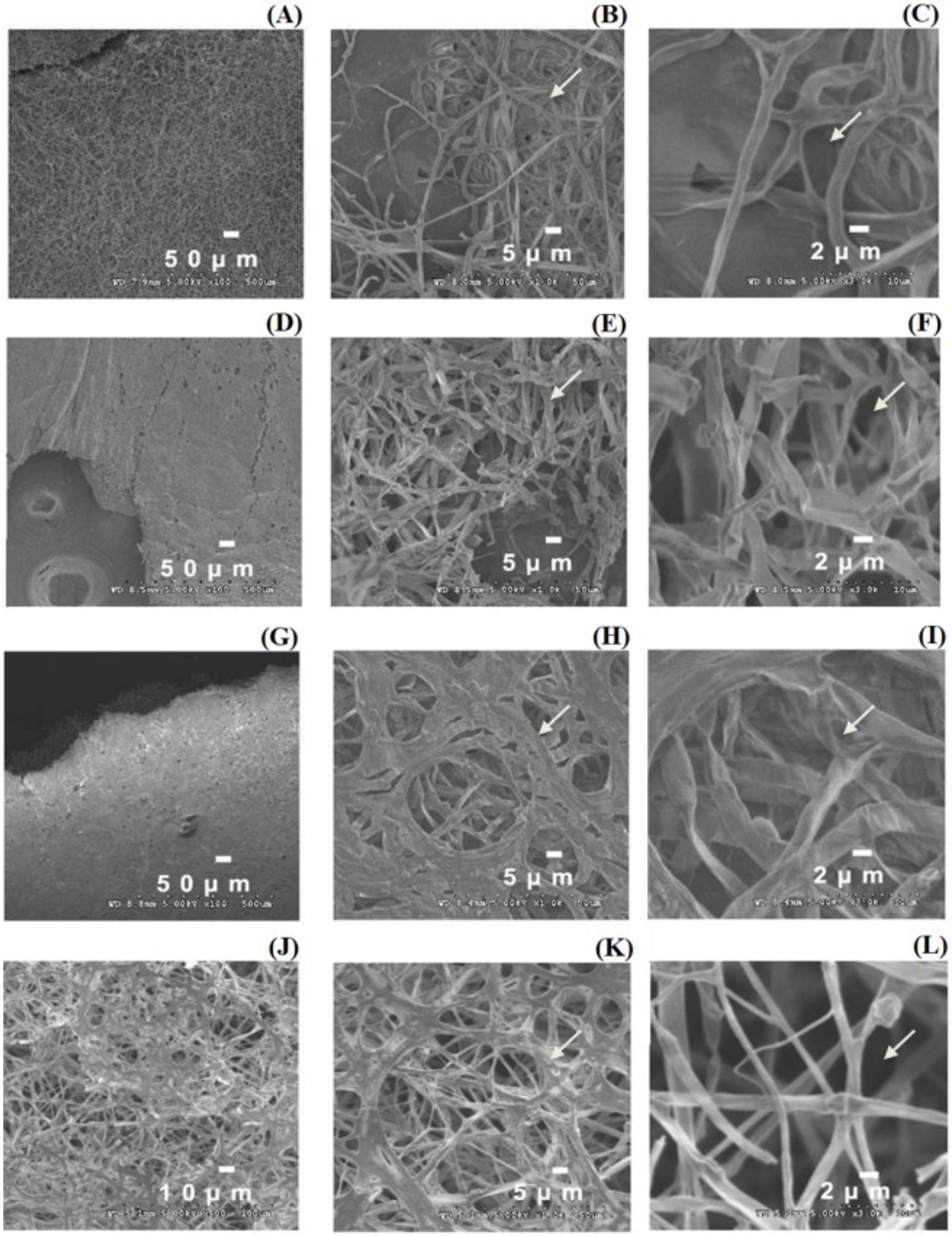

Figure 2. Electron micrographs of mycelium LwMEB sheet (A, B, and C), LwPMPB sheet (D, E, and F), OyPDB sheet (G, H, and I), and OyYEB sheet (J, K and L).

LwMEB sheet, while the minimum value was 0.86 ± 0.54 MPa in the LwPMPB sheet. In another research conducted by Appels et al. (2018), they used *S. commune* to make the mycelium-based composite and tested it for its tensile strength. The resulting test for this was 5.1-9.6 MPa which was around 4-10 times higher than the LwMEB and LwPMPB. The mushroom leather from *Phellinus ellipsoideus* by Bustillos et al. (2020) had a tensile strength of 0.34 MPa and the tensile strengths for the *A. bisporus*, crab shell, and polypore mushroom of Nawawi et al. (2020) were respectively 204.4, 65-204, and 65.3 Mpa, which were also higher than the MS with the highest strength (15.88 Mpa). Research by Bayer et al. (2014) showed bioplastics synthesized from microcrystalline cellulose and plant wastes (parsley and

spinach stems, rice hulls, and cocoa pod husks) powder mixed with trifluoroacetic acid. Our MS could be comparable to parsley (5 MPa) and spinach stems (1 MPa).

To improve the flexibility of the mycelium sheet, Appels et al. (2020) incorporated glycerol to enhance the flexibility of mycelium fiber, making it comparable to leather or rubber. The ultimate tensile strength of the mycelium sheet was 1.8 ± 0.1 MPa with 32 % glycerol, 12.3 ± 1.2 MPa with 2 % glycerol, and 5.0 ± 0.1 MPa for the sheet without the glycerol addition. Additionally, 20 % polyethylene was proved to increase the tensile strength of the mycelium leather from brown rot fungi comparable to the real leather (Raman et al. 2022). The interconnectivity of the mycelium leads to the uniform distribution of fungal mycelium throughout the material surface which could increase the capabilities to endure forces (Haneef et al. 2017). Shen et al. (2024) reported that their results could produce the mycelium material with a tensile strength of 0.72 MPa higher than the tensile strength of expanded polystyrene foam. Therefore, there should be other polymers to enhance this property of the mycelium sheets of this study.

Hardness tests

The compression force test or Vickers hardness test was performed and calculated. The hardness value of OyYEB was the highest (25.82 ± 6.05 HV) and significantly different from the others as shown in Table 1, OyPDB (12.39 ± 1.72 HV), LwPMB (6.89 ± 1.76 HV) and LwMEB (5.81 ± 0.96 HV) ($p < 0.05$).

It was found that OyYEB had the highest hardness of 25.83 HV, whereas LwMEB had the lowest hardness (5.81 HV). While the polymer composites obtained from kenaf fiber according to Abdullahi et al. (2018), the result showed a hardness value of 64.5-83 HRL based on the Rockwell hardness test (more than 600 HV) which is higher than the MS in this study. Meanwhile, the hardness value of the orange peel polymer composite with a combination of resin and natural fiber reported by Ojha et al. (2012) was 17.89-20.72 HV, which is comparable to the Oy and Lw sheets.

The Vickers hardness number (HV) is a unit to determine the hardness of the mycelium sheet derived from calculating the loaded force on the surface area (Wu et al. 2022). Materials with natural plant fiber e.g. lignin possess higher hardness because of the rigidity of lignin molecules (Lee & Choi 2021). In this study, the mycelium sheets reveal very low harness values because they comprised only the mushroom mycelium. Haneef et al. (2017) found the mycelium sheets showed different hardness depending on the mushroom species. The density and structure of the mycelium influence the hardness value. Moreover, the additional technique applied during the formation of mycelium sheets like pressing can improve the interconnectivity of the mushroom mycelium, contributing to the evenness of fungal mycelium throughout the sheets (Haneef et al. 2017). Therefore, this technique could be applied in further study to enhance the hardness of the mycelium sheet.

Biodegradability

The ability of the mycelium sheets to be degraded in nature was determined based on the weight loss of the mycelium sheet in 3 different conditions, i.e., being buried in the soil (BS), soaked in water (SW), and left on the soil surface (SS). The initial weight of each sheet was 0.10 g. In the first 3 days, mycelium sheets placed on the soil surface (SS) were found to be covered by other fungi. Every 7 days, all the mycelium sheets were taken, gently cleaned, dried, and weighed. The weight loss of all mycelium sheets in each condition was greater than 50 % after 7 days (Figure 3).

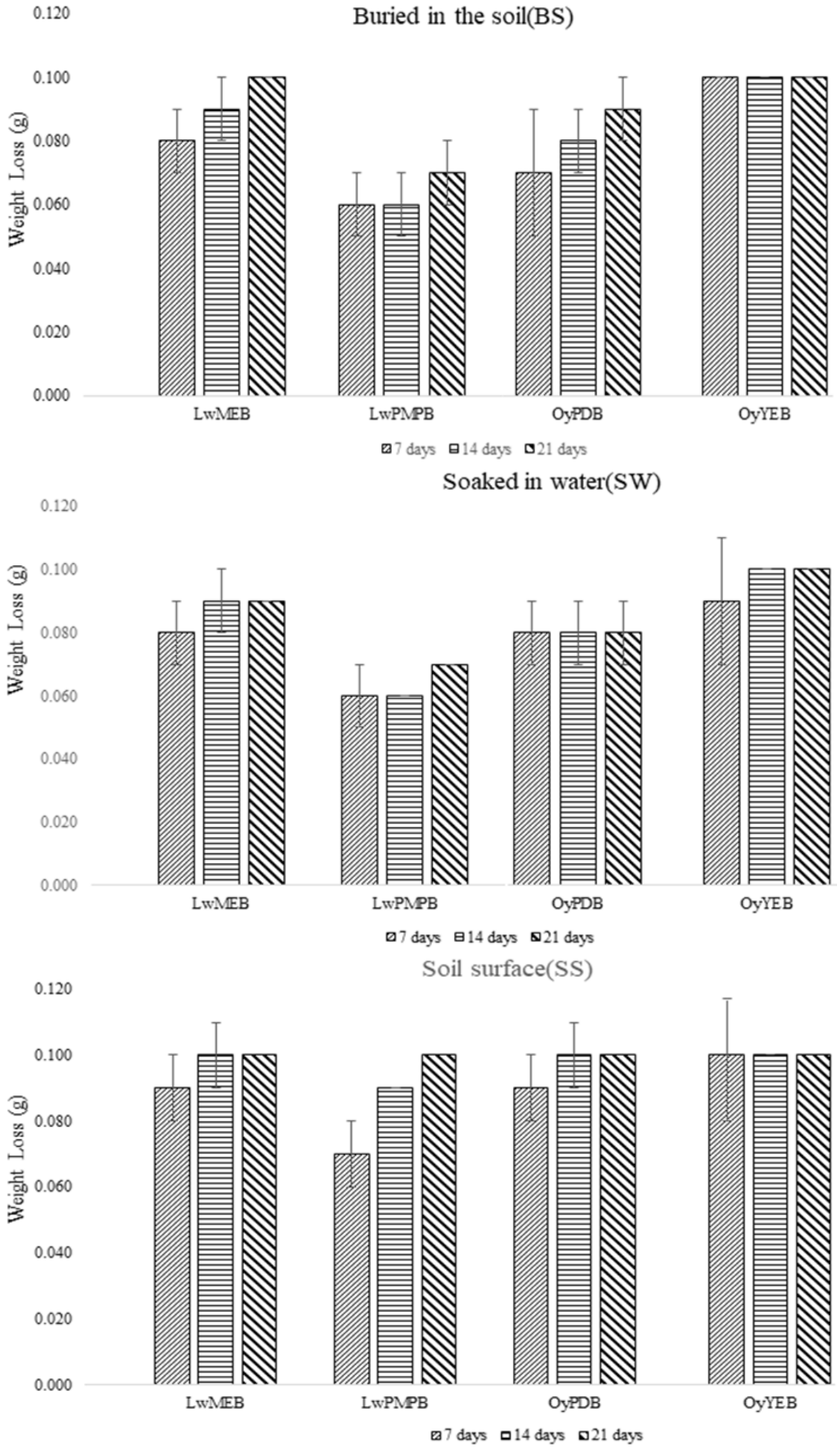

Figure 3. The potential biodegradability of the mycelium sheets in three different environments, being buried in the soil (BS), soaked in water (SW), and left on the soil surface (SS) for 7, 14, and 21 days. Error bars indicate ± SD.

The biodegradability of the mycelium sheets in all conditions was reported as follows. Firstly, in BS condition, at day 7, the OyYEB sheet had completely disappeared according to the weight loss (0.10 ± 0 g) followed by the OyPDB (0.07 ± 0.01g), LwMEB (0.08 ± 0.01 g) and LwPMPB (0.05 ± 0.01). At day 14, the weight loss of OyPDB and LwPMPB were 0.08 ± 0.01g and 0.05 ± 0.01g respectively. After 21 days, only some partial mass of the LwPMPB sheet (60 %, 0.06 ± 0.01 g) was left in this condition. Regarding the SW condition, at day 7, the weight reduction of the OyPDB sheet (0.07 ± 0.01 g) and OyYEB sheet (0.08 ± 01 g) was significantly different from the LwPMPB sheet (0.05 ± 0.01 g) ($p < 0.05$). After being soaked in water for 21 days, the OyPDB lost 0.083 g (83 %), LwMEB lost 0.090 g (90 %) and LwPMPB lost 0.070 g (70 %). In the last environment, which was SS condition, the LwMEB and OyPDB sheets had a weight loss of 0.9 ± 0.01 g, which was significantly different from the LwPMPB sheet (0.06 ± 0.01 g) but non-significantly different from OyYEB (0.10 ± 0) after 7 days of exposure to this condition. Seven days later, the mycelium sheets of OyYEB and LwMEB were completely biodegraded (0.10 ± 0 g) and were not significantly different from the LwPMPB sheet (0.09 ± 0.01 g) and OyPDB (0.09 ± 0.0 g). After 21 days, all samples disappeared, indicating their complete biodegradation.

The ability to decompose in this study, after 7 days of testing in all 3 conditions, the weight loss of every example was more than 50 % and decreased continuously until it could not be measured. We found that out of the four mycelium sheets, OyYEB was rapidly degraded and LwPMPB was the slowest. It demonstrated the MS could be decomposed in a very short period, 1-3 weeks. In congruence with Ounkaew et al. (2018), they invented the polyvinyl alcohol starch film for bio packaging and this film was able to be degraded naturally by 65.28–86.64 % in 30 days. Similarly, the biodegradability of the pectin/polyvinyl alcohol films prepared by Linn et al. (2022) was 50-75 % within 7 days. This common approach to determine the biodegradability of materials is the soil burial test and the loss weight of the material is periodically quantified. This method is very practical for biodegradability determination because it includes environmental factors from the soil or water such as temperature, natural microbes and moisture that have impacts on the rate of biodegradability activities (Vandelook et al. 2021). In another report related to this issue, the bioplastics synthesized by Bayer et al. (2014) took 1 week to fragment the film into smaller pieces and 1 month to completely disintegrate in water. However, no research solely reported the potential biodegradability of mycelium sheets from mycelia.

CONCLUSIONS

This research reports the simple method of mycelium sheet production but there are points to be improved. The fresh mycelium sheet collection from the flask should be more convenient but there was a limitation with the container used to make the mycelium sheet. Fibers grown in flat bottles could form the mycelium sheets more quickly because of the lower water level compared to using a high level of broth. In the flasks, after the mushroom plugs were inoculated, the plugs sunk to the bottom of the flask. Once the mycelia started to grow, they floated to the surface of the liquid medium and began to form the fiber covering the water surface as the mycelium sheets because of the inter-weaving of the mycelia, which was most evident in Lw and Oy. However, the method of harvesting the mycelium sheets from the flasks in this study was too difficult because of the narrow neck of the culture flasks. Specific culture bottles should be obtained to meet both the technical and biological protocols, being simple to harvest and able to prevent contamination. To improve its mechanical properties in the future, the mycelium sheet needs to be enhanced with additional materials. For example,

it could be co-cultured with other mushrooms or coated with polymers such as resin, rubber or other polymers to increase flexibility and provide more stiffness to the texture. However, since the mycelium sheets in this study were made purely from the mushroom mycelia that assured the friendliness to the environment. In conclusion, this study provides the first light to achieve the practical alternative material from mushroom mycelia.

AUTHOR CONTRIBUTION

C.P. designed, conducted the research, collected and analyzed data and drafted the manuscript. W.J. designed the research and supervised all the processes, proofread and reviewed the manuscript. J.T.N.K. designed the research, supervised the experiments, and reviewed the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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