

Research Article

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Biocomposites from agricultural wastes and mycelia of a local mushroom, *Lentinus squarrosulus* (Mont.) Singer

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Abstract: Biomaterials from mushrooms and natural fibers have been used to reduce environmental impacts. This study aimed to develop the mycelium composite from four local mushrooms, *Pleurotus ostreatus*, *Auricularia auricula-judae*, *Lentinus squarrosulus*, and *Lentinus polychrous* and three substrates derived from agricultural wastes (coconut husk, rice husk, and rice straw). The mycelia of all mushrooms were cultured on potato dextrose agar and measured for the growth for 9 days. It showed that *L. squarrosulus* had a colony size significantly greater than the other species. Then, *L. squarrosulus* was selected to form the biocomposites and tested for material properties, e.g., morphology, compressive strength, water and moisture absorption, and biodegradability. Microscopically, the mycelium colonized almost all parts of the rice husk while it colonized less in the rice straw and coconut husk. However, the rice straw absorbed significantly more moisture than rice husk and coconut husk, but the coconut husk absorbed significantly more water than the rice husk and rice straw. Moreover, the rice straw composite showed the highest compressive strength value and the greatest biodegradability according to the highest weight loss. This research provided the results of the mycelium biocomposite production and mechanical tests for future biomaterials.

Keywords: agricultural wastes, biodegradability, green materials, mushrooms, mycelium

1 Introduction

The construction sector helps to develop the country and earn benefits, but construction waste is harmful to the environment and human society, and nonrenewable resources are also rapidly decreasing. This challenge has been taken for solutions. One of them is to create biocomposites from living organisms, e.g., algae, fungi, and bacteria to reduce the use of nonrenewable materials. Mycelium-based composite as an alternative is the result of the structure of filamentous fungi or mushroom species that grow on different natural fibers to produce biomaterials [1]. Mycelium is an association of interwoven and sting-like fungal hyphae which compose the vegetation part of mushroom growth by stretching and splitting their hyphae into substrates. Mycelium-based materials can be applied to industrial materials as a biodegradable alternative resource to develop a broad scope of production in the fields of architecture and industrial design, such as bricks [2].

Agriculture waste is defined as the non-product output of production from cultivating and agricultural activities such as grains, vegetables, or crops [3]. Agriculture has expanded three times over the past 50 years due to the development of land, agricultural needs, mechanical improvement, and population expansion which approximately result in 23.7 million tons of agriculture products per day globally [4]. Around 80% of solid waste is from farms in the form of organic decay, so agricultural waste such as straw, cotton stalks, sunflower shells, corn, rice hulls, coconut shells, and others are contributing varieties of helpful materials for many purposes [5]. Coconut production was estimated at around 1.8 million tons which gives coconut husk 80–85% of the total weight of coconut [6]. Production of rice husk was estimated at around 180 million tons worldwide while rice production was approximately 750 million tons [7]. Rice straw was the result of the rice production of lignocellulosic waste and estimated around 731 million tons [8]. There are biomaterials from living

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organisms such as fungi using these agricultural wastes as substrates.

Various types of mushrooms have been used to produce composites. For example, *Pleurotus ostreatus* was cultured with supplements for cultivating the mycelium on wheat bran and tested mycelium-based composite for compressive strength to produce materials as masonry units for architectural construction [9]. These mushroom species had already been used as mycelium composites to produce the biomaterials for replacing nonrenewable products, and there are more mushrooms locally found that are more interesting to study in this aspect. Therefore, this research aimed to make the mycelial biocomposites from four mushroom species: *P. ostreatus*, *Auricularia auricula-judae*, *Lentinus squarrosulus*, and *Lentinus polychrous*. Particularly, the *Lentinus* species are local to the region which allowed the study reporting the first record. Due to the effective promising properties of agriculture waste and mushrooms, the preparation of biocomposites of local mushroom mycelia using agricultural waste, rice husks, coconut husks, and rice straws as growth substrates was completed. Next, the effect of mushroom species and substrates on material properties of the mycelium composites was also evaluated to obtain the best biocomposite materials.

2 Materials and methods

2.1 Culture media preparation

Two different media were prepared in this experiment for growing mycelium. First, potato dextrose agar (PDA) was prepared: potatoes 200 g were diced into small pieces, mixed with dextrose 20 g, agar 20 g, and cooked with distilled H₂O 1,000 mL until melting. Then they were poured into glass bottles and autoclaved for 30 min at 121°C to sterilize the medium. Next, PDA was poured into Petri dishes and solidified at room temperature. Secondly, potato dextrose broth (PDB) was prepared with the same process without agar. After that, PDB was poured 150 mL per glass bottle and autoclaved for 30 min at 121°C. Dextrose in PDB was added to be proportional to the quantities of potato and water.

2.2 Mushroom isolation and culture

Four mushroom species collected from the local markets in Muang, Khon Kaen, Thailand, *P. ostreatus*, *A. auricula-judae*, *L. squarrosulus*, and *L. polychrous* [10,11], were

isolated for the pure mycelia. Small tissues (1 mm² × 1 mm²) inside the fresh mushrooms were taken using sterile surgical blades. Then, the tissues were placed in Petri dishes containing PDA. The plates were kept at room temperature for 7 days to let the fungi grow under dark conditions. The mycelia growing from the mushroom tissues were isolated on the new PDA to obtain the pure culture for another 7 days and stored at room temperature for further experiment. A mycelium plug was inoculated in a Petri dish containing PDA (four mushroom species, five plates each). Then the plates were kept in the dark condition at room temperature. The radial growth of the mycelia was measured daily to determine which mushrooms exhibited the highest growth.

2.3 Production of mycelium biocomposites in substrates

Bottles containing the substrates (10 g), rice husk, rice straw, and coconut husk, were autoclaved for 30 min. Before the mushroom mycelia were transferred in substrate bottles, the mycelial plugs from the previous experiment were propagated in PDB and placed on the shaker (Brunswick Scientific) at 160 rpm for 7 days at room temperature. Next, the mycelia suspension in the broth (20 mL) was transferred to each screw top culture bottle containing the autoclaved substrates. After 3 months at room temperature, the mycelium composites from each substrate were formed as the blocks. Then, they were taken out from the bottles and dried in the hot air oven at 60°C for 2 days before testing the material properties.

2.4 Scanning electron microscopy (SEM)

Small pieces of the mycelium composites dissected from the blocks of each substrate were bounded over a carbon tape on the copper coins and transferred into Emitech sputter coater model: K500X to coat the surface of the specimen (gold-coated and voltage of 5.00 kV). Then, they were examined under the SEM, S-3000N. The samples were observed for the morphological characters of mycelium.

2.5 Moisture exposure

The blocks were added to a moisture chamber to control humidity with a UNI-T digital temperature humidity meter. Then, the mycelium blocks of each substrate were put in a

moisture chamber to determine moisture uptake at 28°C under a relative humidity (RH) of 85% and the weight of the blocks were measured after 1, 2, 3, and 4 h to monitor the blocks' rapid weight gain in the first few hours of moisture absorption. Then the blocks were measured every 24 h up to 12 days until the weight of the samples was stable. This part was conducted in three replicates. The moisture exposure was expressed in percentage of weight gain [12]: $M(\%) = [(W_w - W_d)/W_d] \times 100$, where M is the moisture content, W_w is the sample mass after immersed moisture, and W_d is the mass of the dry samples.

2.6 Water absorption

The prepared mycelium biocomposite blocks of each substrate were placed in three different chambers containing distilled H₂O at 27°C to measure the different mass of mycelium blocks every 10 min of the first hour then 2, 3, 4, and 5 h to monitor the blocks' rapid weight gain in the first few hours of water absorption. Next, the blocks were measured every 24 h up to 9 days to analyze the water absorption of composites until the weight of the samples was stable. Before the samples were weighed, they were wiped using filter paper to remove the excess water on the surface for 1 min. This part was conducted in four replicates. The water absorption was also expressed as a percentage derived from the formula [13]:

$$\text{water absorption (\%)} = [(W_2 - W_1)/W_1] \times 100, \quad (1)$$

where W_1 and W_2 are the weights before and after soaking in water, respectively.

2.7 Compressive strength

Mycelium blocks of each substrate were performed for the compressive test on Universal Testing Machine, Model LR 30k with 1,000 N, the test speed was at a rate of 5 mm/min and stopped at 40% strain in the specimen. This part was conducted in three replicates. Then, the stress value was recorded and calculated using the formula [12]: compressive strength (σ) = F/A , where F is the compressive effort (N) and A is the specimen pattern cross-section (mm^2).

2.8 Biodegradability

Mycelium biocomposite of each substrate was cut into small pieces and put into nylon bags and buried 6 cm

underneath sand and natural soil mixture (1:1) at 24°C for 5 weeks. Then samples were cleaned with distilled H₂O and dried overnight at 60°C and weighed to determine their weight loss using the formula [14]:

$$\text{weight loss (\%)} = (W_0 - W_1)/W_0 \times 100, \quad (2)$$

where W_0 is the sample weight before biodegradability testing and W_1 is the sample weight after biodegradability testing. This part was conducted in five replicates.

2.9 Statistical analysis

Data derived from the mushroom growth were compared using ANOVA ($p < 0.01$). The values derived from material property tests were compared using ANOVA ($p < 0.05$) to find significant differences. The summary of research methodology is shown in Figure 1.

3 Results

3.1 Growth evaluation and production of mycelium biocomposites

Four mushroom species were isolated on the PDA plate and measured for their growth from the second day until the ninth day. Among the four mushroom species,

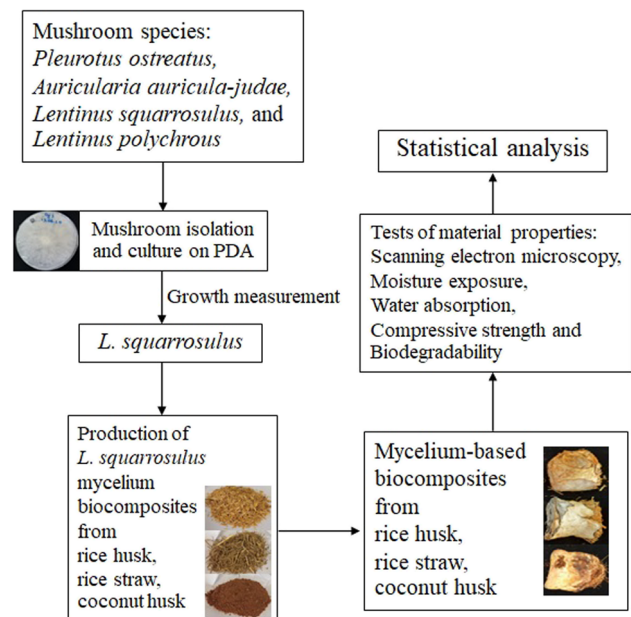


Figure 1: Research methodology employed in this study.

L. squarrosulus, *L. polychrous*, *P. ostreatus*, and *A. auricula-judae* showed different growth (Figure 2). Based on ANOVA, *L. squarrosulus* (8.96 ± 0.4 cm) was significantly greater than *L. polychrous* (8.26 ± 0.2 cm), *P. ostreatus* (7.33 ± 0.28 cm), and *A. auricula-judae* (6.23 ± 0.32 cm) ($p < 0.01$) on the ninth day. It can be said that *L. squarrosulus* proved to be a significantly better grower than the other three species. Therefore, *L. squarrosulus* was chosen to grow with substrates to produce the biocomposites for testing the material properties.

3.2 SEM

The surface of rice husk, rice straw, and coconut husk of *L. squarrosulus* mycelium composites are portrayed in Figure 3. The right pictures represent the micrograph of mycelium which colonized each substrate differently in the images. The pictures show the highest growth density of mycelium on the rice husk compared to the other substrates. The mycelia also spread on the coconut husk, but the growth was weak and limited to the surface. Due to the filamentous structure of the mushroom mycelia extending throughout the substrates, it could bind the substrate parts together to form mycelium blocks (Figure 4).

3.3 Moisture exposure and water absorption

The mycelium blocks of each substrate with *L. squarrosulus* were measured before and after the moisture exposure at 85% RH to calculate the increased weight. The result suggested

that after the first hour of moisture exposure until the twelfth day (Figure 5), the rice straw block had the significant increased weight ($34.76 \pm 1.74\%$) compared to the rice husk ($13.19 \pm 1.95\%$) and coconut husk ($30.11 \pm 2.84\%$), $p < 0.05$. The weight increased rapidly in the first 3 days and kept increasing slowly. The rate of water absorption of *L. squarrosulus* mycelium of different substrates is shown in Figure 6. It shows the increase in weight of the substrates after immersed in water from the first 10 min until the ninth day. The graph shows the water absorption as percentages of coconut husk ($609 \pm 58.62\%$) was statistically better than the rice husk ($229.08 \pm 68.77\%$) and the rice straw ($229.08 \pm 44.00\%$), $p < 0.05$. Therefore, the coconut husk ($237.90 \pm 113.18\%$) absorbed water and its weight increased more significantly than the rice husk blocks ($150.92 \pm 38.37\%$) and the rice straw blocks ($69.94 \pm 43.35\%$) from the first 10 min, $p < 0.05$. The rice straw ($206.45 \pm 50.93\%$) started increasing more weight than the rice husk ($184.88 \pm 40.29\%$) at the first hour while the weight of the rice husk dropped ($264.08 \pm 57.37\%$) on the seventh day because it could no longer hold water and broke into pieces.

3.4 Compressive strength

The results regarding the compressive strength of *L. squarrosulus* mycelium composites using rice husk, rice straw, and coconut husk are shown in Table 1. It revealed the force resistance of the samples. The rice straw blocks showed the highest compressive strength (0.54 ± 0.06 MPa) while the rice husk (0.46 ± 0.10 MPa) and coconut husk (0.47 ± 0.20 MPa) had a similar value due to the statistical analysis ($p < 0.05$). The rice husk and coconut husk blocks

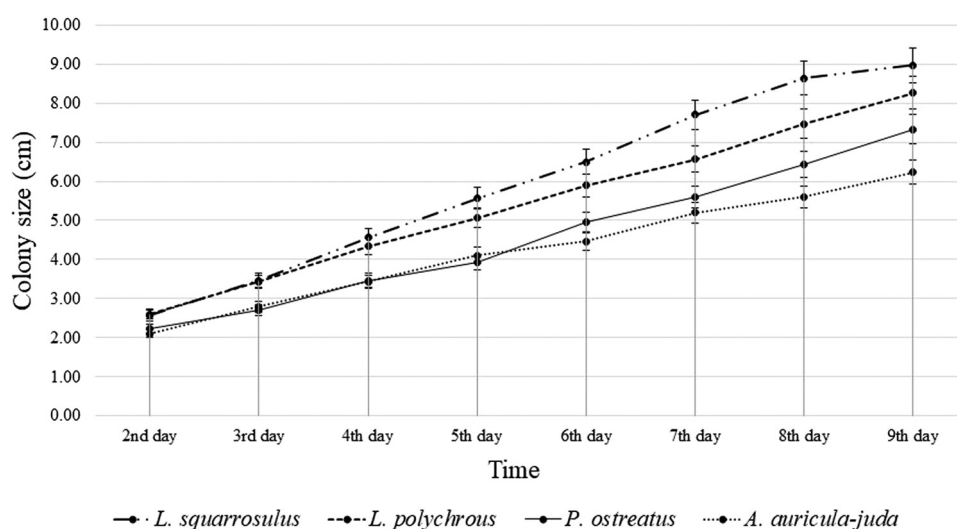


Figure 2: Average growth of four mushroom species in PDA plates from the second day to the ninth day.

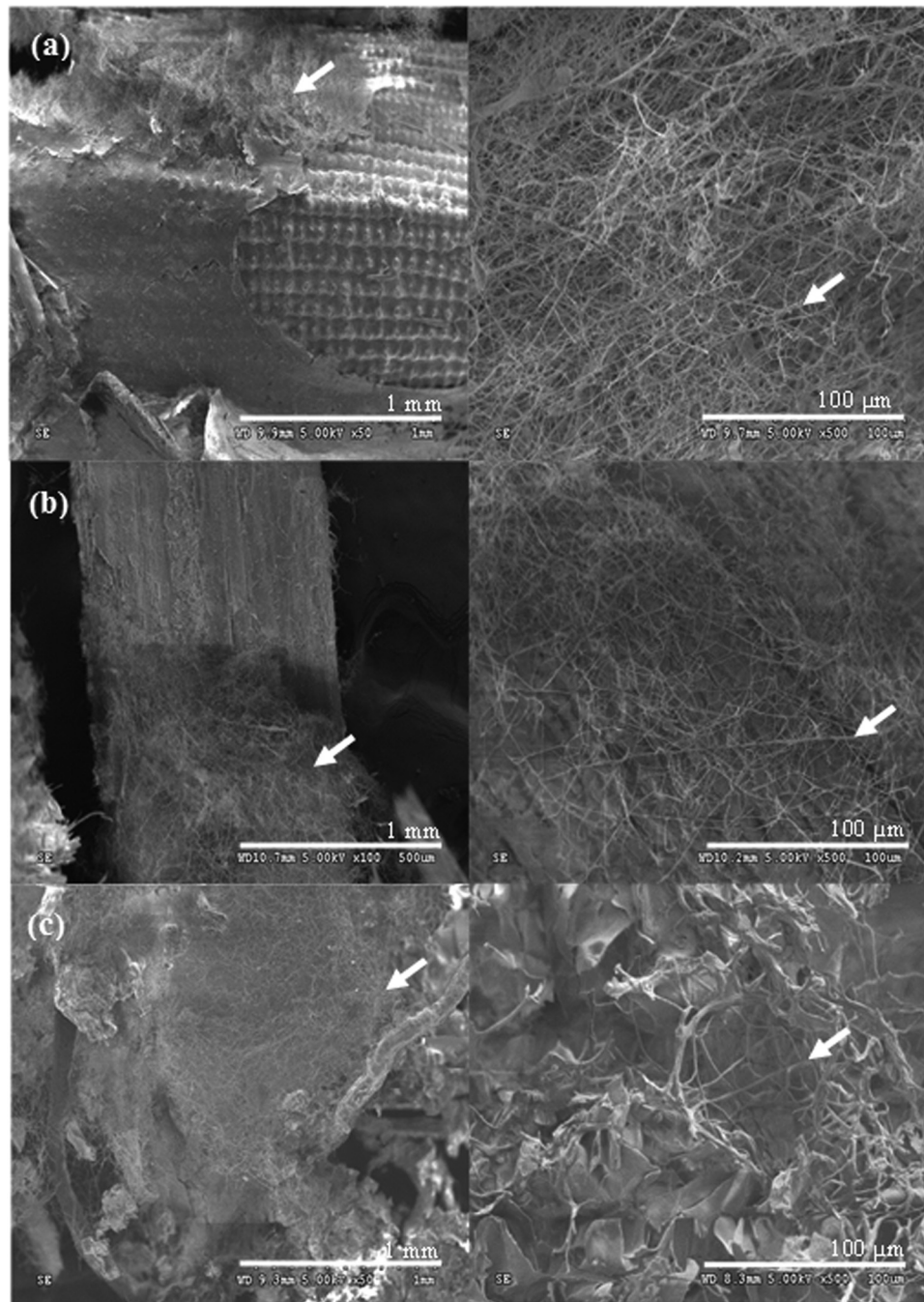


Figure 3: SEM micrographs of *L. squarrosulus* mycelium on the substrates (arrow heads): (a) rice husk, (b) rice straw, and (c) coconut husk.

were fractured into pieces after testing while the rice straw blocks could maintain the block shape.

3.5 Biodegradability

The biodegradability is determined from the loss of the weight of the blocks. This study determined the weight

loss (%) of the blocks after being buried in the soil mixture for 4 weeks. The result suggested that the rice straw's weight loss was significantly higher ($46.98 \pm 4.03\%$) than that of the rice husk ($13.19 \pm 3.13\%$) and coconut husk ($15.31 \pm 7.81\%$) as shown in Figure 7. After 4 weeks underneath the soil mixture, the rice husk and coconut husk blocks were shattered into small fragments, but the rice straw still could retain its shape.

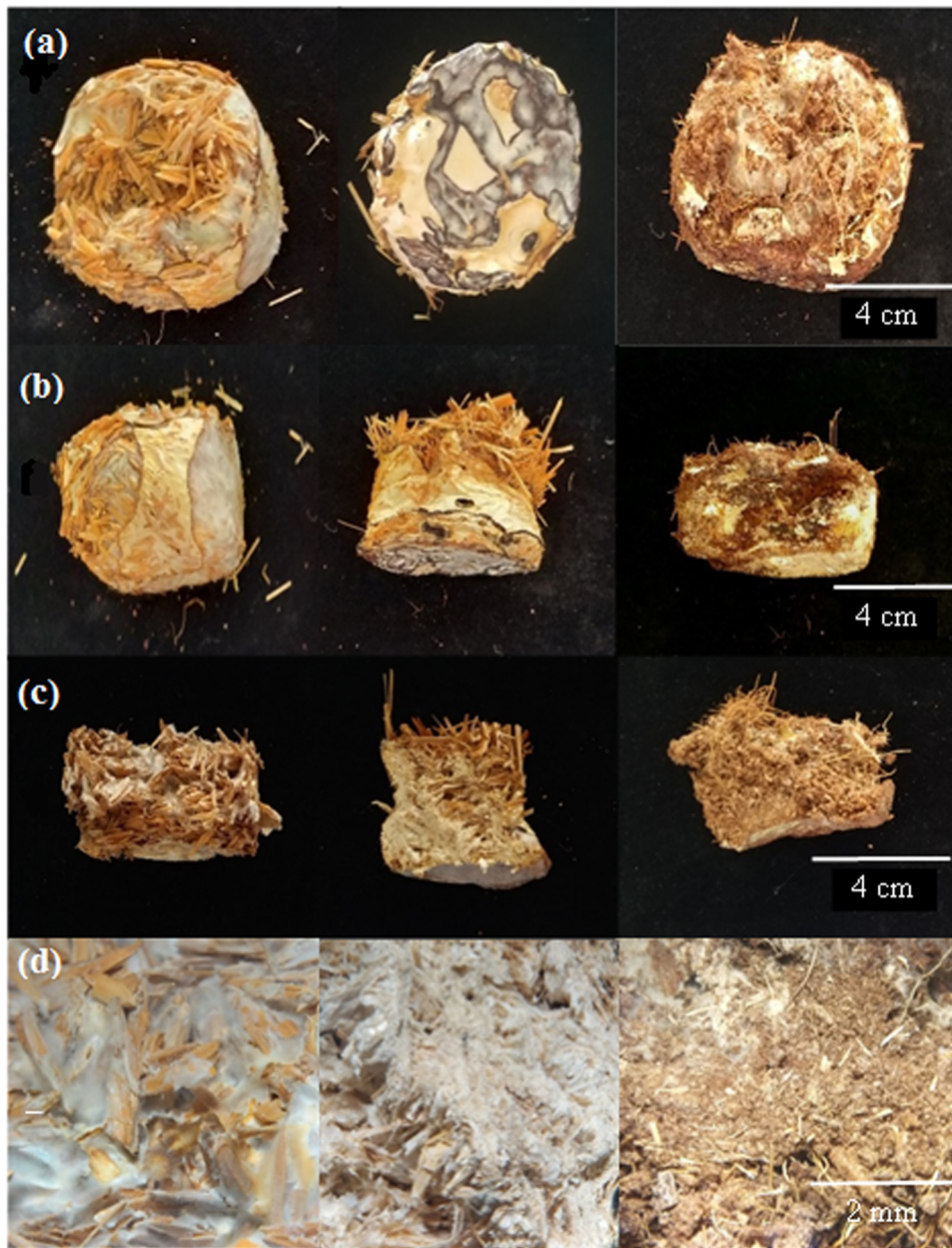


Figure 4: Mycelium composites from *L. squarrosulus* using rice husk (left), rice straw (middle), and coconut husk (right) in different dimensions: (a) top view, (b) side view, (c) inner view, and (d) stereomicroscopic images.

4 Discussion

According to the results, *L. squarrosulus* was the mushroom among the four species exhibiting the highest growth performance. It continued to produce the mycelium blocks with three different substrates. The visual observation (Figure 4) showed the appearances of mycelium blocks of each substrate. The rice husk had large particles to provide oxygen and contained amorphous silica (20%) and organic substances such as cellulose

and lignin (80%). They then became the nutrients for the mycelia to grow and colonize in the rice husk particles more efficiently [15,16]. Rice straw contains a favorable source of carbon for mushrooms such as cellulose (39%), lignin (12%), and dust (11%), which show potential for mycelium growth [17]. The coconut husk contains lignin (45.8%), cellulose (43.4%), hemicellulose (10.25%), and pectin (3%), which are similar to that found in the mushroom's natural habitat and also can keep humidity for the mycelia to grow [18]. These conditions of rice husk,

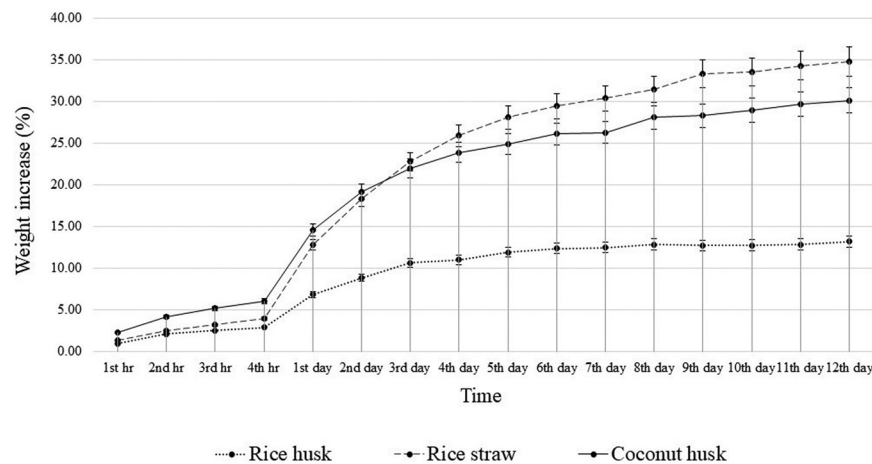


Figure 5: Average weight increase of *L. squarrosulus* mycelium-based composite of each substrate when exposed to moisture at 85% at 28°C from the first hour to the twelfth day.

rice straw, and coconut husk improved *L. squarrosulus* mycelium growth and formed the network with these three substrates as the block composites. The mushroom mycelia were successful growing into the inner parts of the substrates, especially in the rice husk (Figure 4). The rice husk had appropriate particles which let the mycelia attach to and bond inner parts with the nutrients from the substrate to improve the growth [16]. A study by Yang et al. [19], reports about the physical nature and high carbon to nitrogen ratio of rice straw with lower biological efficiency, and it states that the mycelium could not colonize the inner parts of rice straw very well. Also, the

coconut husk would affect the mycelium yield and colonization, so mycelium could not bond the inner parts of this substrate [20]. The property and condition of rice husk allowed mycelium to colonize into the inner parts and were better than rice straw and coconut husk due to the visual observation (Figure 4). However, these conditions did not suggest that the rice husk composite was better than the other two substrates in terms of properties.

As a result of the moisture exposure, Figure 5 shows the increase in weight of the rice straw blocks; it was greater than the coconut husk and rice husk because the

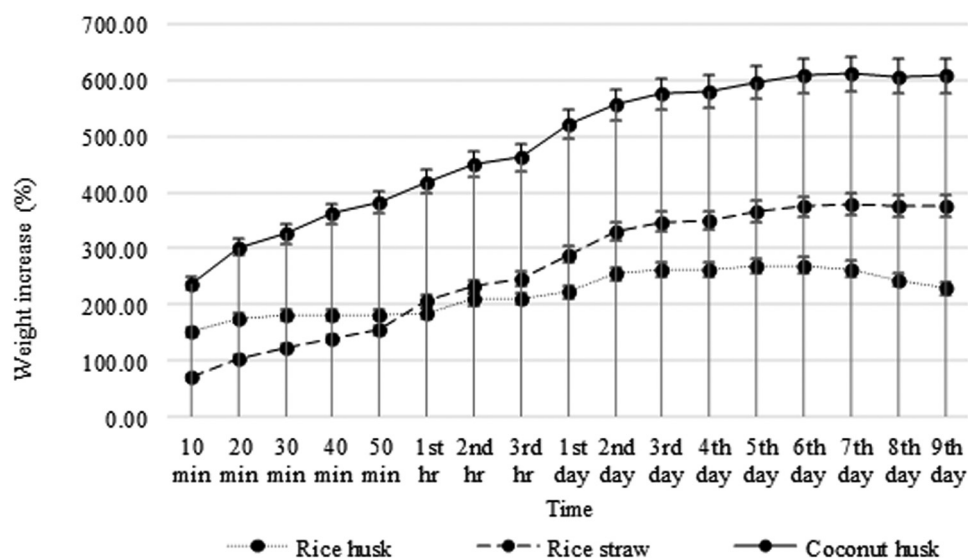


Figure 6: Average weight increase (%) of *L. squarrosulus* mycelium composite after placed in water at 27°C from the first 10 min until the ninth day.

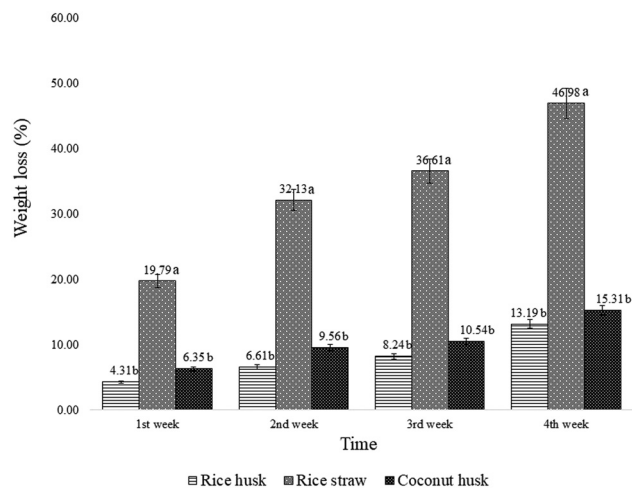


Figure 7: Average weight loss (%) of *L. squarrosulus* mycelium composites using three substrates after buried in the soil mixture for 4 weeks. Different letters indicate significant differences ($p < 0.05$).

rice straw had a low lignin content which is key to the durability and stability including storing and binding the moisture [21]. Meanwhile, the coconut husk structure had the characteristic of high water detention [22]. In contrast to water absorption, coconut husk could maintain water longer than the rice husk and rice straw because it is a natural fiber that has a high content of lignin and hemicellulose. It is able to absorb water and store for a longer time, so the weight increases more than the other two substrates after being immersed into water [23]. The rice straw was reported as a lignocellulosic fiber, leading to a reduction in water absorption by the reason of hydrophobic properties which repel the mass of water from the substrate [24]. Comparatively, the rice husk was a cellulose-based fibrous material with low bulk dimension, toughness, and water resistance. For this reason, this agricultural waste could not absorb water and moisture fast [25]. The coconut husk has cellulose, hemicellulose, and lignin which are able to absorb a high amount of water [26]. Accordingly, the biocomposites from the coconut husk are favorable to applications associated with the water

Table 1: Compressive strength of *L. squarrosulus* mycelium blocks on rice husk, rice straw, and coconut husk

Substrates	Compressive strength (MPa)
Rice husk	0.46 ± 0.10^b
Rice straw	0.54 ± 0.06^a
Coconut husk	0.47 ± 0.20^b

Different letters indicate significant differences ($p < 0.05$).

absorption but they are not an option as the materials used to protect water or moisture unless the waterproof ability of the blocks is further enhanced.

The conclusion of the compressive test among the three substrates showed that the rice straw took a lead (0.54 MPa) compared to rice husk (0.46 MPa) and coconut husk (0.47 MPa). Three of them were known as the lignocellulosic materials, but their properties were different during the compressive test, i.e., the composite blocks of rice husk and coconut husk were broken after compressing, and their compressive strength was lower than the rice straw because the rice husk had small and thick particles and the coconut husk body was similar to powder which could be shattered easily. In contrast to the rice straw blocks, they had longer and thinner components to maintain the shape with higher stress strength during compression. This study is compared to the article by Elsacker et al. [12], in which *Tarmites versicolor* mycelia was grown on different substrates like hemp, pine wood, and flax waste, and tested for the compressive strength to measure the resistance of composite. The combination of the mycelia and hemp received the highest compressive stiffness (0.51 MPa) which is similar to the finding in this study, followed by flax waste (0.31 MPa) and pine wood (0.14 MPa). The article also described that the small-sized fiber of hemp had an influence on the ability to tolerate the compression. Rice straw and hemp had similar fiber conditions and also gave the highest compressive strength among the following substrates. Also, the wood fiber composite had the highest compressive strength (39.78 ± 8.71 MPa) [27]. Moreover, a study by Bamigboye et al. [28] evaluated the compressive strength on concrete cubes incorporated in coconut husk, cement, and sand. The value strength of the specimen was obtained at 25.59 N/mm^2 . Due to the results, the blocks produced in this study were obviously not as strong as other materials in the literature. Therefore, the improvement of the block related to this issue should be sought out.

The biodegradability test conducted in sand and soil mixture was determined by the weight loss of the mycelium biocomposites [29]. The rice straw lost more weight than the rice husk and coconut husk because the microbes in the soil mixture could degrade this type of cellulosic substrate better [30]. The rice husk structure was bigger, and the coconut husk structure was in a powder-like form [31]. They both contained lignin as a hydrophobic component which reduced the rate of biodegradation in the soil. These three natural fibers had similar components, but they had different abilities to be degraded in soil. In this study, it can be said that the most biodegradable biocomposite was the rice straw. Similarly, the kenaf composites

were buried in organic soil for 22 days and the degradation rate of kenaf composite was at 43% [32].

5 Conclusion

The finding of this research provided important information on structure, water and moisture absorption, and other properties. The mycelium-based biocomposite was achieved from the mushroom species and different substrates. As the results, it could be concluded that *L. squarrosulus* was the most favorable mushroom species to form the biocomposite with rice straw as the most suitable substrate because of its potential properties including biodegradability. Also, the mycelium composite from this study could be used as an economical, degradable, and sustainable alternative material to reduce the solid wastes causing pollution to the environment.

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Author contributions: LL: literature search, methodology, experiments, data analysis, original draft; WJ: conceptualization, supervision, original draft, review and editing.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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