

**Evaluation of Ramie Bark and Albasia Sawdust Substrates for Mycelium-Based Composites Using *Leiotrametes lactinea***

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**Abstract.** Mycelium-based composite (MBC) has significant potential to utilize agricultural biomass waste. The use of fungi that are commonly used as MBC materials is still very limited to certain types. This study aims to test the MBC characteristics of the growth of mycelium *Leiotrametes lactinea* MYCL-3 on three different media substrates containing 72% Albasia (AS) sawdust, 72% ramie bark (RB), and 30% Albasia + 47% ramie mixture (AS-RB). The resulting MBC has dimensions of 20 x 5 x 5 cm and is yellowish-white in color. The structure of MBC shows that the density is higher (RB 0.28%). The absorption percentages for AS, RB, and AS-RB were 100.88%, 135.2%, and 199.34% respectively. The biodegradability of MBC showed that by day 10, the sample had degraded by about 14.14–17.46%. Mechanical testing for compression strength determined values for AS (117 kPa), RB (350 kPa), and AS-RB (140 kPa), with a final strength at 464 kPa, and the effect of the mixed media reduced the tensile strength of the composite to 277 kPa. The IR spectral results showed that the three composite samples did not show much different structures, but AS composites were known to have more types of constituent compounds not found in RB and AS-RB composites such as C=C (alkenes) bending, O-H (alcohol) associations, C-O-C (glycosidic) stretching, O-H (acid) bending, and C=C (benzene) stretching. Future investigations may focus on improving aggregate interlocking to increase strength and flexibility, tailoring MBC for specific applications.

**Keywords:** Albasia; Composite; *Leiotramaetes lactinea* MYCL-3; Mycelium; Ramie.

**Type of the Paper:** Regular Article.

## 1. Introduction

Mycelium-based composites (MBCs) are emerging as innovative biomaterials, utilizing lignocellulosic by-products from agricultural biomass combined with fungal mycelium. These materials present significant promise from an environmental perspective, providing renewable and biodegradable alternatives to traditional engineering materials. The versatility of MBCs has led to their applications in various industries, including automotive, aerospace, construction, furniture, and biomedicine [1].

In particular, the automotive industry increasingly incorporates natural fiber composites in components like panels and trim parts, due to their cost-effectiveness and ability to meet specifications for elongation, ultimate breaking force, flexural properties, impact strength, and acoustic absorption [2]. Mycelium-based composites exhibit physical and mechanical characteristics similar to certain types of polystyrene, serving as thermal insulators under various conditions [3–5]. Additionally, their production costs are very low, making them a potential alternative for producing biodegradable composites that can contribute to a more circular and sustainable economy [6,7]. Despite their advantages, MBCs still face performance challenges compared to conventional engineering materials. Factors such as mechanical strength, durability, and moisture resistance require further development to make these composites more competitive. Addressing these limitations can be achieved through the careful selection of substrate types, fungal species, and manufacturing technologies [1]. Furthermore, utilizing mycelium as a binding material within biocomposites not only enhances structural integrity but also promotes the formation of strong bonds with natural fibers [8,9]. The combination of high cellulose content materials with fungal mycelium represents a promising avenue for improving the performance characteristics of biocomposites [10].

Among various mycelium species, *Ganoderma* has been researched for its potential in biocomposite development, resulting in a composite with strong physical strength (0.741 MPa) [3]. Studies have highlighted the growth of *Ganoderma* on diverse substrates, such as guayule and aromatic plant biomass, underscoring the importance of substrate selection in mycelium growth [3,4]. However, to our knowledge, research involving the combination of *Leiotrametes lactinea* mycelium with ramie chips (*Boehmeria nivea*) remains unexplored.

Ramie fiber, rich in lignocellulose (comprising 72–89% cellulose, 10–13% hemicellulose, 4–7% lignin, and 2–4% pectin) [11], offers significant potential as a substrate for MBCs. Even ramie waste contains approximately 3.67% cellulose and 56.25% hemicellulose [12], further supporting its use in biocomposite production. This study aims to evaluate the biocomposite created from the growth of *Leiotrametes lactinea* MYCL-3 mycelium on ramie substrate and albasia powder. Comprehensive evaluations will include morphological analysis, FTIR for chemical functional group identification, mechanical testing for compressive strength, water absorption testing, and biodegradability assessments, allowing for a thorough understanding of the performance and characteristics of the resulting biocomposites.

## 2. Materials and Methods

### 2.1 Fungal inoculum

The *Leiotrametes lactinea* strain MYCL-3 fungus was obtained from the MyCL-Lab.

Mycotech, PT Miko Bahtera Nusantara, Indonesia. *Leiotrametes lactinea* (Berk.) Welti & Courtec., a tropical white-rot fungus from the Polyporaceae family, produces lignin-degrading enzymes like laccase, lignin peroxidase, and manganese peroxidase [13]. It belongs to the newly proposed genus *Leiotrametes*, recorded in regions such as the Neotropics and New Caledonia [14]. Its genome sequencing, part of the JGI CSP project on Polyporales, offers insights into biocatalysts for lignocellulose degradation, with applications in biofuel production and sustainable waste management [1,15]. Small fragments of *L. lactinea* mycelium were taken from the solid media stock PDA medium (oxoid, Basingstoke, Hampshire, United Kingdom) for pre-culturing in fresh media during an incubation period of 5–7 days at room temperature ( $\pm 27^{\circ}\text{C}$ ). The starter of *L. lactinea* was prepared by inoculating the mycelium into a bag log spawn medium containing corn seeds and sawdust, then incubating it for 10–14 days.

## 2.2 Biomass preparations

The lignocellulolytic materials used as biomass substrates were formulated as follows: stem bark of ramie (*Boehmeria nivea*) was supplied by PT Haramai Jaya Nusantara, Indonesia, and wood of *Albasia chinensis* (*Falcataria moluccana*) was obtained from PT Miko Bahtera Nusantara, Indonesia. The stem bark of ramie was cut into chips measuring 10 mm to 50 mm, while *Albasia* was prepared in powder form. The lignocellulosic composition of ramie typically includes 68–76% cellulose, 13–16% hemicellulose, and 0.6–1.5% lignin [16]. In contrast, the lignocellulosic composition of *Albasia* sawdust primarily consists of cellulose, hemicellulose, and lignin, with cellulose content averaging around 40-50%, which provides structural strength, while hemicellulose ranges from 25-30%, contributing to flexibility and moisture retention. Lignin, which offers rigidity and resistance to decay, constitutes about 20-30% [17]. The choice of these two materials is based on their chemical compositions, which enhance the properties of the resulting biocomposite. All materials were sterilized using an autoclave for 45 minutes at  $121^{\circ}\text{C}$ , followed by incubation overnight, or approximately 12 hours [18].

## 2.3 Mycelium composite manufacture

The method for producing biocomposites was modified from César et al. [8] and Angelova et al. [10]. To prevent the substrate formulation from clumping, the materials were crushed to their original particle size and wetted with water until reaching 55% humidity. A starting inoculum amounting to 10% (w/w) of the medium's weight was added [6]. Once the mixture was well combined, it was formed into a chamber. The room temperature was maintained between  $22$  and  $28^{\circ}\text{C}$ , with humidity controlled at 85%, good air circulation, and no direct sunlight exposure. The mixture was removed from the mold after seven days and kept inside for an additional seven days.

The composites were then compressed at a pressure of 600 g/cm<sup>2</sup> [19]. The mycelium was left to grow in the humid chamber for an additional two days before being placed in an air dryer set at 45°C for 48 hours.

#### 2.4 Morphological analysis

The composite samples were subjected to morphological analysis using a binocular microscope (XSZ107BN, Olympus CX23) and a Scanning Electron Microscope (SEM) (JEOL JSM-IT300). Each sample was coated with Au-Pd and then exposed to high vacuum and a 15 kV acceleration voltage for analysis [8].

#### 2.5 Mechanical test: Compressive strength

The physical and mechanical properties were evaluated through compressive strength testing using a Shimadzu AG-IS autograph 10 kN universal testing machine (UTM). Biocomposite samples with dimensions of 14 cm in length and 3 cm in width were prepared for testing. The procedure involved applying a gradually increasing load directly onto the sample, concentrating on the center until it fractured or broke. The maximum load at the point of fracture represents the compressive strength of the material. The compressive strength ( $\sigma_c$ ) of the material is calculated using the following equation, where F = compressive force [N], A = original cross section of the specimen [mm<sup>2</sup>] (equation 1) [5]:

$\sigma_c \text{ (MPa)} = \frac{F}{A}$	(1)
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#### 2.6 Fourier Transform Infra-Red (FTIR) analysis

Fourier Transform Infrared (FTIR) (Shimadzu, Prestige 21) analysis was conducted on AS, RB, and AS-RB, as well as the mycelium-based ramie and albizia powder biocomposites, using a Shimadzu Prestige 21 FTIR spectrometer. Spectra were recorded in the range of 4000–400 cm<sup>-1</sup> with 50 scans and a spectral resolution of 4.0 cm<sup>-1</sup>. Additionally, spectra were recorded in the range of 400–4000 cm<sup>-1</sup> with 132 scans and a spectral resolution of 2 cm<sup>-1</sup> [10]. Three samples of each type were measured to ensure reproducibility.

#### 2.7 Water absorption

The analysis of water absorption capacity of the biocomposite samples was conducted using the methods outlined in NBN EN ISO 15148 and ASTM C 1585–04. Biocomposite samples were cut into dimensions of 5 cm × 5 cm for the width (a) and length (b) and weighed to obtain the initial weight (mdt). After recording the initial weight, the samples were immersed in water for

one minute, lifted, and then gently dried with tissue to remove excess water. Subsequently, the biocomposite samples were reweighed to determine the final weight ( $mw,c$ ). The final weight was compared to the initial weight, and the amount of absorbed water was calculated using the following equation 2 [5]:

$\text{Water absorption (\%)} (Wc) = \frac{mw,c - mdt}{a \times b} \times 100\%$	(2)
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### 2.8 Biodegradable analysis

This test involved soil fertility conditioning using EM4, a mixed microbe culture comprising bacteria such as *Lactobacillus*, *Actinomyces*, *Streptomyces*, fungal yeast, and photosynthetic bacteria, which mutually support each other in the simultaneous decomposition of organic matter. Samples were cut into dimensions of 2.5 cm × 5 cm, then placed in a desiccator and weighed to obtain the initial weight ( $W_o$ ). Subsequently, the samples were immersed in a box containing soil with a depth of 20 cm and stored for 14 days. After this period, the samples were cleaned of any remaining soil, then reweighed and recorded as the final weight ( $W_i$ ). The degradation or decomposability of the samples was calculated as the percentage of weight loss using the following equation 3 [5]:

$\text{Degradability (\%)} = \frac{W_o - W_i}{w_o} \times 100\%$	(3)
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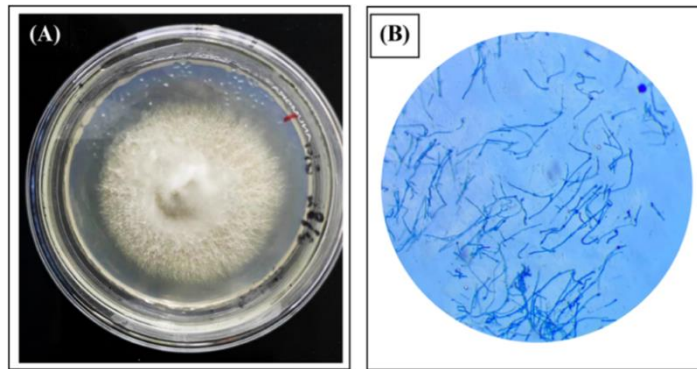
## 3. Results and Discussion

### 3.1 Morphology of *Leiotrametes lactinea* strain MYCL-3

The mycelium isolate of *L. lactinea* exhibits non-septate hyphae, categorizing it under coenocytic hyphae (Fig. 1a, b). Additionally, parts of the conidia form fork-like structures arising from the branching of fungal hyphae [20]. Recent studies have highlighted the genus *Leiotrametes* for its capability in degrading lignin, cellulose, and hemicellulose. It is known to thrive on various lignocellulosic materials such as cotton seed hulls, corn cobs, peanut shells, cotton from the textile industry, coffee pulp, paper [21], and leaves [22] as well as low-quality organic waste streams like sawdust and straw [3,23].

Observations under a binocular microscope (Fig. 1b) revealed the initial structure of the biocomposite. Further magnification using a Scanning Electron Microscope (SEM) provided clearer insight into how the mycelium spreads throughout the substrate (Fig. 2). SEM characterization of the biocomposite aimed to observe the morphological structure and analyze the distribution of the MYCL-3 fungal isolate binding to ramie fibers. The SEM micrographs of the biocomposite at 1000x magnification are depicted in Fig. 2, showing that the mycelium of the MYCL-3 isolate forms elongated, non-septate, twisted hyphae, resembling a white woven pattern

in the RB, AS-RB, and AS biocomposites (Fig. 2a, 2b, 2c). The growth of the mycelium appears irregular yet consistent with previous studies on mycelium-based composites [19].



**Fig. 1.** (a). Colony Mycelium of *L. lactinea*; (b). Microscopic Hyphae of *L. lactinea* (Mag. 400x)

The *Leiotrametes lactinea* isolate MYCL-3 (Fig. 1a) demonstrates the classic features of white-rot fungi, which are known for their potential to degrade lignin and other lignocellulosic materials. In the petri dish (Fig. 1a), the mycelium forms a dense, radial growth pattern, indicative of healthy fungal colonization. This dense structure is characteristic of the fungus's aggressive growth on lignocellulosic substrates such as ramie and albasia sawdust, commonly used in biocomposites.

The dense white appearance of the mycelium suggests that MYCL-3 effectively spreads and occupies the substrate, covering it entirely. This uniform colonization plays a critical role in binding the biomass into a composite structure. Mycelium's filamentous network not only degrades the organic components of the substrate but also serves as a natural binder, weaving through and around the fibrous elements to form a cohesive biocomposite.

Under the microscope (Fig. 1b), the hyphae of MYCL-3 can be seen as non-septate, indicating that the fungal isolate forms coenocytic hyphae. Coenocytic hyphae lack cross-walls, allowing for the free flow of cytoplasm and nutrients throughout the fungal structure, which can enhance growth rates and colonization efficiency. This feature supports MYCL-3's ability to spread aggressively across the substrate, forming long, uninterrupted filaments that can effectively bind the biocomposite.

In some regions, the hyphae form fork-like structures, which could represent branching points where new growth extends, allowing the fungus to explore and utilize more of the available substrate. These branching structures are crucial for the formation of an interconnected mycelial network that enhances the mechanical properties of the biocomposite.

### 3.2 Mycelium composite of *Leiotrametes lactinea* strain MYCL-3

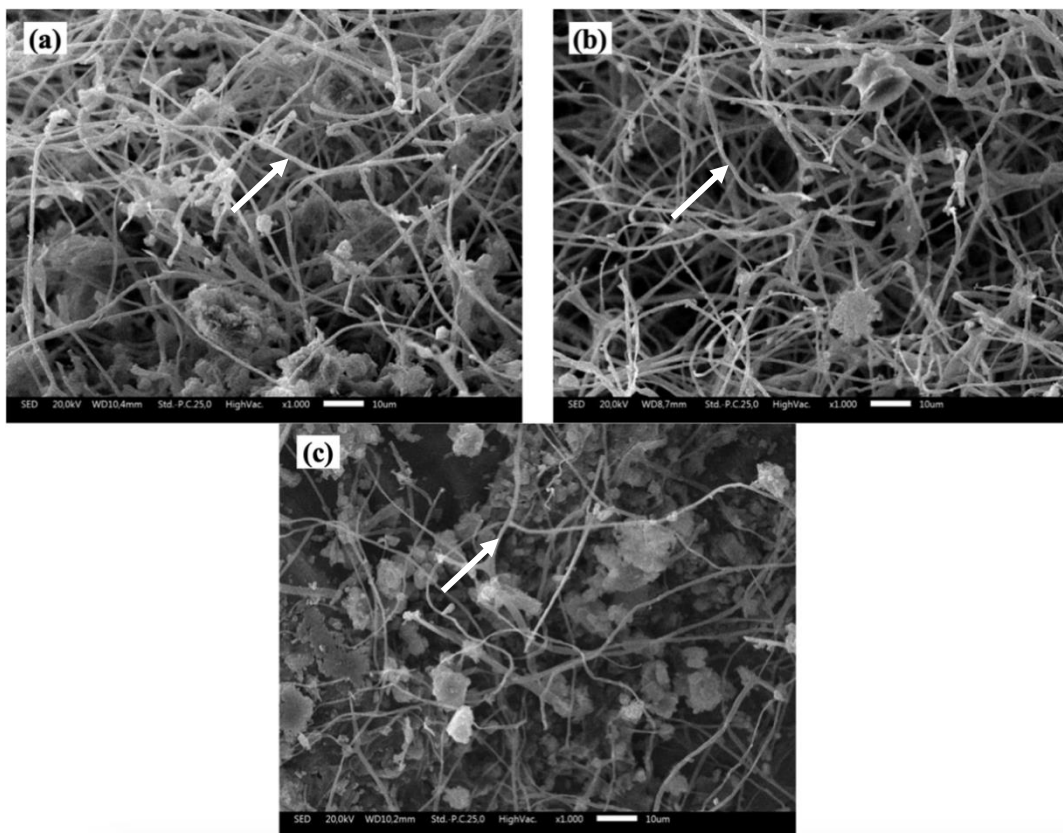
During the baglog growth phase, ramie was demonstrated to be a viable growth medium for the MYCL-3 isolate. However, it is important to note that the formulation used in this trial may



not represent the optimal composition for utilizing ramie as a growth medium for the MYCL-3 isolate. Another critical factor in this trial is the particle size of the ramie used. Both formulations utilized coarsely chopped ramie with an average particle length ranging from approximately 2 to 4 cm and a diameter between 0.5 and 1 cm. Larger particle sizes have the potential to enhance the structural strength of the composite. However, they may also affect substrate colonization times during the initial stages of baglog growth. Excessively large particle sizes could further limit the substrate's applicability in more intricate composite molds.

Key fungal traits that enhance their efficacy in engineering materials include rapid hyphal development, elevated pathogenicity, a dimittic or trimitic hyphal system, white rot decay type, high nutritional adaptability, resistance to environmental conditions and deleterious elements, ease of management, simple deactivation, saprophytic behavior, absence of mycotoxins, and synthesis of natural active ingredients.

For the dried specimens (Fig. 3), visible distinctions were observed among specimens from all formulations. RB produced a denser composite with a rougher surface that adhered closely to the substrate's texture. In contrast, AS-RB felt lighter, resembling styrofoam, with a thicker layer of mycelium covering its surface, resulting in a smooth and soft texture similar to AS. These differences in characteristics allow each formulation to be suitable for different applications.

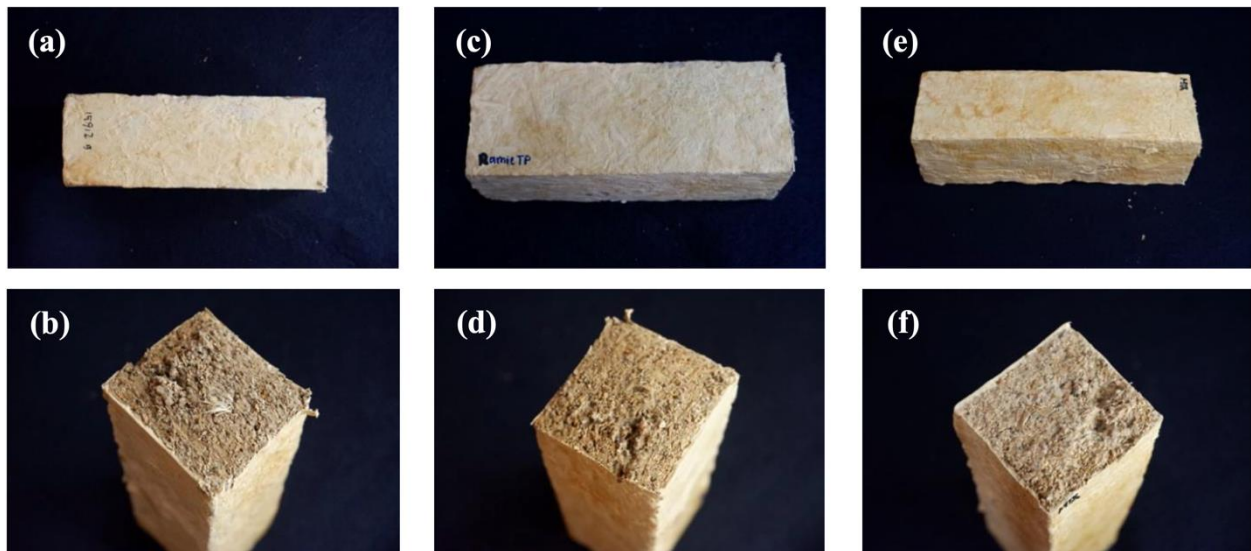


**Fig. 2.** The results of SEM microscope 1000x of sample biocomposite MYCL-3. (a) RB; (b) AS-RB; (c) AS

Based on the appearance of the biocomposite sample, characterized by its yellowish-white

color (Fig. 3), morphological analysis was conducted on the mycelium composite derived from the cultivation of *L. lactinea* MYL-3 on AS-RB medium. The external surface of the block clearly exhibits a developed mycelium layer. Upon cross-section examination, the internal structure reveals a biomass substrate entangled in the process of mycelium growth during development.

Micrographs at a magnification of 1000X (Fig. 2) depict the internal cross-sectional area, demonstrating the even distribution of mycelium growth among the ramie biomass fibers. The biocomposite maintains its structural integrity due to the formation of a matrix that binds the aggregated materials. The indicated section in Fig. 2, refers to clusters of mycelium bonds distributed within the substrate. Previous studies on mycelium composites produced with macrofungi species, such as *Pycnoporus sanguineus* and *Pleurotus albidus*, utilizing pine sawdust and wheat bran, have demonstrated a similar matrix formation [24].



**Fig. 3.** Comparison among AS, RB, AS-RB, by morphology physic appearance. (a) AS horizontal section, (b) AS vertical section; (c) RB horizontal section, (d) RB vertical section; (e) AS-RB horizontal section, (f) AS-RB vertical section

### 3.3 Physical mechanical analysis (compressive strength)

The mechanical characterization results of mycelium composites, including density, compression strength, and ultimate strength shown in (Table 1). RB exhibited a density of  $0.28 \text{ g/cm}^3$ , a compression strength of 355 kPa, and an ultimate strength of 459 kPa. AS-RB showed a density of  $0.19 \text{ g/cm}^3$ , a compression strength of 140 kPa, and an ultimate strength of 277 kPa. Meanwhile, for AS (Albasia-based), the density was  $0.24 \text{ g/cm}^3$ , with a compression strength of 117 kPa, and the ultimate strength was not observed. These results provide insight into the mechanical properties of the mycelium composite, demonstrating variations in density and strength characteristics among different formulations.



**Table 1.** Compressive strength of difference biocomposite formulations

Sample	Density (g/cm <sup>3</sup> )	Compression strength (kPa)	Ultimate strength (kPa)
RB	0.28	355	459
AS-RB	0.19	140	277
AS ( <i>albasia based</i> )	0.24	117	-

The test results (Table 1) indicate that RB exhibits superior compressive strength compared to AS-RB and AS. In contrast to the albasia-based AS control formulation, RB demonstrates three times greater compressive resistance. This difference is evident in the SEM observations (Fig. 2), where the mycelium in the RB biocomposite appears denser and more thoroughly integrated into the substrate compared to AS-RB. RB displays characteristics of brittleness and rigidity, contributing to its robust mechanical properties. Conversely, the AS-RB specimen exhibits more resilient properties.

There is a difference in particle size between the ramie chips and albasia wood powder used as substrate media. However, according to Islam et al. [25], the compressive properties of the grown composite were found to be largely independent of the particle size of the substrate filler phase. Although there is a significant variation in the characterization results of mycelium composites, particulate substrates such as sawdust appear to provide higher compressive strength in composites compared to fibrous substrates like straw [26]. Furthermore, the compressive performance of pre-grown composites will depend on the compressive properties and porosity of the substrate filler, the composite itself, and the extent to which the fungus digests the filler, increasing its porosity in the process [25].

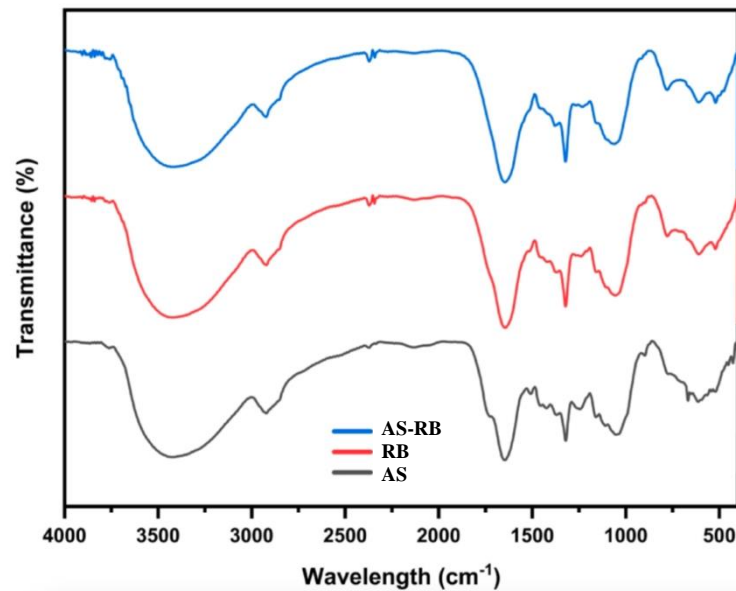
As a result, both formulations offer potential for diverse applications. The strong and brittle nature of RB, coupled with its superior strength, makes it suitable for composite products requiring robust final outcomes, such as construction panels, structural components, and furniture [27]. Research by Haneef et al. [21] has shown that denser, more rigid substrates, like those found in RB, contribute to higher mechanical strength, making them ideal for load-bearing applications. Furthermore, Islam et al. [25] noted that the compressive strength of mycelium composites is significantly improved by the inclusion of denser particulate substrates like sawdust, further supporting RB's suitability for heavy-duty applications.

In contrast, AS-RB shows promise as a substrate for composite products requiring standard strength while maintaining a lightweight and soft texture, such as packaging containers, acoustic panels, or insulation materials [22,26]. Mycelium-based composites with mixed substrates like AS-RB have been found to offer flexibility and improved workability, making them suitable for lightweight applications where ease of processing and transportation are important factors [21]. Additionally, these composites exhibit excellent biodegradability and can be customized for use

in sustainable packaging solutions, promoting eco-friendly alternatives to plastic packaging [23].

### 3.4 FTIR spectra

FTIR spectroscopy in Fig. 4 was employed to characterize the functional groups present in biocomposite samples. AS exhibits more pronounced peaks associated with glycosidic bonds, alkenes, and acids, reflecting the higher content of polysaccharides and lignin in albasia sawdust. In contrast, RB and ASRB show fewer peaks in these regions, indicating that the ramie component alters the chemical structure, potentially making the composite less rigid but more flexible due to lower lignin content. The infrared absorption spectra of mycelial biocomposites are influenced by the biomolecules comprising them. Specifically, lipids (3000-2800  $\text{cm}^{-1}$ , ester bonds around 1737  $\text{cm}^{-1}$ ), proteins (amide I and II at 1700-1600  $\text{cm}^{-1}$ , amide III at 1575-1300  $\text{cm}^{-1}$ ), nucleic acids (1255-1245  $\text{cm}^{-1}$ ), and polysaccharides (1200-900  $\text{cm}^{-1}$ ) contribute to the spectra, consistent with previous studies [10].



**Fig. 4.** FTIR spectra of composite mycelium (AS, RB, & AS-RB)

The typical functional groups identified in the IR spectra and their associated compounds are summarized in Table 1. It is noted that the biomass components—Albasia substrate (AS), Ramie substrate (RB), and Ramie-Albasia substrate (ASRB)—consist of aromatics, esters, alcohols, alkanes, amines, ketones, carbonyls, and carboxyls. The IR spectra reveal that the composite samples exhibit similar structural features, although ATP composite shows additional constituent compounds not found in RB and ASRB composites, such as C=C bending (alkene), O-H stretching (alcohol), C-O-C stretching (glycosidic), O-H bending (acid), and C=C stretching (benzene). The interaction between mycelium and the combined substrate in ASRB composites potentially leads to a reduction in certain functional groups. Angelova et al. (2021) [10] reported

that IR bands in mycelium-based composites can vary depending on the substrate due to biodegradation of chemical compounds like lignin and cellulose by mycelium.

The FTIR spectrum of albasia sawdust exhibits a broad absorption band around  $3400\text{ cm}^{-1}$ , attributed to the stretching vibrations of hydroxyl (O-H) groups in cellulose and hemicellulose. Lignin, the aromatic polymer found in wood, contributes significantly to the chemical signature of albasia sawdust. The absorption bands around  $1510\text{ cm}^{-1}$  and  $1600\text{ cm}^{-1}$  correspond to C=C stretching vibrations of the aromatic rings in lignin. The presence of acetyl groups in hemicellulose and the carbonyl groups in lignin are indicated by a peak around  $1730\text{ cm}^{-1}$ . This C=O stretching band is typically stronger in species with higher hemicellulose content. In the case of albasia sawdust, the prominence of this peak provides insights into the chemical modifications that might occur during thermal or chemical treatments, such as those used in bio-composite manufacturing. The fingerprint region (below  $1500\text{ cm}^{-1}$ ) of albasia sawdust displays several overlapping bands, corresponding to C-O, C-C, and C-H deformations. For instance, the peak around  $1030\text{ cm}^{-1}$  is attributed to the C-O-C stretching of the cellulose backbone, reflecting the glycosidic linkages in polysaccharides. This region is highly complex and reflects the intricate nature of the sawdust's biochemical composition [28–30].

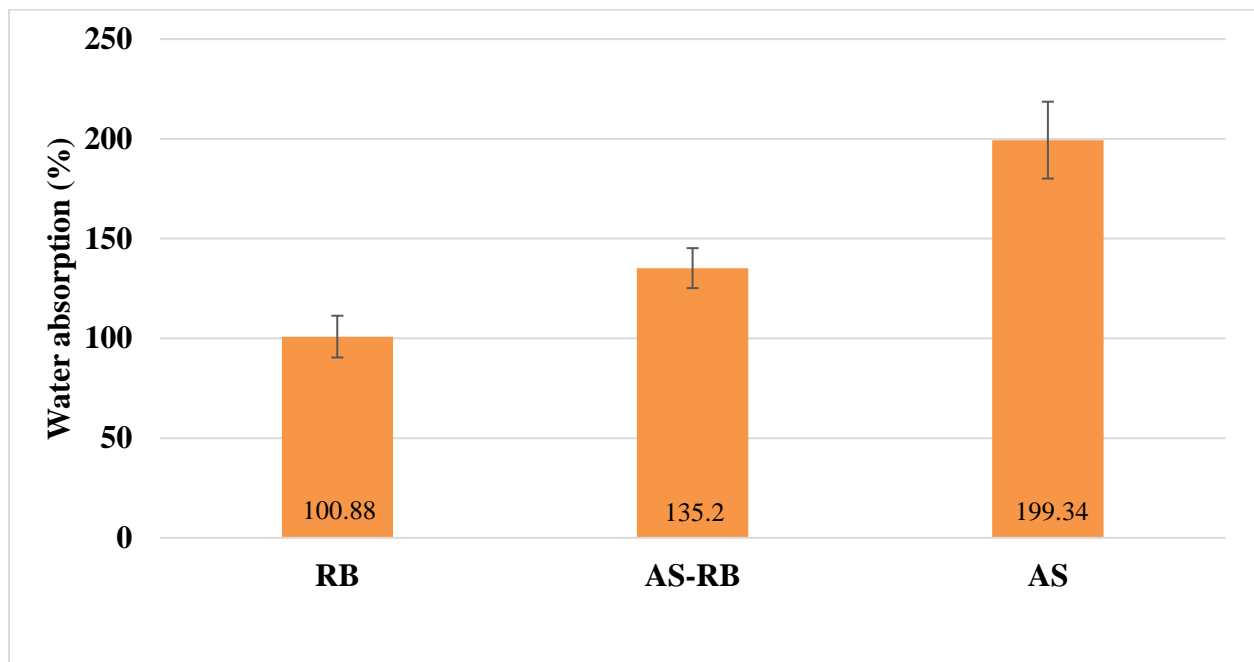
Fourier Transform Infrared (FTIR) spectroscopy on *Boehmeria nivea* (ramie fiber) primarily highlights the presence of cellulose, hemicellulose, and lignin in the fiber. Significant peaks typically include the O-H stretching vibration around  $3400\text{ cm}^{-1}$ , representing hydroxyl groups in cellulose and hemicellulose. Other notable peaks include the C-H stretching at  $2900\text{ cm}^{-1}$  and the C=O stretching at  $1735\text{ cm}^{-1}$ , indicating ester and carboxyl groups in hemicellulose [28,30].

AS uniquely contains alkene ( $898.83\text{ cm}^{-1}$ ) and benzene ring vibrations ( $1508.33\text{ cm}^{-1}$ ), indicating a higher content of unsaturated compounds and aromatic lignin in the albasia sawdust composite. AS shows a distinct O-H association ( $1109.07\text{ cm}^{-1}$ ) and O-H bending ( $1425.40\text{ cm}^{-1}$ ), suggesting a greater presence of hydroxyl and acid groups compared to the ramie-based composites. AS exhibits unique glycosidic (C-O-C) stretching at  $1159.22\text{ cm}^{-1}$ , implying that albasia sawdust has a richer cellulose or hemicellulose content than the ramie composites. C-O stretching (ester) and C=O stretching (ketone and carbonyl) are consistent across all biocomposites, highlighting similar functional group contributions from lignin or hemicellulose regardless of fiber type. This comparison reveals that the albasia sawdust-based composite retains more characteristic features of lignin and cellulose, while the ramie-based composites (RB and AS-RB) show differences likely due to the properties of ramie fibers. These results also confirm ramie fiber's composition of lignocellulosic materials, similar to the findings on albasia sawdust.

### 3.5 Characterization of water absorption

Based on the water absorption testing results, it is evident that the RB biocomposite exhibits lower water absorption compared to AS-RB and AS. The average water absorption percentages for AS, RB, and AS-RB are 100.88%, 135.2%, and 199.34%, respectively (Fig. 5). The higher water absorption in AS-RB is influenced by the addition of albasia wood powder in its formulation. This addition increases the susceptibility of AS-RB to water absorption, possibly due to incomplete mycelium coverage on the biocomposite surface, allowing water to penetrate the ramie composite fibers easily.

Suliswati et al. [31] noted that the mycelium surface possesses hydrophobic properties, attributed to hydrophobin proteins coating the aerial hyphae surface. However, in AS-RB, sparse mycelium coverage may reduce this hydrophobic effect, contributing to higher water absorption. Additionally, the presence of pollard (bran) in the formulation can affect mycelium density and further influence water absorption. Bran is known to enhance mechanical properties and reduce water content in products.

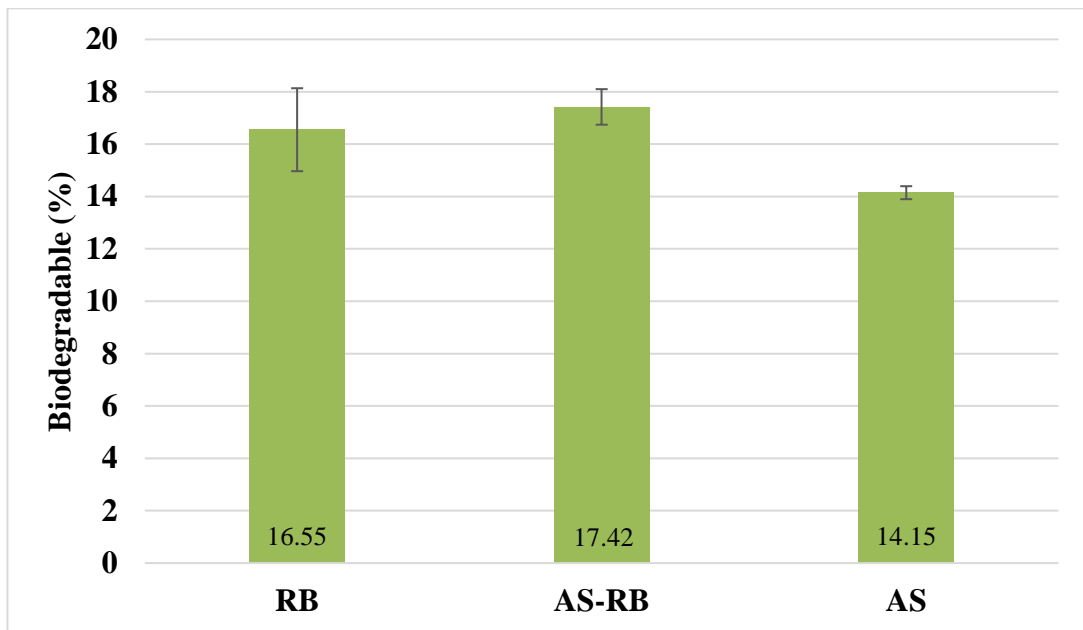


**Fig. 5.** Water absorption of Composite Sample: RB, AS-RB, and AS.

The long-term water absorption (24 hours) of the biocomposites investigated by Elsacker et al. [5] demonstrated higher absorption capacities compared to the results obtained in this study. Specifically, Elsacker et al. [5] reported values of 126% for the HERF-based biocomposite and 245% for the SDLS-based biocomposite. These findings align with literature references on biocomposites, where water absorption values can reach up to 350% [32]. Appels et al. [22] also documented a wide range of water absorption capacities for various lignocellulosic mycelium-based biocomposites, spanning from 43% to 508%.

### 3.6 Biodegradable test

The biodegradable composite chip ramie is illustrated in Fig. 6. The results reveal that AS-RB exhibits the highest biodegradation among the biocomposites, with an average degradation value of 17.46%. Following AS-RB, RB samples showed a degradation of 16.55%. According to international standards such as ASTM 5336, packaging materials take approximately 60 days to completely degrade (100%). This indicates that by day 10, the samples have already degraded by at least 16%.



**Fig. 6.** Biodegradable test of Composite Sample: RB, AS-RB, and AS.

The AS-RB composite, which combines the beneficial components of both ramie and Albasia, demonstrates a balanced lignocellulosic profile conducive to microbial degradation. The high cellulose content from ramie enhances biodegradability, while the hemicellulose from both substrates promotes microbial action [33,34]. This synergistic interaction likely facilitates a more effective microbial breakdown compared to either substrate alone [35]. The lower density of the AS-RB composite suggests a more porous structure, enhancing microbial accessibility to the substrate and facilitating quicker biodegradation. Based on its density, the AS-RB composite allows for more efficient degradation compared to the other samples [36]. In contrast, the higher density of the RB sample may limit microbial penetration, thereby slowing down the degradation process [37].

This value demonstrates that after its lifespan as packaging material or in other applications, mycelium-based materials can be buried in the soil and decompose within a few weeks. These materials are significantly more cost-effective when produced on a large scale and much more easily degradable compared to environmentally damaging polyester. Polyester poses challenges in



natural degradation because microorganisms struggle to break down its polymer bonds. In contrast, biocomposites, being organic materials, undergo chemical structural changes as they naturally degrade through enzymatic actions of microorganisms such as bacteria, algae, and fungi. This enzymatic breakdown allows for the degradation of organic material polymer chains [38].

#### 4. Discussion

This study produced results similar to previous research, with a comparison of the findings to several existing biocomposite studies presented in Table 2. One example is the *Pleurotus albidus* composite with *Pinus* sp. substrate, which has a compressive strength of 0.4 MPa [24]. However, the composite formulation of *Leiotramete lactinea* MYCL-3 and ramie chips has a lower compression value compared to *Ganoderma curtisii* on guayule (bagasse) with a compression result of 0.740 MPa [8]. Meanwhile, when we compared with compression values for composites of *Trametes versicolor* (0.023-0.199 MPa) [33] and *P. ostreatus* (0.020–0.14) [39] with sawdust from fir, the composite formulation produced in this study still has higher compression values.

The results of the water absorption tests indicate that all samples exhibit high moisture retention values, with Elsacker et al. [5] reporting the lowest absorption rate of 24%. While this figure suggests good performance due to its low absorption capacity, it contrasts with findings from Ziegler et al. [32] where significantly higher absorption rates of 325% and 277% were observed. In comparison, the present study demonstrates a lower absorption capacity of 100.88% (RB) for the mycelium composites derived from, positioning it favorably among these studies. Furthermore, when compared to the water absorption result of 112% reported by César et al. [8] the mycelium composite developed in this research exhibits superior performance. This outcome underscores the potential for further optimization of water absorption characteristics in mycelium-based composites, enhancing their applicability in moisture-sensitive environments.

Regarding biodegradability, the mycelium composites from this study show superior degradation performance relative to the findings of Ziegler et al. [32]. After 14 days, the degradation rates for samples RB, AS-RB, and AS were 16.55%, 17.42%, and 14.15%, respectively. In contrast, the biodegradation rates for the composites utilizing cotton gin waste and flax fibers were reported at lower values of 13.8% and 13% [32]. This demonstrates the effectiveness of the mycelium composites in biodegradation, suggesting their potential as sustainable materials in various applications.

Therefore, the materials obtained in this research have broad potential for further applications and warrant further study to enhance their mechanical properties. In addition, the utilization of lignocellulosic materials such as ramie chips (*B. nivea*) and *L. lactinea* MYCL-3 in the conversion into bio-composites can result in lower environmental impact and reduce

dependency on petroleum-based products.

Issues like moisture absorption, insufficient toughness, and decreased long-term stability for outdoor applications need to be further investigated. To be more specific, the product's service life is impacted by various weathering conditions like temperature, humidity, and UV radiation. This alternate method of producing bio-composites can be especially useful for meeting the everyday needs of the general public, such as light-weight car parts or sports equipment, as well as furniture for the home, fencing, decking, and flooring. The key factors influencing the shift from the dependent present to a sustainable future will be their affordability, accessibility, and beautiful designs. A lot of research is being done right now to address and get past the aforementioned challenges all over the world.

**Table 2.** Comparative compressive strength, density values, water absorption, biodegradable of mycelium composites made of *Leiotrametes lactinea*/ramie chip biomass/albasia wood powder

Material	Fungi/substrate	Compression strength (MPa)	Density ( $\text{gcm}^{-3}$ )	Water absorption (%)	Biodegradable (%)	Reference
Mycelium composite	<i>Leiotrametes lactinea</i> /Ramie Bark	0.35	0.28	100.88	16.55	This work
	<i>Leiotrametes lactinea</i> /Albasia wood powder	0.14	0.19	199.34	14.15	This work
	<i>Leiotrametes lactinea</i> /Albasia wood powder+Ramie Bark	0.11	0.24	135.2	17.42	This work
Mycelium composite	<i>Ganoderma curtisii</i> /guayule bagasse	0.740	0.469	112	-	[8]
Mycelium composite	<i>Ganoderma resinaceum</i> /beech wood sawdust	0.48-1.29	0.13-0.14	24	-	[5]
Mycelium composite	<i>Trametes versicolor</i> /spruce wood sawdust	0.023-0.199	0.10-0.18	-	-	[33]
	Expanded polystyrene	0.096	0.013-0.018	-	-	
Mycelium composite	<i>Pleurotus ostreatus</i> /spruce wood sawdust	0.020-0.14	0.19-0.55	-	-	[3]
Mycelium composite	<i>Ganoderma lucidum</i> /cotton gin waste	0.71	0.54	325	13.8	[32]
	flax fibers	0.52	0.63	277	13	

## 5. Conclusions

The mycelium in the RB formulation demonstrated a denser distribution compared to the AS-RB formulation. In the compressive strength test, RB exhibited three times the strength of AS. Both formulations show potential for various applications. The robust and brittle nature of RB, along with its superior strength, makes it suitable for composite products requiring high strength, such as furniture. This is attributed to the aggressive growth of *L. lactinea* MYCL-3 on cellulosic materials, with the ramie substrate, which contains a higher percentage of cellulose, promoting

denser mycelium growth than the albasia sawdust substrate, which has a lower cellulose content. The *L. lactinea* MYCL-3 fungus shows promise as an ideal candidate for mycelium-based materials, ensuring further development potential. Sustainable materials play a crucial role in reducing environmental pollution, and the biocomposites proposed in this study are well-aligned with this strategy, being natural polymers that require minimal energy for production. This research, for the first time, explores the creation of composites using ramie bark and albasia sawdust, which have proven to be suitable substrates for these materials. In the compressive strength test, RB exhibited three times the strength of AS. However, further research is necessary to enhance the compressive strength of the composites for broader industrial applications, particularly in the construction sector.

### **Data availability statement**

Data will be made available on request.

### **CRedit authorship contribution statement**

APW: conceptualization, supervision, writing-review and editing. DRS: investigation, performed the data collection, analyzed the data, and writing – original draft preparation. All authors have read and agreed to the published version of manuscript. SSK: data curation, and validation. MNF: investigation, and validation. AR: resources, and project administration.

### **Declaration of Competing Interest**

The authors declare that have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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