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Identification and characterization of *Ganoderma enigmaticum*, *Ganoderma casuarinicola*, and *Polyporus thailandensis* to fabricate mycelium-based composites

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Abstract

Mycelium-based composites (MBCs) are biodegradable materials produced from fungal species grown on lignocellulosic residues. These composites have characteristics that could replace synthetic plastics used for packaging by different manufacturing companies. In this study, three fungal species (*Ganoderma enigmaticum*, *Ganoderma casuarinicola*, and *Polyporus thailandensis*) were isolated and characterized morphologically. Polymerase chain reaction (PCR) was used to amplify the internal transcribed spacer (ITS) region of the fungal isolates using the Qiagen TopTaq Master Mix Kit (Qiagen, Hilden, Germany). Two of the three fungi (*G. enigmaticum* and *P. thailandensis*) were grown on lignocellulosic residues (sawdust and cornhusk) to fabricate MBCs. Some physical properties of the MBCs were analyzed, and the results showed that the substrate type and fungal species affected the properties of the MBCs produced. MBCs obtained from *G. enigmaticum* exhibited better physical properties than those obtained from *P. thailandensis*. Moisture contents were 60.03 and 63.24%, and densities were 110.11 and 121.13 kg/m^3 for *G. enigmaticum* and *P. thailandensis*, respectively. Water absorption was highest in MBCs produced by *P. thailandensis* (136%) compared with MBCs produced by *G. enigmaticum* (106%). With respect to substrate combinations, MBCs produced from combined substrates had improved physical properties, including higher density and lower water absorption and moisture content.

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1. Introduction

Designs from fungal mycelium have been a core area of interest for more than two decades [1]. Initially, these applications began when farmers grew fungi on agricultural waste to produce simple molds. Over time, further innovations have resulted in the production of various items from fungal mycelium, ranging from household materials to furniture and building materials [2, 3]. Recent technological progress in bio-based research, including bioplastics and related biotechnological innovations, has led to the

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development of mycelium-based leather suitable for clothing production. Ongoing innovations have also enabled the creation of useful products for packaging food, cosmetics, and pharmaceutical items [4]. The use of mycelium-based materials for packaging is expanding rapidly, further increasing the potential industrial design applications of mycelium. One major company in this sector is Ecovative, which manufactures several packaging products using mycelium-based composites (MBCs), including MycoComposite and a mycelium foam product marketed as MycoFlex.

Using agricultural raw materials, such as corn stalks or wheat straw, as substrates for selected fungal strains has several benefits, including biodegradability and low weight. It also supports the recycling of agricultural products and by-products and the replacement of existing packaging materials, thereby reducing environmental pollution. There is an urgent need for sustainable packaging solutions that can decompose naturally and reduce the environmental footprint of packaging materials. This study was carried out within the localities of interest and used a natural biofabrication process to produce biodegradable materials with potential commercial applications and feasible solutions for waste problems caused by rapid industrialization, population growth, and intensified agriculture [5–7]. The production of *Ganoderma* and *Polyporus* mushrooms began in 1969 in China before spreading to other Asian countries such as Japan and South Korea. The process involved inoculating several meters of unsterilized natural tree trunks and burying them in soil to allow fungal growth [8]. Agricultural wastes commonly used to produce mycelium-based composites with *Ganoderma* and *Polyporus* include sawdust, cornhusks, tree stems, and rice residues. These substrates are preferred because of their high lignin and cellulose contents [9]. The identification of *Ganoderma* and *Polyporus* species in West Africa is primarily based on cultural and morphological characteristics. However, these methods are influenced by pleomorphic and environmentally induced traits, limiting accurate species identification [10]. Recent studies suggest that Africa is home to a large variety of *Ganoderma* and *Polyporus* species [7].

Darwana *et al*. [11] and Adotey *et al*. [7] separately contributed to the identification and characterization of *Ganoderma* and *Polyporus* species. Adotey *et al*. [7] characterized five *Ganoderma* species to improve understanding of the growing importance of *Ganoderma* as a health-promoting biomedical fungus. Darwana *et al*. [11] conducted an extensive study on the characterization and identification of polypore fungi and the use of polypore fungi as biological agents against phytopathogens.

There is a need to properly characterize and establish the phylogenetic positions of *Ganoderma* and *Polyporus* species in West Africa in comparison with species from other regions. Synthetic plastics have become central to modern life. However, their widespread use has led to significant accumulation, posing serious risks to human health and the environment [12–15]. This is of great concern because the recalcitrant nature of plastics causes accumulation and saturation in the environment [16]. Bioplastics are derived from renewable sources and provide an eco-friendly alternative to synthetic plastics. These MBCs are clean, safe, strong, and biodegradable, with the potential to replace polyethylene and other synthetic plastic products. They may also have better recycling efficiency and environmental benefits through reduced toxic emissions and improved land use [3, 17, 18]. This study aligns with global efforts to reduce plastic use and promote sustainable biodegradable materials.

This study integrated biotechnological tools to investigate the potential of saprophytic fungi. The aim was to develop novel MBCs for sustainable packaging materials. The fabrication process involved growing *Ganoderma* and *Polyporus* mycelia on sawdust and cornhusk substrates. During incubation, the mycelium penetrated the substrate matrix, where it functioned as both a reinforcing fiber and a natural binding agent. After a few days of incubation, the MBC was assembled and dried, forming a naturally produced biodegradable composite. Producing this biodegradable composite on a large commercial scale could provide a lasting solution to waste-management and environmental-pollution problems caused by rapid industrialization and population growth [11].

MBCs are clean, safe, strong, and biodegradable, with potential to substitute fossil-based and synthetic materials such as polyurethane and polystyrene. They have also been described as having better recycling efficiency, which may contribute to a circular economy alongside environmental benefits such as lower emissions and better land use [17]. MBCs can be used for a wide range of packaging applications, including food packaging, protective packaging for fragile goods, and disposable containers. They are fully biodegradable and can break down naturally in the environment without leaving harmful residues. Previous research has shown the potential for developing a wide range of products from MBCs for packaging and insulating materials [19]. However, based on the reviewed literature, limited research has focused on improving the physical properties of the final product. This study therefore characterized *Ganoderma* and *Polyporus* fungi to produce mycelium-based composites with improved physical properties using sawdust and cornhusk as substrates.

2. Materials and methods

2.1. Collection of samples

The fruiting bodies (basidiocarps) of *Polyporus* sp. and *Ganoderma* sp. were collected from decaying logs, tree trunks, and stumps found in forested areas of Kuje, Abuja. Specimens showing characteristic bracket-like morphology were carefully detached using sterile scalpels and placed in clean, labeled paper bags to prevent moisture accumulation during transport to the laboratory (Figure 1).

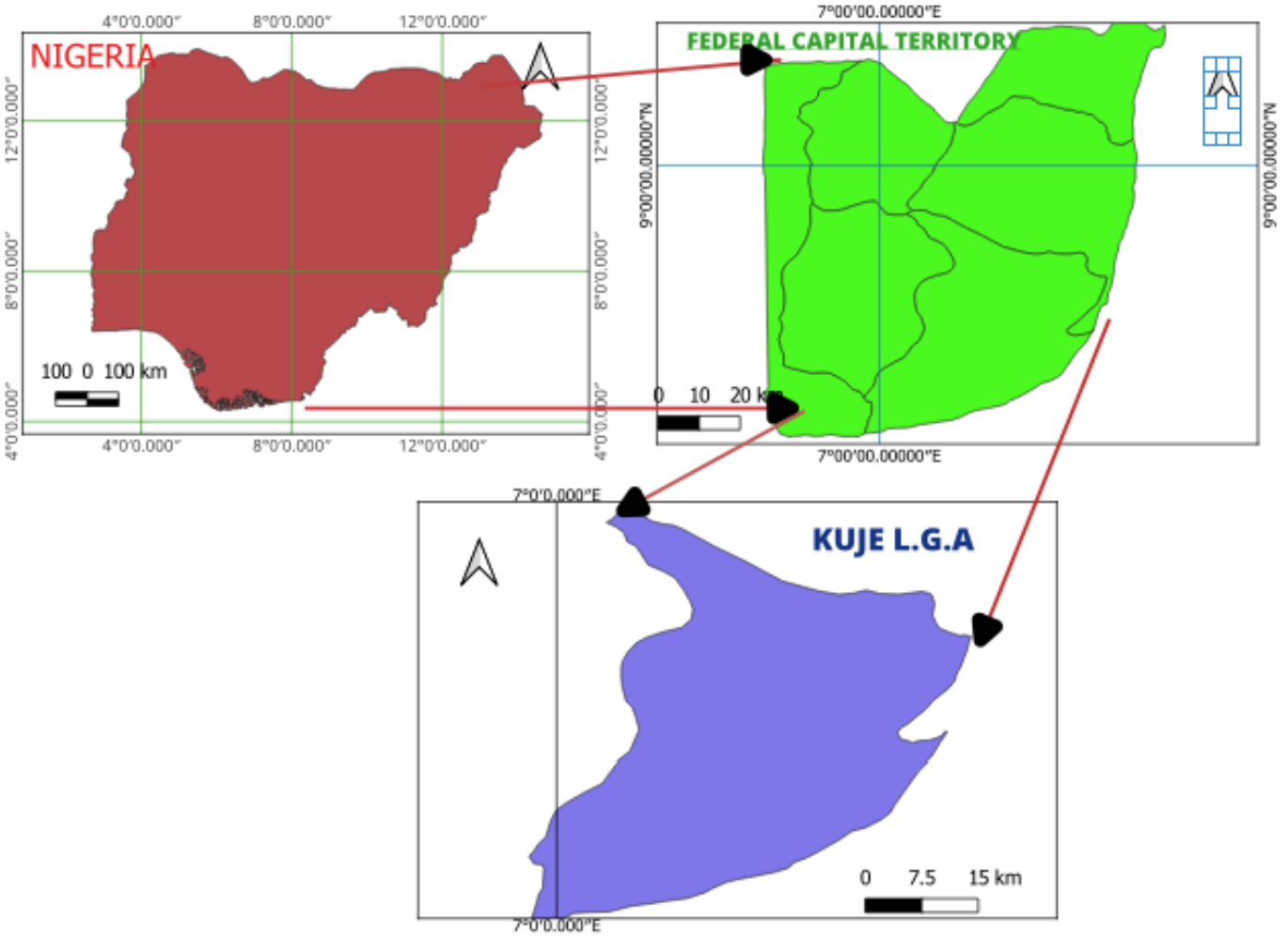

Figure 1: Map of the study area.

2.2. *Isolation of Polyporus sp. and Ganoderma sp.*

In the laboratory, the collected samples were washed twice with sterile distilled water to remove surface debris and then blotted dry using sterile Whatman No. 1 filter paper. Under aseptic conditions in a laminar-flow hood, small tissue pieces (approximately 3–5 mm) were excised from the inner context tissue of each basidiocarp using sterilized forceps and scalpels to minimize contamination [20]. The fruiting bodies resembling *Ganoderma* species and *Polyporus* species are shown in Figure 2.

The tissue fragments were inoculated onto potato dextrose agar (PDA) supplemented with chloramphenicol (50 µg/mL) to inhibit bacterial growth. The inoculated plates were sealed with parafilm and incubated at 25 ± 1°C for 7 days to allow fungal mycelial development. Emergent white, cottony, or filamentous mycelial colonies characteristic of *Polyporus* and *Ganoderma* were purified by subculturing onto fresh PDA. The purified isolates were then transferred to PDA slants and incubated under the same temperature conditions until full growth was achieved. Fully grown cultures were stored at 4°C for further analysis.

2.3. *Morphological characterization of Polyporus sp. and Ganoderma sp.*

Macroscopic examinations of the collected fruiting bodies were carried out to determine morphological characteristics such as the presence or absence of a stipe, color, size, pileus surface texture, and pore-surface characteristics, including pore shape, size, and color [11]. Microscopic observations were performed using a compound light microscope. Sections of the hymenial layer were mounted in lactophenol cotton blue and examined for hyphal structure, basidiospore shape, and spore dimensions. Measurements were made using an eyepiece graticule calibrated with a stage micrometer [21]. Pure isolates were maintained on PDA slants at 4°C for short-term storage. For long-term preservation, small discs of actively growing mycelia were stored in 15% glycerol solution at −20°C or in sterile distilled water at ambient temperature.

Figure 2: Fruiting bodies of *Ganoderma* sp. and *Polyporus* sp. Keys: GAN1 and GAN2, two fruiting bodies resembling *Ganoderma* fungi; POL1 and POL2, two fruiting bodies resembling *Polyporus* fungi.

2.4. DNA extraction of Ganoderma sp. and Polyporus sp.

Genomic DNA was extracted from fungal isolates using a modified Qiagen QIAamp Mini spin-column protocol (Qiagen, Hilden, Germany). Before the procedure, all samples and reagents were equilibrated to room temperature. Buffer AW1 and Buffer AW2 were prepared according to the manufacturer's instructions by adding 25 and 30 mL of absolute ethanol, respectively. A direct suspension of the fungal isolate was prepared by suspending the fungal material in 200 μL of nuclease-free water in a 1.5 mL nuclease-free microtube and vortexing thoroughly. Subsequently, 30 μL of Proteinase K was added, and the mixture was vortexed for 15 s to ensure adequate mixing. Thereafter, 200 μL of Qiagen lysis buffer was added to the tube, and the mixture was incubated at 55°C for 20 min to facilitate cell lysis. To separate cellular debris and proteins, 250 μL of chloroform was added to the lysate. The mixture was incubated at room temperature for 5 min and mixed thoroughly by inversion. The tubes were centrifuged at 14,000 rpm for 3 min to achieve phase separation. The upper aqueous layer, containing DNA, was carefully transferred to a newly labeled 1.5 mL nuclease-free microtube. Absolute ethanol (200 μL) was then added to the transferred aqueous layer to optimize DNA-binding conditions [22]. The entire content of the microtube was carefully transferred into a QIAamp mini-column placed in a 2 mL collection tube, taking care not to wet the rim of the column. The column was centrifuged at 8,000 rpm for 1 min after closing the cap. The QIAamp

mini-column was then transferred to a clean 2 mL collection tube, and the flow-through from the previous centrifugation step was discarded. For washing, 500 µL of Buffer AW1 was added to the spin column, followed by centrifugation at 8,000 rpm for 1 min, after which the flow-through was discarded. A further 500 µL of Buffer AW2 was added to the column, and it was centrifuged at full speed (14,000 rpm) for 3 min, with the flow-through discarded.

2.5. Amplification of the ITS region of Ganoderma sp. and Polyporus sp.

To amplify the internal transcribed spacer (ITS) region of the fungal isolates, PCR was performed using the Qiagen TopTaq Master Mix Kit (Qiagen, Hilden, Germany). A total reaction volume of 25 µL was prepared for each sample, consisting of 12.5 µL of Qiagen TopTaq Master Mix, 1 µL each of 10 µM forward primer ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and reverse primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), 1.5 µL of CoralLoad PCR buffer, 1 µL of nuclease-free water, and 8 µL of DNA template. After gentle mixing, the reaction tubes were placed into an Applied Biosystems 9700 Thermal Cycler. The thermal cycling protocol began with initial denaturation at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 49°C for 1 min, and extension at 72°C for 1 min. The PCR reaction mixture was then placed in a PCR thermal cycler and amplified using the following parameters: 95°C for 5 min; 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min 30 s; followed by final extension at 72°C for 10 min. The amplicons were separated by running the PCR mixture on a 1% (w/v) agarose gel prestained with ethidium bromide. The gel was viewed on a UV transilluminator and documented using a Bio-Rad XRS gel documentation system. Amplicon sizes were calculated using a DNA 100 bp ladder [23].

2.6. Preparation of fungal mycelium inoculum

The mycelium inoculum of each species was produced using guinea corn grain following the method of Aiduang *et al.* [24]. The grains were washed and boiled for 20 min. After cooling, 100 g of boiled grains were placed in glass bottles, sealed with cotton-wool plugs, and autoclaved at 121°C for 20 min. The bottles were allowed to cool for 24 h at room temperature, and an 11 cm mycelial plug of each species from a PDA plate was aseptically placed on the grains, with five plugs added to each bottle. After 2 weeks, the guinea corn grains were completely covered with fungal mycelium and were used as inoculum [25].

2.7. Fabrication of mycelium-based composites

Each substrate was supplemented with 5% rice bran, 1% calcium carbonate, 2% calcium sulfate, and 0.2% sodium sulfate on a dry-weight basis, as recommended by Kupradi *et al.* [26]. Water was added to achieve a final relative humidity of 60% in the mixtures. Five hundred grams of the resulting mixed substrate were placed into polypropylene bags measuring 3.5 inches in width and 12.5 inches in length. The bags were sealed using cotton-plugged polyvinyl chloride pipe rings, covered with paper sheets, and autoclaved at 121°C for 60 min. After the bags cooled to room temperature over 24 h, 5 g of each mycelial inoculum was deposited onto the top of the substrate in each bag, producing an inoculum-to-substrate mass ratio of 1:100 (w/w). The inoculated bags were then incubated at 30°C in darkness for 21 days [26].

Once each substrate was colonized by its respective fungal mycelium, it was placed into a unidirectional mold, subjected to molding pressure, and incubated inside a plastic box for an additional 3 days until the mycelium had grown over the sides that were in contact with the mold. The resulting MBCs were dried in an oven at 70°C for 24–72 h until their mass became stable [5].

2.8. Assessment of physical properties

2.8.1. Moisture content

The moisture content of the MBCs was measured in accordance with the ASTM D 644 standard method [24] using a desiccator (Shimadzu Corporation, Japan). Percentage moisture content was determined by calculating percentage mass loss using Eq. (1):

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100, \tag{1}$$

where W_1 is the initial weight of the sample and W_2 is the weight of the dried sample after oven drying. Ten replicates were performed for each sample under all treatments. After drying, the MBCs were stored in desiccators pending analysis.

2.8.2. Density

The density of the MBCs was tested using the MBCs prepared for the compression test according to the protocol of Aiduang *et al.* [24] using a compression-testing machine (Shimadzu Corporation, Japan). Density was calculated using the ISO 9427 standard method by measuring the mass and volume of dried MBCs after drying at 70°C for 24–72 h, with 10 replicates per sample per treatment.

2.8.3. Water absorption

Water absorption tests were conducted according to the ASTM C272/C272M-18 standard method. Before the test, MBC specimens were dried in an oven at 70°C until constant mass was achieved and then cooled in a desiccator for 24 h. The initial mass of each sample was recorded. The samples were then fully submerged in deionized water for a total of 96 h. Weighing was performed at 24, 36, 48, 60, 72, 84, and 96 h after submersion. Percentage increase in mass was determined using Eq. (2):

$$\text{Mass increase (\%)} = \frac{W - D}{D} \times 100, \tag{2}$$

where W is the wet mass and D is the dry mass. Each treatment was applied to each sample across 10 replications.

2.9. Assessment of mechanical properties

2.9.1. Compression strength

Compression strength was evaluated in accordance with ASTM D 3501 [9]. Testing was performed using a Hounsfield H10Ks universal testing machine (New York, NY, USA) configured as a load bench with 10 kN capacity and a 1 kN load cell. Experiments were conducted under ambient laboratory conditions (25°C, 40–50% relative humidity) with a controlled displacement rate of 5 mm/min. The resulting load–displacement curves were converted into stress–strain plots using Eq. (3) and Eq. (4):

$$\sigma = \frac{F}{A}, \tag{3}$$

$$\varepsilon = \frac{\Delta L}{L_0}, \tag{4}$$

where F is the compressive force (N), A is the original cross-sectional area of the specimen (mm^2), ΔL is the displacement of the loading surfaces (mm), and L_0 is the original height of the test piece (mm). Compression strength was expressed in MPa, with 10 replicates performed for each treatment to ensure statistical reliability.

2.9.2. Tensile strength

Tensile strength was measured in accordance with ASTM D 638-14 [9]. The experiments were conducted using a Hounsfield H10Ks universal testing machine (New York, NY, USA). The setup used an elongation rate of 2 mm/min and a maximum force capacity of 1 kN. Data collected from the machine were processed to generate stress–strain plots, from which tensile-strength values were obtained. Each treatment was replicated 10 times to ensure statistical validity.

2.9.3. Flexural strength

Flexural strength was determined in accordance with ASTM D 790-10 [9]. Testing was carried out using a Hounsfield H10Ks universal testing machine (New York, NY, USA), configured in a three-point bending setup. The machine was operated with a cross-head speed of 2 mm/min and a clamp support distance of 40 mm. Each treatment was replicated 10 times to ensure statistical reliability.

2.9.4. Statistical data analysis

Data obtained from each experiment were subjected to one-way analysis of variance (ANOVA) using SPSS version 16.0 for Windows. To detect significant differences among mean values, Duncan's multiple range test was subsequently applied at a significance level of $p \leq 0.05$.

3. Results

3.1. Isolation of Polyporus sp. and Ganoderma sp.

Four isolates designated GAN1, GAN2, POL1, and POL2 were morphologically established to the genus level based on their shapes, sizes, colors of the fruiting bodies, internal tissues, and basidiospores. Figure 3 shows the growth of the fungal isolates on PDA [27].

3.2. DNA extraction of Ganoderma sp. and Polyporus sp.

The ITS region of the fungal isolates was amplified using PCR with the Qiagen TopTaq Master Mix Kit (Qiagen, Hilden, Germany) [28]. The results showed that the sample labeled 1 ITS 4 was *Ganoderma enigmaticum*; 2 ITS 4 was *Ganoderma casuarinicola*; and 3 ITS 4 and 4 ITS 4 were *Polyporus thailandensis*. Figure 4 shows gel electrophoresis of the amplified ITS region of the fungal isolates.

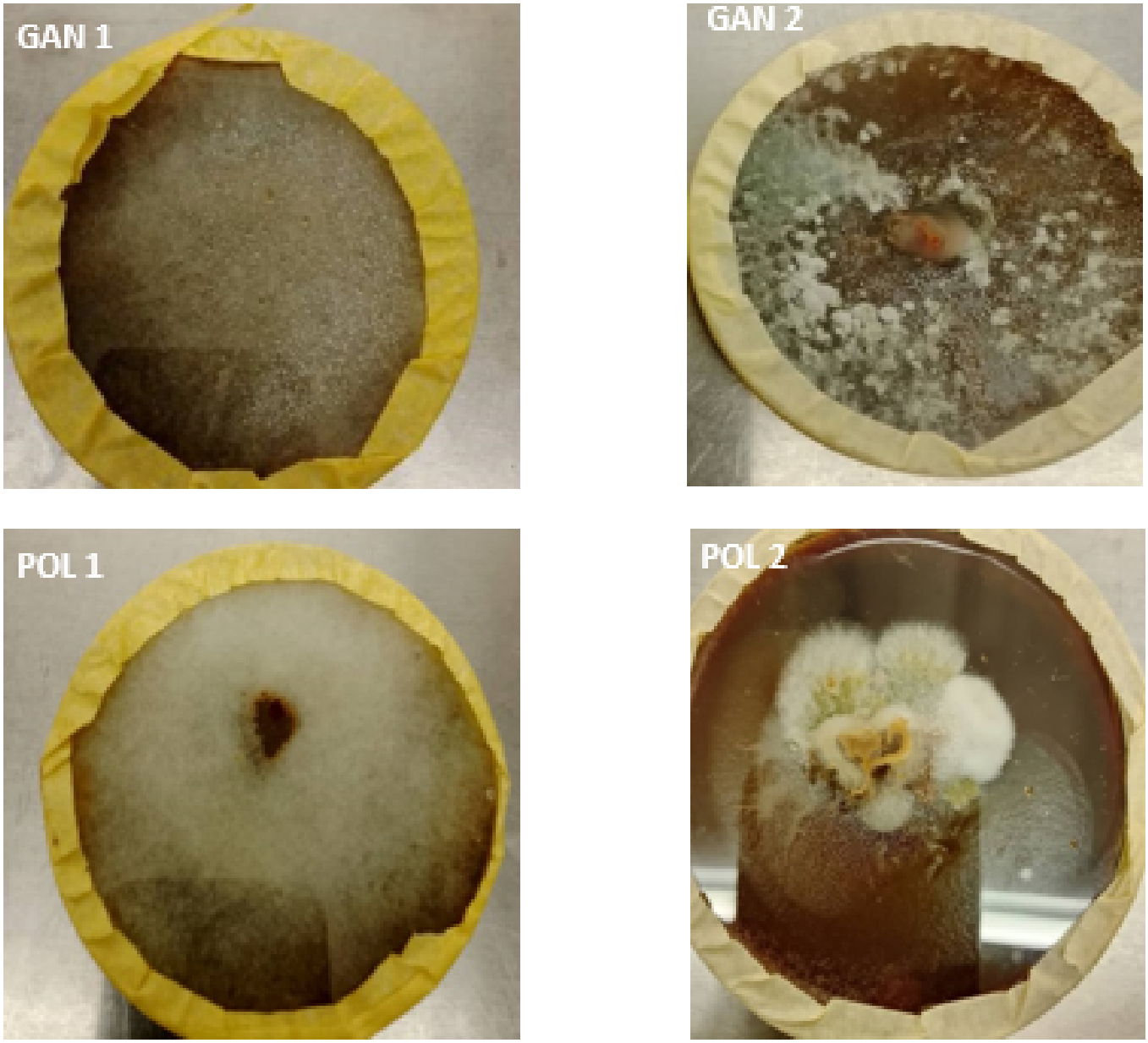

Figure 3: *Polyporus* sp. and *Ganoderma* sp. growing on potato dextrose agar.

3.3. BLAST analysis of the fungi

Table 1 shows the four query sequences (1 ITS 4, 2 ITS 4, 3 ITS 4, and 4 ITS 4) representing DNA fragments of different fungal isolates. Each was compared against the NCBI nucleotide database using BLAST, and the closest species matches were retrieved. The *Ganoderma* sequences (197 and 615 bp) were relatively short but sufficient for species-level identification when they represented conserved gene regions such as ITS or LSU rDNA. The *Polyporus* sequences were of similar length (608 and 621 bp), suggesting that they amplified similar regions of fungal ribosomal DNA. The maximum score measured the quality of the query–database alignment; the highest scores in the groups were 1052 for 2 ITS 4 and 812 for 4 ITS 4, suggesting high similarity between the query and database sequences in the respective groupings. Combined with very low or zero E values, these results support true homology rather than chance alignment. The E value represents the likelihood that the query–database alignment occurred by chance; values of zero for 2 ITS 4, 3 ITS 4, and 4 ITS 4, and 1e-99 for 1 ITS 4, indicate extremely reliable matches. The 1 ITS 4 sequence was 100% identical to *G. enigmaticum*; 2 ITS 4 was 99% identical; and 3 ITS 4 and 4 ITS 4 were 96.45% and 97.91% identical to *P. thailandensis*, respectively. The query coverage was 100% and 99% for 1 ITS 4 and 2 ITS 4, respectively, suggesting that almost the entire sequenced regions aligned, whereas coverage for 3 ITS 4 and 4 ITS 4 was lower at 79% and 77%, respectively. This may have

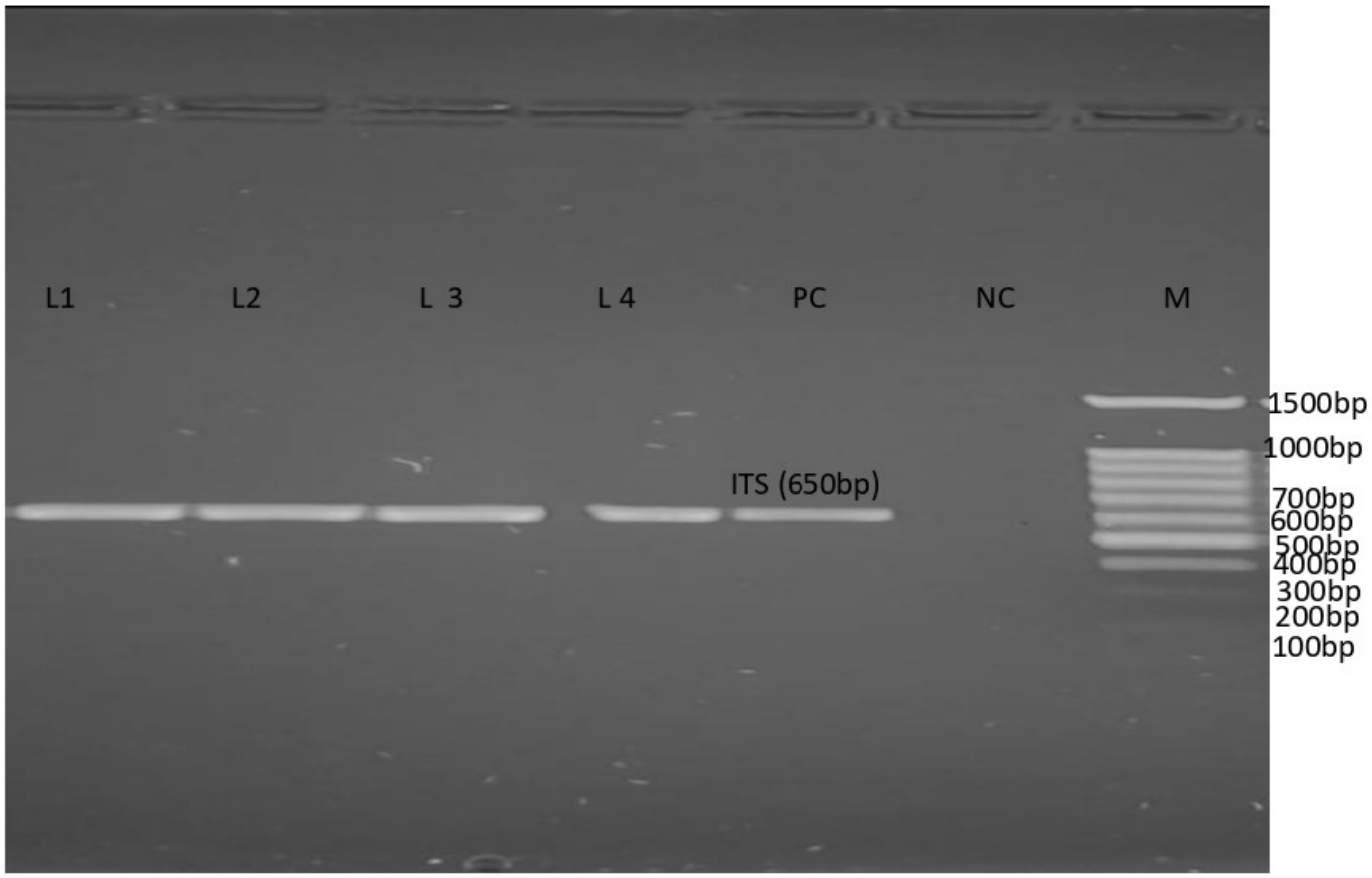

Figure 4: Gel electrophoresis of the internal transcribed spacer region of the fungal isolates. Keys: NC, negative control; PC, positive control; Lane 1, *Ganoderma* sp. (1); Lane 2, *Ganoderma* sp. (2); Lane 3, *Polyporus* sp. (1); Lane 4, *Polyporus* sp. (2).

Table 1: BLAST analysis for the fungal sequence overview of queries and results.

Query	Length (bp)	Top-hit species	Max score	E value	Identity (%)	Accession No.
1 ITS 4	197	*Ganoderma enigmaticum*	356	1e-99	100	NR_132918.1
2 ITS 4	615	*Ganoderma casuarinicola*	1052	0	99	NR_158432.1
3 ITS 4	608	*Polyporus thailandensis*	792	0	96.45	NR_155033.1
4 ITS 4	621	*Polyporus thailandensis*	812	0	97.91	NR_155033.1

Table 2: Confidence levels for the fungal isolates.

Genus	Query IDs	Likely species identity	Confidence level	Notes
Ganoderma	1 ITS 4, 2 ITS 4	*G. enigmaticum*, *G. casuarinicola*	High	High identity and full coverage support species-level matches.
Polyporus	3 ITS 4, 4 ITS 4	*P. thailandensis*	High	High identity with lower coverage; probably the same species and/or strain.

resulted from amplification of only a section of the gene or from the amplified gene region being longer than the section covered by the reference sequence [29]. The confidence levels for each fungal isolate are shown in Table 2.

3.4. Phylogenetic analysis of the fungal isolates

In a phylogenetic analysis of the ITS and ribosomal RNA gene regions, the isolates from this study were nested within the *Ganoderma* clade. ITS 4 from isolate 1 branched with *G. enigmaticum* isolates (OQ601588.1, PQ452369.1, and OQ601578.1) with bootstrap support of 93% and demonstrated a close evolutionary relationship to *G. enigmaticum*. The ITS 4 sequence from isolate 2 branched with *G. enigmaticum* TN2 (PQ452370.1) and *G. casuarinicola* (MT126492.1), but at a lower bootstrap value of

Figure 5: Top views and side views of the fabricated MBCs used for different tests.

60%, indicating less correlation and greater variability within the ITS 4 sequence than isolate 1. The clear distinction between *G. pakistanica* isolates (bootstrap 100%) and the remaining *Ganoderma* species supports the proposed phylogenetic arrangement of *Ganoderma* and indicates that isolate 1 ITS 4 is closely related to *G. enigmaticum*, whereas isolate 2 ITS 4 may be considered a strain of, or closely related to, *G. enigmaticum* [30].

The phylogenetic analysis based on ITS and ribosomal RNA sequences also indicated that the study isolates clustered within the *Polyporus thailandensis* clade. Isolate 3 ITS 4 grouped strongly with *P. thailandensis* reference sequences (AF516547.1, PV108362.1, and AJ132941.1), with bootstrap support values of 89–100%, confirming its identity as *P. thailandensis*. Isolate 4 ITS 4 also clustered with *P. thailandensis* strain CIRM-BRFM 1432 and *Polyporus* sp. isolate SA23 with moderate bootstrap support (74–76%), suggesting a close relationship to *P. thailandensis* but possibly representing a different strain or subpopulation. Overall, the tree topology supports the assignment of both isolates to *P. thailandensis*, with minor genetic divergence observed among strains [31, 32]. Both study isolates (3 ITS 4 and 4 ITS 4) clustered with *P. thailandensis* reference sequences. Isolate 3 ITS 4 was strongly supported within the *P. thailandensis* group, while 4 ITS 4 showed close affinity to *P. thailandensis* with slightly lower bootstrap support, suggesting potential intraspecific variation or a closely related lineage.

3.5. Morphological characteristics of the MBCs

The morphological properties of the MBCs were investigated, and the surfaces of all produced MBCs were covered with fungal mycelia, as shown in Figure 5. Examination of the cross-sections revealed that the fungal mycelia interconnected the substrate particles through networks of mycelial strands and air spaces within the composites. The morphological features observed in the MBCs in this study were consistent with those reported by Gou *et al.* [33].

3.6. Physical properties of the MBCs

3.6.1. Moisture content

Figure 6 presents the moisture contents of the MBCs examined in this study. The moisture content of the MBCs ranged from 56.27 to 62.34% on a wet-mass basis, varying with fungal species and substrate type. When a single substrate was used, the moisture contents fell within the 60–80% wet-mass range reported in earlier research [34]. In contrast, moisture content was notably lower than this range when a mixed substrate was used. These findings indicate that the moisture content of MBCs depends on the fungal species, substrate type, and number of substrates combined [35].

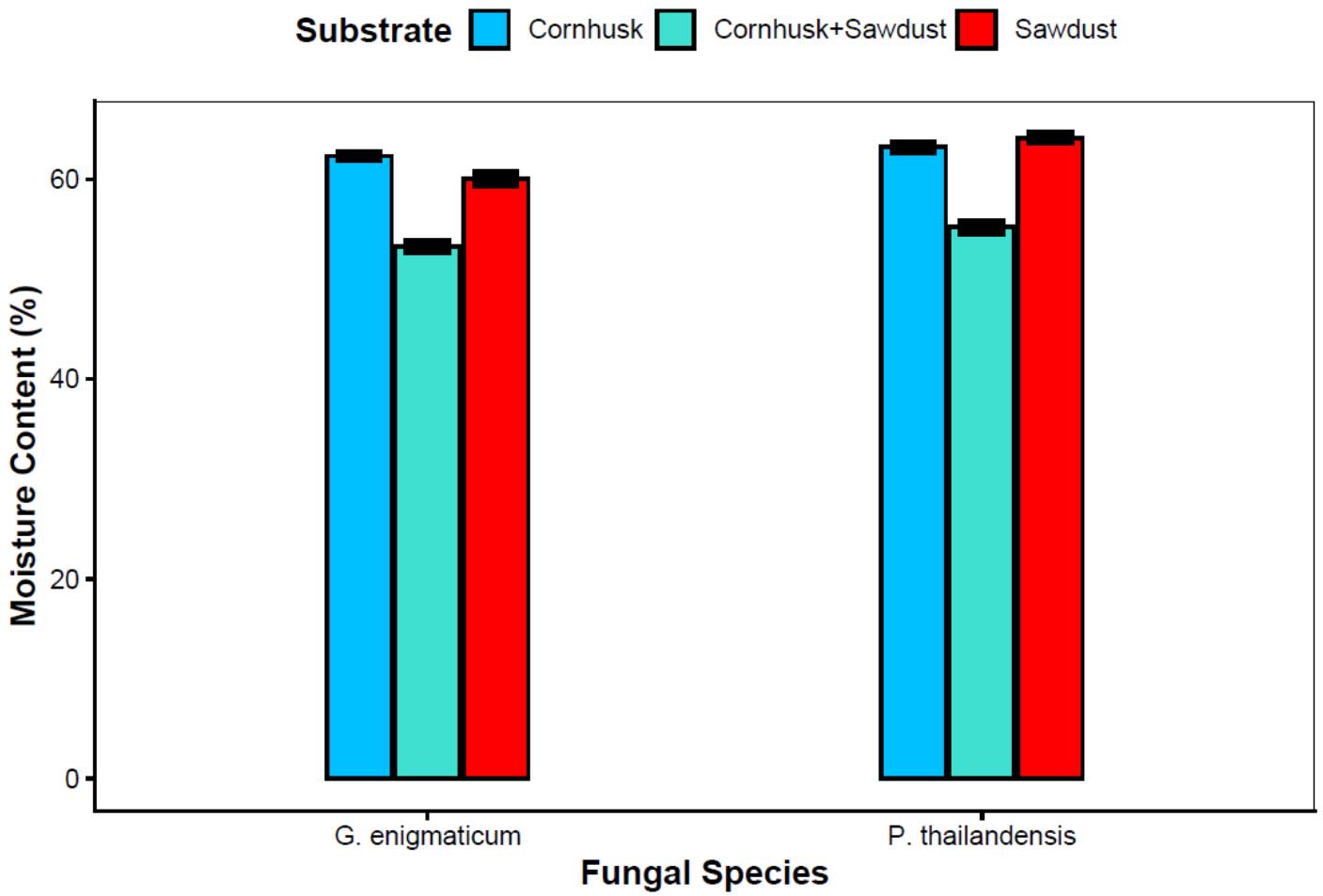

Figure 6: Moisture content of MBCs obtained in this study. Results are mean ± standard deviation. Different letters in the same column within each substrate type are significantly different according to Duncan's multiple range test ($p \leq 0.05$).

3.6.2. *Density*

Figure 7 shows the density values of the MBCs obtained in this study, which ranged from 110.11 to 130.24 kg/m^3. The highest density was observed in MBCs produced from a combination of sawdust and cornhusk (130.24 kg/m^3), while the lowest density was observed in MBCs made from sawdust alone with *G. enigmaticum* (110.11 kg/m^3). These density values fall within the 25–954 kg/m^3 range reported in earlier studies [36, 37]. The results align with previous findings indicating that substrate type and fungal species significantly influence MBC density [33]. The densities also lie within the typical range for lignocellulosic materials (94–1560 kg/m^3) and are comparable to those of polyimide foam (50–400 kg/m^3) [36].

3.6.3. *Water absorption*

To determine the water absorbency of the MBCs produced, samples were immersed in water for 96 h. The water absorption capacity of cornhusk MBCs increased rapidly up to 36 h and stabilized after 48 h of immersion in water. In contrast, MBCs made from sawdust continued to absorb water for up to 48 h, with stabilization occurring slowly after 60 h. Among all samples, cornhusk-derived MBCs exhibited the highest water absorption capacity, while sawdust-derived MBCs showed the lowest. The results also indicated that MBCs produced using *G. enigmaticum* had lower water absorption across all substrate types, whereas those produced with *P. thailandensis* displayed significantly higher water absorption regardless of substrate type. This study found that water absorption decreased as density increased. This finding is consistent with multiple previous studies, which reported that higher density in MBCs correlates with reduced water absorption [38]. In addition, the obtained MBCs showed water absorption capacities similar to those of lignocellulosic composites (53.6–148.8%) [39]. Nevertheless, the high water absorption capacity of MBCs remains a significant obstacle to their effective application and use. Figure 8 shows the water absorption of the MBCs obtained in this study.

Compression strength. The compression strength of the obtained MBCs varied depending on fungal species and substrate type (Table 3). The MBCs produced by *G. enigmaticum* and *P. thailandensis* using sawdust showed relatively low compressive strength,

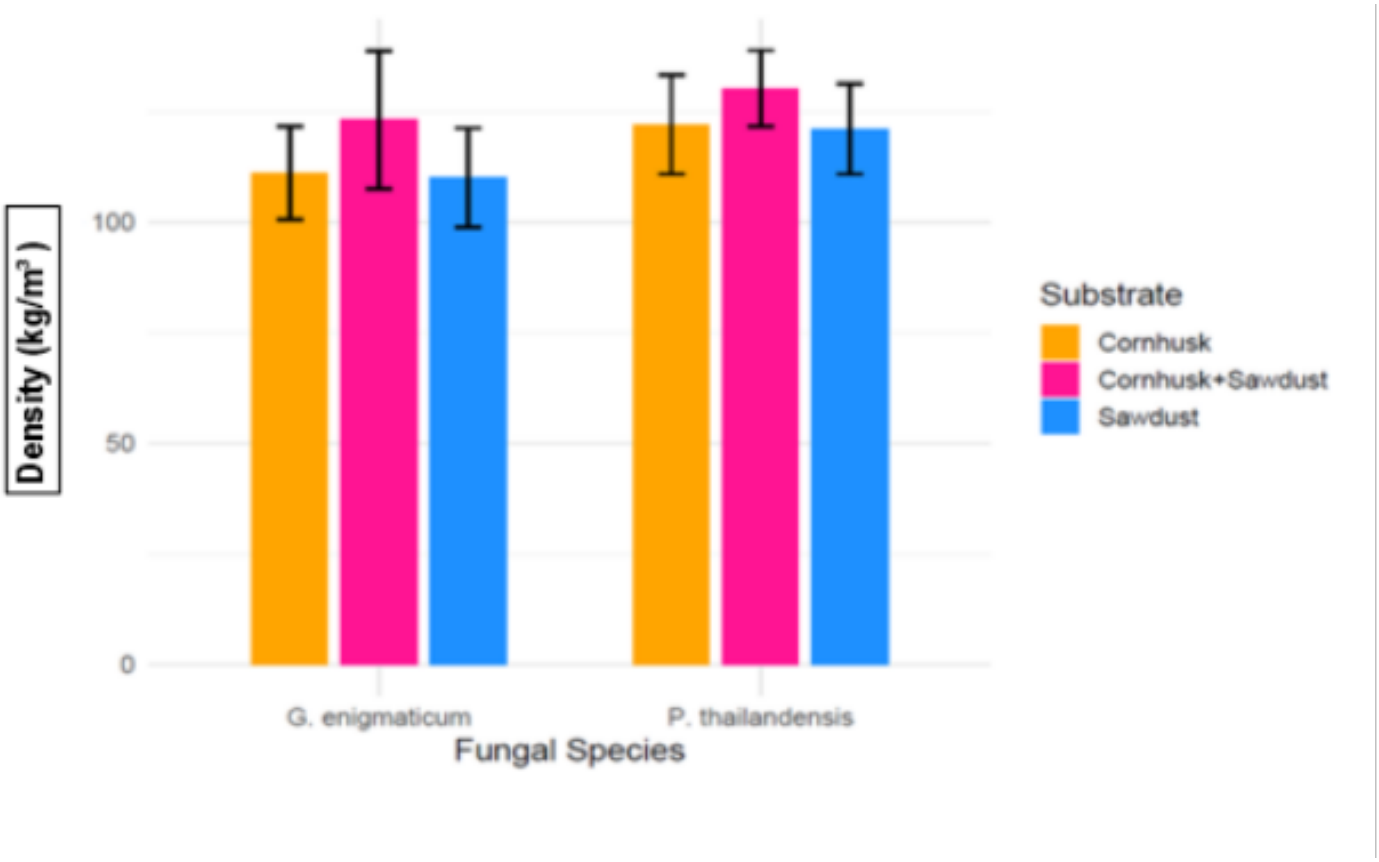

Figure 7: Density of MBCs obtained in this study. Results are mean ± standard deviation.

Table 3: Compression, tensile, and flexural strengths of MBCs obtained in this study.

Substrate	Fungal species	Compression strength (MPa)	Tensile strength (MPa)	Flexural strength (MPa)
Sawdust	*G. enigmaticum*	0.62 ± 0.01^{a}	0.75 ± 0.06^{b}	0.28 ± 0.03^{a}
Sawdust	*P. thailandensis*	0.62 ± 0.02^{a}	0.87 ± 0.06^{a}	0.32 ± 0.02^{a}
Cornhusk	*G. enigmaticum*	1.59 ± 0.02^{c}	0.20 ± 0.01^{c}	0.06 ± 0.01^{c}
Cornhusk	*P. thailandensis*	0.58 ± 0.02^{b}	0.63 ± 0.06^{c}	0.18 ± 0.04^{b}
Sawdust + cornhusk	*G. enigmaticum*	1.85 ± 0.01^{a}	0.42 ± 0.01^{a}	0.09 ± 0.02^{ab}
Sawdust + cornhusk	*P. thailandensis*	1.87 ± 0.03^{a}	0.44 ± 0.03^{a}	0.11 ± 0.02^{a}

approximately 0.62 MPa. This indicates that sawdust alone does not provide sufficient density or bonding for high compressive resistance. A marked difference was observed in MBCs produced by *G. enigmaticum* and *P. thailandensis* using cornhusk as the substrate; *G. enigmaticum* achieved 1.59 MPa, while *P. thailandensis* decreased to 0.58 MPa. This highlights the importance of fungal physiology in determining mechanical outcomes. In the mixed substrates (sawdust + cornhusk), both fungi reached the highest compressive values, approximately 1.85–1.87 MPa. This suggests a synergistic effect whereby substrate combination enhances density and bonding, producing composites comparable to synthetic foams. Overall, compressive strength was maximized in mixed substrates, confirming that substrate blending can significantly improve mechanical performance. These values fall within the range of synthetic foams, making the composites suitable for packaging and insulation applications. The findings align with prior research indicating that the compression strength of MBCs is strongly dependent on both fungal species and substrate type [24, 40]. For example, Tacer-Caba *et al*. [38] reported that MBCs produced from *Ganoderma lucidum* cultivated on oat husk and rapeseed cakes exhibited higher compressive strength than those produced by *Agaricus bisporus* and *Pleurotus ostreatus* grown on the same substrates. Similarly, MBCs derived from *Pleurotus sanguineus* grown on pine sawdust demonstrated superior compression strength compared with those produced by *Pleurotus albidus*.

Tensile strength. MBCs from sawdust produced moderate tensile-strength values of 0.75–0.87 MPa, with *P. thailandensis* slightly outperforming *G. enigmaticum*. This suggests that sawdust provides a favorable matrix for fungal binding. MBCs from cornhusk produced much lower tensile strength (0.20–0.63 MPa), indicating weaker bonding capacity. The fibrous structure of cornhusk may hinder the formation of a strong mycelial network. MBCs from the mixed substrates produced intermediate tensile strength, approx-

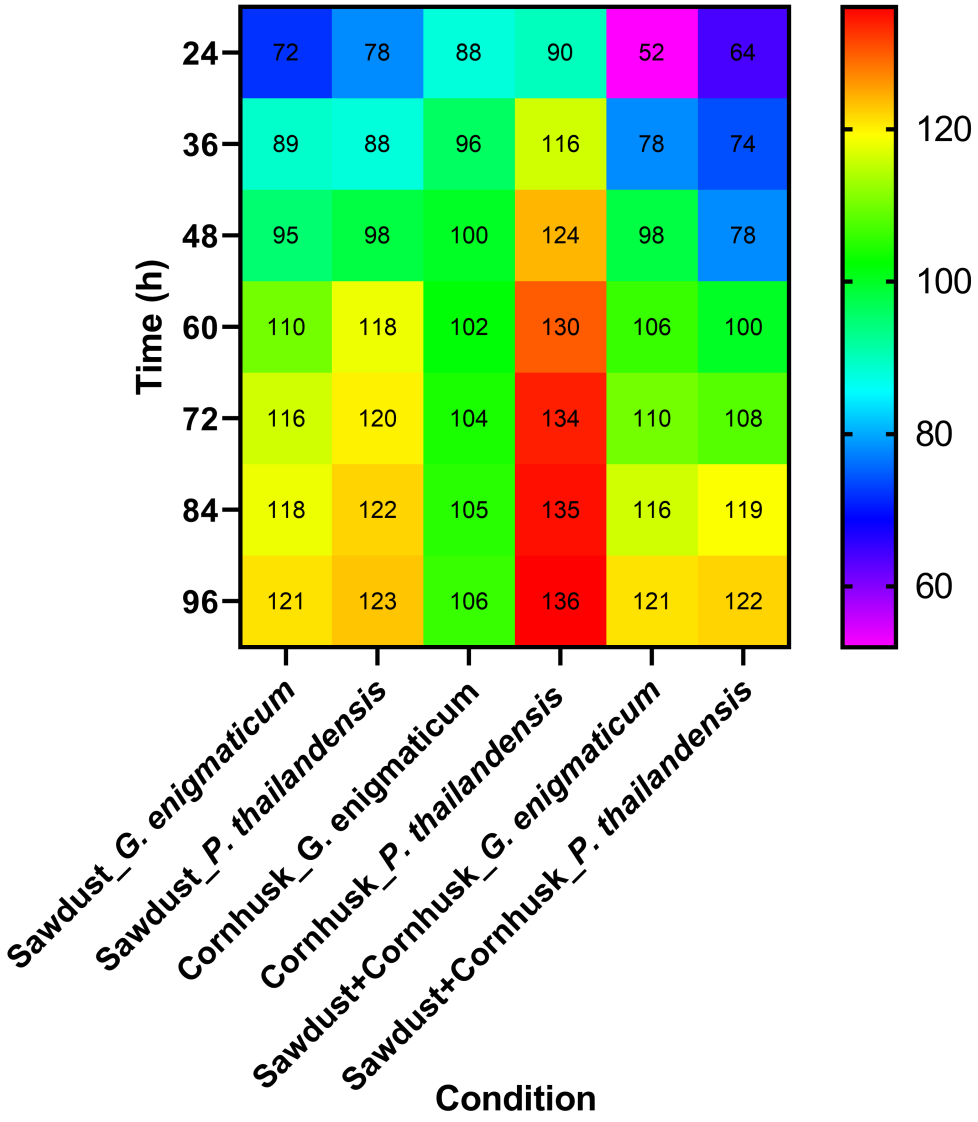

Figure 8: Water absorption of MBCs obtained in this study.

imately 0.42–0.44 MPa, lower than pure sawdust but higher than cornhusk alone. This indicates that substrate blending improves compressive strength but does not necessarily optimize tensile properties. Tensile strength was most favorable in sawdust-based composites, aligning with previous studies linking stronger tensile resistance to denser and more uniform substrates. These values are comparable to those of polystyrene and phenolic foams, reinforcing their potential in packaging. These results are consistent with earlier research showing that the tensile strength of MBCs is strongly influenced by substrate type and the architecture of the mycelial binder network [40]. In particular, Appels *et al.* [9] reported that MBCs produced from cotton substrates with *Pleurotus ostreatus* exhibited higher tensile-strength values than those derived from rapeseed straw or beech sawdust.

Flexural strength. The flexural-strength values were moderate (0.28–0.32 MPa) in the MBCs produced from sawdust. Both *G. enigmaticum* and *P. thailandensis* performed similarly, suggesting that sawdust provides a relatively uniform matrix but lacks the structural reinforcement needed for bending resistance. The fibrous arrangement of sawdust may allow good compression and tensile bonding but does not translate into high flexural performance. Cornhusk composites showed a wider range of 0.06–0.18 MPa. Interestingly, *P. thailandensis* achieved higher flexural strength (0.18 MPa) than *G. enigmaticum* (0.06 MPa). This indicates that fungal species selection plays a critical role in bending resistance, possibly owing to differences in hyphal density and binding capacity. The fibrous and less compact structure of cornhusk may hinder stiffness, explaining the generally lower values. In the mixed substrates (sawdust + cornhusk), flexural strength was very low (0.09–0.11 MPa), even lower than the values from single substrates.

This suggests that while substrate mixing improved compressive strength, it disrupted the uniformity required for flexural resistance. The heterogeneity of particle sizes and fiber orientations likely weakened bending capacity. These obtained flexural-strength values were within the ranges reported in previous studies, from 0.05 to 4.40 MPa [24]. Appels *et al*. [9] suggested that the flexural strength of MBCs depends on the type of mycelial network, substrate type, and pressing method used.

4. Discussion

The search for biodegradable materials that can replace plastic has continued for several decades. Recently, biofabrication technology involving the growth of fungi on agricultural waste materials has been developed for the production of MBCs. This study examined fungal species, substrate types, and their effects on the final properties of MBCs, particularly regarding their potential to replace polystyrene materials. MBCs have gained increasing popularity as alternative materials in packaging, fashion, and architecture because of their advantages over synthetic materials, including affordability, safety, and biodegradability. In addition, MBC production provides the benefit of using lignocellulosic waste materials to create products capable of replacing synthetic alternatives. Previous research found that the physical properties of MBCs are influenced by the fungal species used in production, which affects the binding network and colonization within the substrate [41, 42].

In this study, two lignocellulosic residues (sawdust and cornhusk) and two fungal species (*G. enigmaticum* and *P. thailandensis*) were used to develop MBCs. With the main aim of improving MBC properties, two different substrates were combined rather than using one substrate, as reported by Aiduang *et al*. [24]. The results indicate that MBCs produced by *G. enigmaticum* exhibited better physical properties than those produced by *P. thailandensis*. Moisture contents were 60.03 and 63.24%, and densities were 110.11 and 121.13 kg/m^3 for *G. enigmaticum* and *P. thailandensis*, respectively. The greatest degree of water absorption was observed in MBCs of *P. thailandensis* (136%) compared with MBCs of *G. enigmaticum* (106%). The water absorption of MBCs in this study was within the range of 25–560% reported in previous studies [17]. According to several other studies, MBCs are classified as hydrophilic materials, and substrate type and fungal species can greatly influence water absorption [43]. Jinanukul *et al*. [44] reported that the size of MBCs can affect final density. Their findings showed that the decrease in MBC density varied with the amount of cornhusk added; as the substrate ratio increased, thickness also increased.

With respect to the substrates used, MBCs obtained from combined substrates showed improved physical properties, including the highest density and the lowest water absorption and moisture content, compared with MBCs obtained from single substrates. Although none of the physical properties of the obtained MBCs were similar to those of polyimide and polystyrene, the quality of the physical properties improved in this study compared with the results obtained by Aiduang *et al*. [24].

The problem of low density and high water absorption compared with polystyrene remains a major challenge. Expanded investigations of the components of the various substrates could be developed and applied to understand which components influence the quality of the final products. In addition, more refined analytical methods could be used to better evaluate the suitability of MBCs for packaging materials.

Producing an ideal MBC capable of replacing synthetic plastics while maintaining a balance among strength, durability, scalability, cost-effectiveness, user acceptance, and environmental sustainability remains a major challenge. Variations in production processes, substrate types, growth conditions, and manufacturing techniques make it difficult to achieve consistent physical properties in the resulting MBCs. In addition, large-scale production remains a significant issue that requires further improvement through future research. Despite these challenges, biocomposite materials show strong potential as substitutes for expanded polystyrene in packaging applications because of their strength and biodegradable nature. Furthermore, because many products packaged with expanded polystyrene are dry and do not contain liquids, the limitations of MBCs related to moisture content and water absorption may be less critical.

5. Conclusion

One major advantage of MBC production is the use of agricultural waste to manufacture high-value materials. In this study, two agricultural wastes (sawdust and cornhusk) were used to develop MBCs, and the physical properties were investigated. *Ganoderma* and *Polyporus* fungi were characterized and identified molecularly. Both organisms produced substantial mycelium biomass for composite production. The mycelium-based composite showed commendable physical properties and, with appropriate additive incorporation, could be adopted for food and food-product packaging.

Data availability

The authors confirm that all data supporting the findings of this study are contained within the article.

Declaration of competing interest

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

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