A Comparative Study of Mycelium Films from Nine Fungal Species for Biocomposite Applications

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Mycelium-based biocomposites (MBC) offer a sustainable alternative to synthetic materials due to their biodegradability and low environmental impact. This study examined the structural and mechanical properties of mycelium films produced from nine fungal species representing monomitic, dimitic, and trimitic hyphal systems. These species were selected following preliminary screening of 21 strains for growth characteristics and mechanical performance. Growth rates varied significantly, with *Irpex lacteus* exhibiting the fastest growth (8 mm/day), while *Fomes fomentarius* and *Daedaleopsis confragosa* grew more slowly but exhibited superior mechanical strength. Tensile testing identified *D. confragosa* as the strongest fungus (6.51 MPa), followed by *F. fomentarius*, although considerable variability was noted. *Ganoderma* spp. and *Trametes* spp. showed moderate to low tensile strength. No consistent correlation was found between mycelium density and tensile strength, nor did chitin content alone explain mechanical performance. For instance, *I. lacteus* had the highest chitin content but weak tensile properties. Scanning electron microscopy revealed differences in hyphal diameter, density, and cell wall structure, indicating that factors such as glucan-chitin interactions and hyphal morphology influence mechanical behavior. These findings highlight the potential of less investigated fungal species in advancing MBC development.

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INTRODUCTION

Due to the increased demand for sustainability in material production and consumption (Despeisse *et al*. 2012; Hami *et al*. 2015), significant effort has been invested in the exploitation of natural materials with lower energy production demands and higher biodegradability than synthetic polymers (Ling *et al*. 2018; Dhali *et al*. 2021; Sangmesh *et al*. 2023; Rao *et al*. 2024). One such prominent precursor is mycelium, the fibrous structure of a fungus consisting of a mass of branching, hair-like hyphae (Gooday 1995; Islam *et al*. 2017). The mycelia of different *Agaricomycetes* fungi have been used to develop and manufacture a new class of sustainable materials since the 2000s (Kuribayashi *et al*. 2022).

They offer a promising sustainable alternative for product design and manufacturing, as agricultural and wood processing wastes can be used in mycelium cultivation (Appels *et al*. 2019; Yang *et al*. 2021; Aiduang *et al*. 2022a). Mycelia are grown on a lignocellulosic substrate, leading to the production of a mycelium-based composite (MBC). The MBC lignocellulosic substrate is partially decomposed by fungal metabolism, while the hyphae simultaneously strengthen it by their extensive growth (Appels *et al*. 2019; Elsacker *et al*. 2019). The physical and mechanical properties of mycelium are determined by its fibrous structure and the chemical components of the hyphae. In most species, the inner cell wall consists of covalently attached, branched chitin, chitosan, and glucan, and these are mainly responsible for its load-bearing properties (Nawawi *et al*. 2019). Due to its versatility, MBC can be used in a number of different applications, such as packaging (Vaishnav and Choudhary 2021; Rajendran 2022), acoustic and thermal insulation (Zhang *et al*. 2023; Weinland *et al*. 2024), construction material (Voutetaki and Mpalaskas 2024), and furniture (Sydor *et al.* 2022a). Thanks to its material properties, clean mycelium sheets can also be used as a leather alternative (Amobonye *et al*. 2023).

The mechanical properties of fungal mycelium are strongly influenced by the hyphal structure, which varies with species (Ryvarden and Gilbertson 1993; Porter and Naleway 2022). According to their hyphal systems, wood fungi can be classified as monomitic, dimitic, and trimitic (Ryvarden and Gilbertson 1993). A monomitic structure consists of a system of solely generative hyphae, produced as the basidiospore germinates. This ancestral hyphal type later developed into a skeletal structure, with unbranched and thick-walled hyphae generally oriented in one direction. When skeletal hyphae adopt a highly branched structure, those which bind different hyphae together are called ligative hyphae. A dimitic structure includes both generative and skeletal hyphae, while trimitic species possess all three hyphal types (generative, skeletal and ligative) (Porter and Naleway 2022). Different hyphal structures lead to materials with different mechanical properties.

Due to their potential as natural-based materials, the mechanical properties of MBC have been intensively investigated. Appels *et al*. (2019) tested the tensile and bending properties of *Trametes multicolor* and *Pleurotus ostreatus* mycelium grown on rapeseed straw with and without cold and hot pressing. Chan *et al*. (2021) investigated the tensile, bending, and compressive mechanical properties of *Ganoderma lucidum* grown on a mixture of empty fruit bunch fibre and sawdust. Aiduang *et al*. (2022a) compared the material properties of different lignocellulosic substrates inoculated separately with four fungal species. Kuştaş and Gezer (2024) tested the bending, compressive, and internal bonding mechanical properties of MBC made from desilicated wheat straw inoculated with *P. ostreatus* and *G. lucidum*. Alaneme *et al*. (2023) published a review of articles concerning MBC since 1960, which showed an exponential increase in number in the last few years, reaching its peak in 2022 with 70 articles. However, the literature on clean mycelium discusses the mechanical properties of only a few fungal species, often focusing on the same model isolates repeatedly.

Haneef *et al*. (2017) examined the difference in material properties of mycelium films of *G. lucidum* and *P. ostreatus* cultivated on pure cellulose and a mixture of cellulose and potato dextrose broth, showing the strong influence of the medium on the morphological features of both species, while a relevant difference in mechanical properties was shown only for *G. lucidum*. César *et al*. (2021) investigated the morphological and physical properties of five fungal species obtained from wild specimens and a commercial *P. ostreatus* culture and found that *Ganoderma curtisii* had the greatest

tensile strength. Islam *et al*. (2017) tested the tensile and compressive properties of mycelium sheets produced by Ecovative Design, LLC, USA (without specifying the species), and showed the strong influence of density on the mechanical performance of the fungal material. Appels *et al*. (2018) tested the material properties of *Schizophyllum commune* mycelium cultivated from wild strains and a genetically modified strain grown in different environmental conditions. Cartabia *et al*. (2021) conducted an anatomical comparison of mycelium plates from 21 fungal species but did not investigate their mechanical properties.

In this study, mycelium films were derived from ten fungal species selected following a preliminary testing of 24 species, including monomitic, dimitic, and trimitic hyphal systems. The material properties of mycelium films were compared. The findings highlight the potential of underexploited fungal species in advancing mycelium-based bio-composite development.

EXPERIMENTAL

Cultivation and Growth Assessment of Fungal Mycelium

Selection of strains and cultivation

The investigated strains were obtained from the culture collection of Mendel University of Brno, Department of Forest Protection and Wildlife Management, Czech Republic. One strain of each species was selected for analysis. The strains were identified according to the morphological characters of their basidiome and the DNA sequence of the internal transcribed spacer of their ribosomal RNA genes. The strain of *Abortiporus biennis* recommended by (Balaeș *et al*. 2023) was obtained from the CCBAS culture collection (Institute of Microbiology, Czech Academy of Science, Prague, Czech Republic). An isolate of *Ganoderma sessile* from a purchased inoculated substrate from GrowBio (Ecovative) was used as the reference strain. The solid-state culture system of malt extract agar (MEA) medium (malt extract agar base by Himedia) in 90-mm Petri dishes was kept in laboratory incubators (Sanyo Mir-153, Japan) at 21 °C and 60% relative humidity. For preliminary testing, 21 species were selected (Table 1).

The species marked with * in Table 1 were those where the mycelium film (a layer of clean mycelium grown across the MEA surface, used afterwards for mechanical testing) could not be removed from the growth medium without damaging it. After preliminary mechanical testing of the remaining strains, nine were selected for further analysis (shown in bold in Table 1). New films of these nine species were cultivated in Petri dishes and removed one week after complete colonisation of the Petri dish. Examples of similar mycelium films have been presented by Cartabia *et al.* (2021) and Haneef *et al.* (2017).

Growth assessment and preparation of mycelium films

A block of MEA with a fresh culture transplantation spot was positioned at the edge of each Petri dish. The radial growth of hyphae was measured with a ruler every two days until the Petri dish was completely colonised by the mycelium (Fig. 1). The growth rate for each fungal strain was assessed as an average of the results from five Petri dishes. When fully covered with mycelium, the dishes were stored in an incubator at the same temperature and relative humidity for an additional one and two weeks. In preliminary tests we compared the mechanical properties of mycelium films collected one and two weeks after complete overgrowth. No significant differences in mechanical properties were

observed in these tests. Therefore, for detailed analysis, all subsequent mycelium films of selected species were collected one week after full overgrowth.

Table 1. Species Investigated Classified According to their Hyphal Structure

MONOMITIC	DIMITIC	TRIMITIC
*Phlebiopsis gigantea**	*Laetiporus sulphureus**	***Ganoderma applanatum***
*Irpex latemarginatus**	*Laetiporus montanus**	*Ganoderma adspersum*
	*Gleophyllum trabeum**	*Ganoderma lucidum*
	Irpex lacteus	***Ganoderma resinaceum***
	Pleurotus ostreatus	*Ganoderma carnosum**
	Abortiporus biennis	*Ganoderma pfeifferi*
		Ganoderma sessile
		Trametes hirsuta
		Trametes gibbosa
		Trametes suaveolens
		Trametes versicolor
		*Fomitopsis pinicola**
		*Fomitopsis betulina**
		Fomes fomentarius
		Fomes inzengae
		Daedaleopsis confragosa
		Trametes betulina
		*Funalia gallica**

Note: Species marked with * are those where removing mycelial sheets without damage was not possible. The nine species selected after preliminary testing are shown in bold.

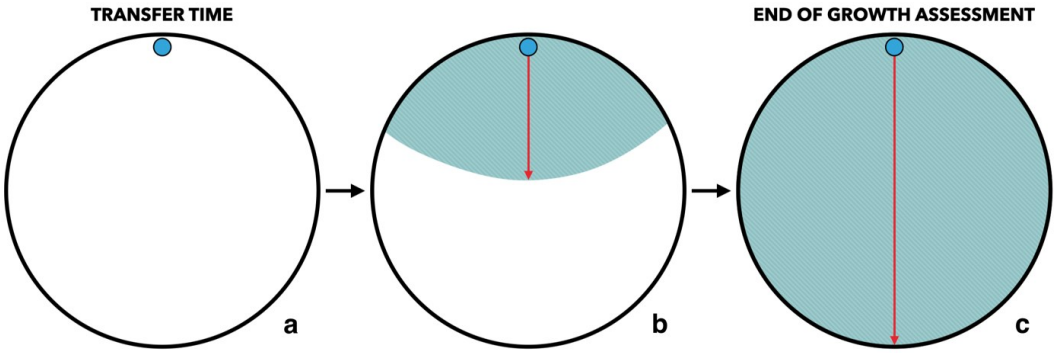

Fig. 1. Schematic representation of the growth of the mycelium film from the transfer time (a), through the initial growth phase (b) until complete overgrowing of the Petri dish (c)

Mechanical Testing of Mycelium Films

To measure the tensile strength of the mycelium, a dog-bone-shaped specimen was cut from each film using a custom-made steel punch, which was oriented so that the inoculation point was as close to the central line of the specimen as possible. Thus, the tensile force was applied in the same direction as the mycelium growth. Even though the mycelia had been kept pressed under a glass sheet during the first phase of the drying process, some mycelium films had warped. In these cases, the specimens were stamped out

to fit the available area of the warped mycelium, regardless of the mycelium growth direction. Preliminary testing did not show any significant difference between samples stamped out parallel and perpendicular to the growth direction. The outstamping was carried out on the part of the sheet with the most homogeneous film thickness.

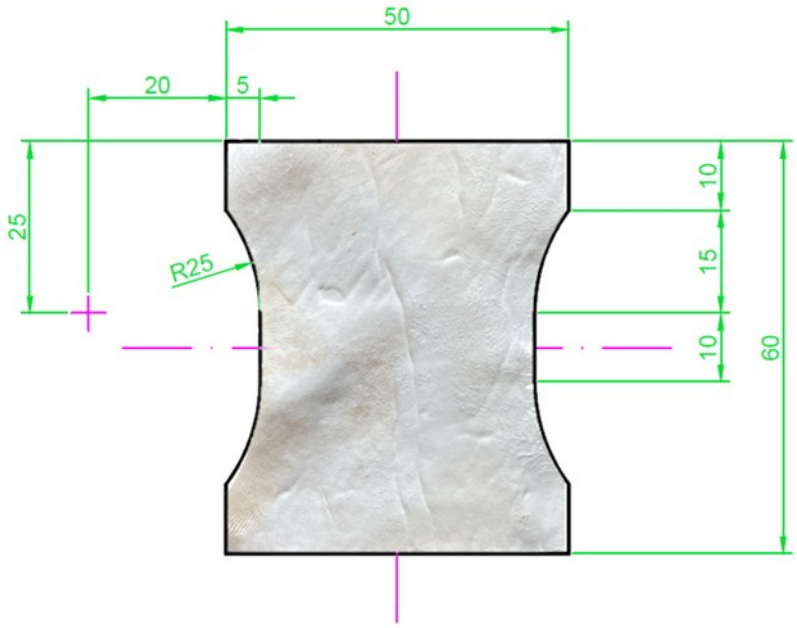

Fig. 2. Shape and dimensions (mm) of tensile-strength test specimens

The tensile test was carried out on a universal testing machine Tinius Olsen 10ST (Tinius Olsen, Ltd, UK) equipped with a 500 N load cell with 0.0001 N readability. The tensile clamping jaws designed for tear testing of textile and paper sheets were used. The specimens were accommodated in the rubber grips using a preload at 0.5 N. The specimens were loaded in the quasi-static displacement-controlled rate of 1 mm·min^{-1} until a visible sign of failure in force-displacement relation was achieved. The tensile displacement was controlled by crosshead movement with 1 μm readability.

The tensile strength (σ_{TS}) was determined as the maximum force related to cross-section area at the specimen waist (the narrowest part of the specimen), according to Eq. 1,

$$\sigma_{TS} = \frac{F_{MAX}}{T \cdot W} \quad (1)$$

where σ_{TS} is the tensile strength in MPa, F_{MAX} is the maximum force in N at failure, T is the thickness, and W is the width of the specimen in mm. The thickness was determined as the mean of six measurements made with a Mitutoyo Series 547 gauge (0.5 μm readability, measuring force ≤ 3.5 N) at points along the failure edge.

Preliminary testing and selection of species for further analysis

For preliminary testing, dog-bone samples were prepared from mycelium films collected from completely overgrown Petri dishes. For each species, at least six samples were tested. The mycelium films of *G. pfeifferi*, *G. adspersum*, and *G. lucidum* were significantly deformed during the drying process, which made it impossible to prepare a sufficient number of tensile specimens. These species were excluded from further analysis.

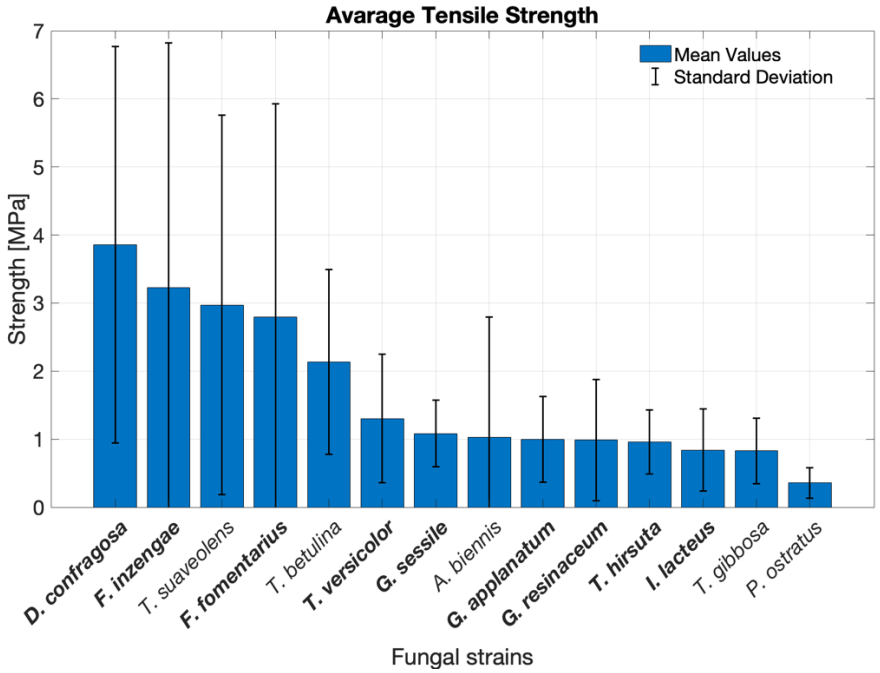

Fig. 3. Results from the preliminary tensile strength testing of 14 fungal species; the nine species selected for further testing are shown in bold.

The nine species selected for further assessment were chosen not only based on their preliminary mechanical performance (Fig. 3) but also on other factors, such as the variability of the measured mechanical data, deformation of mycelium sheets during the drying process, the heterogeneity of the film thickness, and the relative novelty of the species in the literature. For further mechanical analysis, at least 12 samples were tested for each selected species.

Scanning Electron Microscopy (SEM) and Image Analysis

SEM

All test high-quality cross-sections were prepared using the following procedure. Mycelium films were cut with a razor blade to produce samples 5 mm wide and 10 to 15 mm long. The samples were glued into the rebate of a wooden holder using a cyanoacrylate glue. To facilitate easy removal of the mycelium, a thin HDPE foil was placed as a separator, with only the ends of the samples glued directly to the holder.

A drop of ethanol was applied to each sample to thoroughly soak them, after which they were immediately immersed in demineralised water. The leaching process lasted 10 min, with one water change. Each holder, bearing two samples, was placed in a plastic zip-lock bag filled with water and then frozen. Excess ice was removed by thawing on a warm aluminium block. The cross-section of the sample embedded in ice was smoothed using a small GSL1 microtome (Gärtner *et al* 2014). The frozen samples were freeze-dried for six hours in a Scanvac CoolSafe 55-4 freeze dryer (LaboGene, Denmark). The middle section of each dried sample was cut out and fixed to an aluminium stub using Leit-C conductive carbon cement. The samples were sputter-coated with a 15 nm thick gold layer using a LUXOR gold coater (APTCO Group, Germany). The mycelium microstructure was observed using a Tescan Vega 4 scanning electron microscope (Tescan Orsay Holding, a.s., Czech Republic).

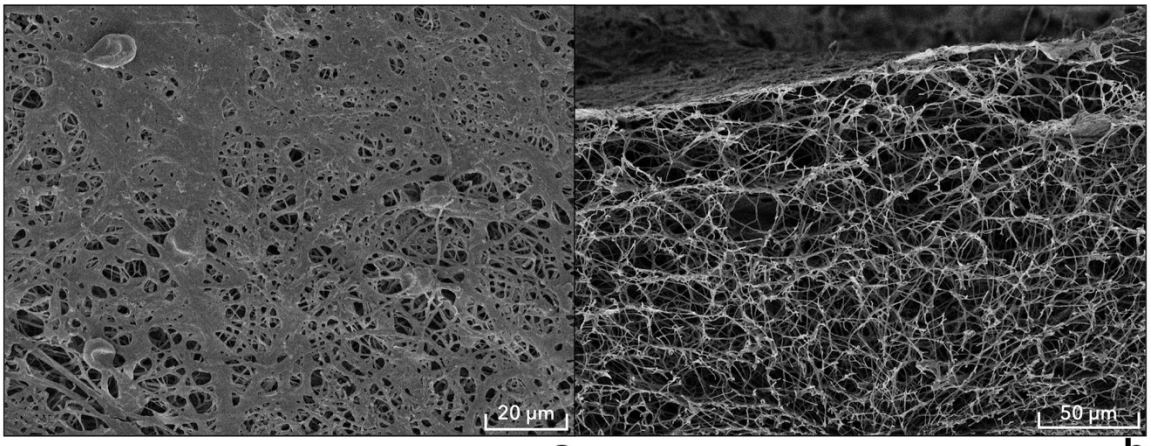

Fig. 4. SEM scans of (a) the surface and (b) the cross section of a mycelium film

High-vacuum scanning in resolution mode was performed with a secondary electron detector under the following settings: landing energy of 7 keV, beam current of 30 pA, scan speed of 3 (1 µs/pixel), and a magnification of 1,000×. The imaging quality was enhanced by averaging 20 accumulated images at a resolution of 1,024 × 768 pixels.

Image analysis

All The SEM images of mycelium were collected at 1,000× magnification. Because the sample surface was often covered by a uniform layer (Fig. 4; Cartabia *et al*. 2021), the assessment of hyphal density was made on the cross-section of the samples. Images were obtained from two samples for each fungal species. A minimum of 10 images were taken from different locations of each sample to ensure statistical relevance.

SEM images were processed and analysed using image analysis software ImageJ. Due to the different thicknesses of the mycelia of different fungi, the original SEM images were first cropped so that the image only captured the mycelium. The reflection of the hyphae in the upper layers of the images was greater than in the deeper layers, resulting in greater pixel intensity of hyphae in the upper layers than beneath. The visibility of individual layers depended on the density and porosity of the mycelium. Image analysis was therefore a function of the image threshold. By changing this, different hyphal layers were observed. The lower threshold was individually set for each scan, based on the peak value from the picture histogram, which was multiplied by a coefficient (0.35), chosen according to the visual assessment of different SEM images.

Once the threshold was set, the differentiation of the image was obtained, where the black colour represented the porous region and the yellow corresponded to the hyphae (Fig. 5). The mycelium surface area [%] (SA) represents the area of hypha-occupied surface (A_h) relative to the total area (A_t) of the analysed image, expressed as a percentage, as shown in Eq. 2.

$$SA = \frac{A_h}{A_t} \times 100[\%] \quad (2)$$

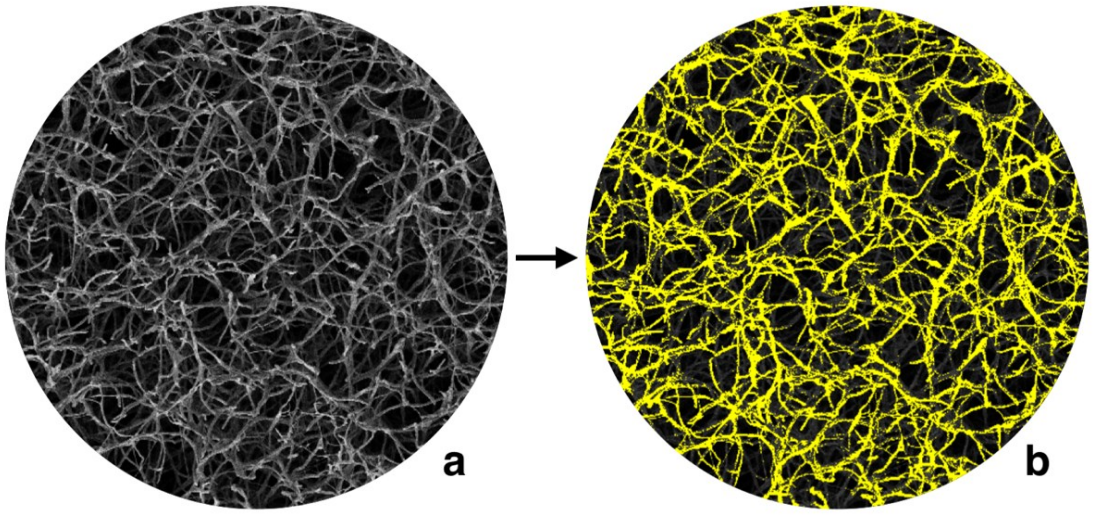

Fig. 5. Representation of a SEM scan (a) before image analysis and (b) highlighted area (hypha region) according to the set threshold.

Determination of Chitin Content in Fungal Mycelium

Chitin contained in mycelium was subjected to acid hydrolysis at high temperatures, which decomposed chitin into its essential building block, which is an amino monosaccharide glucosamine. The method for determining glucosamine hydrochloride in a mycelium sample was based on derivatization with 9-fluorenylmethoxycarbonyl chloride (FMOC-Cl). A rapid, selective and specific HPLC method with fluorescence detection was subsequently used to determine and quantify glucosamine hydrochloride (FMOC-Cl adducts of two anomers of glucosamine hydrochloride) in the fungal mycelium.

Chemicals

Chemicals included hydrochloric acid (anhydrous, 37% v/v, Penta Chemicals, CZ), chitin (practical grade, Toronto Research Chemicals, Altium International Inc., CZ), 9-fluorenyl-methoxycarbonyl chloride (≥99.0%, for HPLC derivatization, LiChropur™, MERCK, CZ), glucosamine hydrochloride (analytical standard, MERCK, CR), borate buffer pH 8 (prepared from boric acid p. A. and sodium hydroxide p. A., both Penta Chemicals, CZ), and acetonitrile (≥99,9%, HPLC gradient grade, MicroCHEM, CZ).

Samples preparation and hydrolysis

Samples of selected fungal mycelia were homogenized in a ball mill (Retsch, MM 400) to a fine powder. For hydrolysis, a modified Araujo *et al*. (2022) method was used. A sample of approximately 0.02 g of mycelium was accurately weighed into a 10 mL screw-cap glass vial. Five mL of 6 M hydrochloric acid was added. The samples were then placed in a laboratory oven (DRYSD30, COLD lab experts, Slovenia), where hydrolysis took place at 103 °C for 2 hours. After this time, the samples were removed from the oven and cooled at laboratory temperature for another hour. Subsequently, an aliquot volume of 20 µL was taken from the hydrolysate into a vial (1.8 mL), which was followed by drying in a nitrogen stream. 500 µL of borate buffer (pH 8) was added immediately. This was followed by derivatization of the thus prepared sample. Chitin standard samples were also processed using the same procedure for method control.

Precolumn derivatization and chromatography analysis

For the derivatization step and chromatographic determination with fluorescent detection, the method according to Zhang *et al*. (2006) and Huang *et al*. (2006) was modified. Derivatization was carried out by adding 30 µL of 1 mg/mL FMOC-Cl (acetonitrile solution). The sample was vortexed briefly. Derivatization was performed at 30 °C for 30 min in a water bath, and then 1 µL of the resulting solution was injected into the HPLC system.

The analysis of glucosamine hydrochloride as FMOC-Cl adducts was carried out using an Agilent Technologies series 1260 modular HPLC system consisting of a quaternary gradient pump with vacuum degasser (G1311B), a 100-sample autosampler (G1329B) a thermostatic column compartment (G1316A), and a fluorescence detector (G7121A). For the evaluation of chromatograms, OpenLab ChemStation data analysis software was used. The chromatographic separation was performed on a RP-C18 column Kinetex C18, 5 µm, 100 Ä (150 mm x 4.6 mm, Phenomenex). A column oven temperature of 30 °C was used for the separation. A suitable protective pre-column (Zorbax Eclipse Plus-C18, 2.1 mm x 2.5mm, 5 µm) was also used. The mobile phase consisted of acetonitrile (ACN) and deionized water (DW), initial ratio 30:70, v/v. The separation was achieved using gradient elution with flow rate set at 0.9 mL/min. The gradient progression was as follows: t=8 min, 30% ACN, 70% DW; t=12 min, 100% ACN, 0% DW; t=15 min, 100% ACN, 0% DW; post time 5 min). The monitoring wavelength of fluorescent detection glucosamine derivatives was set at 263 nm for excitation and 315 nm for emission parameter. The retention times the glucosamine-FMOC-Cl adducts were 4.5 and 5.3 min, respectively. The dry matter was also determined in the mycelium samples. The final results of the glucosamine content were converted into dry matter content.

Calibration

To prepare a stock solution of glucosamine hydrochloride, 1 mg was dissolved in 10 mL of deionized water. The stock solution was stored at 4 °C in the dark. The working solution was prepared daily by diluting the stock solution 10 times. Samples for constructing a calibration curve in the required range were prepared from the working solution of the glucosamine standard. These standard samples were analysed using the same procedure as the mycelium samples, *i.e.*, after FMOC-Cl derivatization followed by HPLC with fluorescent detection. A so-called blank sample was also analysed with each series of samples. All samples were analysed in duplicate.

Statistical Analysis

The relationships between measured parameters were statistically investigated in MATLAB 2024b® (The MathWorks, Inc., California, USA) using the nonparametric Kruskal-Wallis test, the Turey-Kramer multiple comparison test and the coefficient of variance (*CV*). The significance level (α) for ANOVA was set at 0.05, which is the standard threshold for natural materials.

RESULTS AND DISCUSSION

Growth-rate Assessment

As shown in Fig. 6, the highest growth rate was observed for the wood fungus *I. lacteus* – 8 mm/day – which may be compared with the average growth rate (AGR) of 10

mm/day reported by Cartabia *et al*. (2021). Similar agreement was observed for *D. confragosa*, which reached an AGR of 4.4 mm/day in the present study compared to 4 mm/day in a paper by Cartabia *et al*. (2021). However, a large discrepancy was noted in *F. fomentarius*, which showed an AGR of 5.7 mm/day, lower than the 7 mm/day reported by Cartabia *et al*. (2021). In contrast, Rypáček (1957) recorded an AGR for *F. fomentarius* at 20 °C on agar medium of approximately 3.8 mm/day. The variability in these results can be attributed to the natural differences between the strains tested (historically, *F. fomentarius* and *F. inzengae* were not distinguished), the types of growth media used in the studies (malt extract agar and highly moisturised millet grains) and different incubation temperatures. The media content could be considered a factor influencing the growth rate of the fungus. Aiduang *et al.* (2022b) tested the growth rate of five fungal species on potato dextrose agar; however, since they tested different fungal species than those investigated in this study, no relevant comparison is possible. The significant influence of temperature on the AGR of wood fungi was demonstrated by Dresch *et al*. (2015), who found high variability in growth rates across a temperature range of 10 to 37 °C, with the optimal growth temperature being between 25 and 30 °C. This aligns with the earlier findings of Rypáček (1957), who also reported the fastest growth at 30 °C.

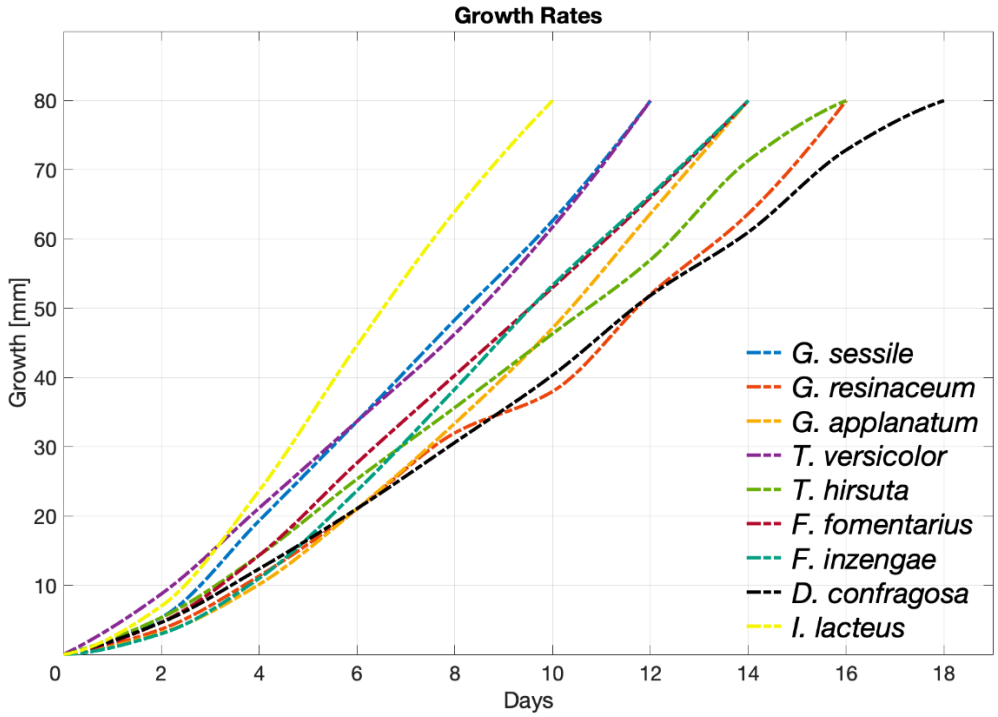

Fig. 6. Growth dynamics in radial direction of the selected fungal species in Petri dishes

According to Fig. 6, there was a noticeable difference in growth rate among the three *Ganoderma* spp., with *G. sessile* found to be the fastest. The growth rate was influenced by the incubation temperature, which was kept constant for all cultures, although optimal temperature for each species can vary. Nguyen *et al*. (2023) studied the fungus *Ganoderma sinense* under different environmental conditions and found an optimal temperature for growth between 25 and 30 °C. A similar range was observed for *Trametes* species, with *T. versicolor* growing faster than *T. hirsuta*. The rapid growth of *T. versicolor* is one of the main reasons for its frequent mention in current literature, making it one of

the most extensively studied species for application in MBC (Nussbaumer *et al*. 2023; Sydor *et al*. 2022b).

The fastest growth rate of *I. lacteus* compared to the other species may be attributed to its highly effective specific metabolism (Cartabia *et al*. 2021; Novotný *et al*. 2009). The cited authors also noted that some dimitic fungi perform rapid growth, such as *Abortiporus biennis*, *Bjerkandera adusta*, *Irpex lacteus*, and *Stereum hirsutum.* These species could be suitable for developing mycelium-based materials due to their high growth rates under favourable conditions. However, these fungi have light and fragile mycelium mats. Another factor influencing the growth rate of *I. lacteus* can be its metabolism

Morphological, Mechanical and Chemical Analysis

The mycelium structure of species varied, as viewed in cross-section (Fig. 7). All three *Ganoderma* spp. (Fig. 7a-c) exhibited a highly branched mycelium with thin hyphae. Güler *et al*. (2011) studied the mycelium structure of *Ganoderma lucidum* and found similar hyphal density and thickness to the three *Ganoderma* spp. examined. While the mycelia of *Ganoderma* spp. appeared visually denser than other species, image analysis (Fig. 8b) showed that the area covered by their mycelia was similar to other species, but that they had a smaller hyphal diameter. This feature of the genus did not occur for the tested *Trametes* species: *T. versicolor* appeared to show a higher hyphal density than *T. hirsuta* (Fig. 7d and e). However, as indicated in Fig. 8b, no statistically significant difference in hyphal density was found between the two *Trametes* spp. ($p > 0.05$). *F. fomentarius* and *F. inzengae* had a similar mycelium structure (Fig. 7f and g), which is further supported by their similar growth rates (Fig. 6) and hyphal densities (Fig. 8b). These two species also showed the same wood degradation ability, as reported by (Cristini *et al*. 2023).

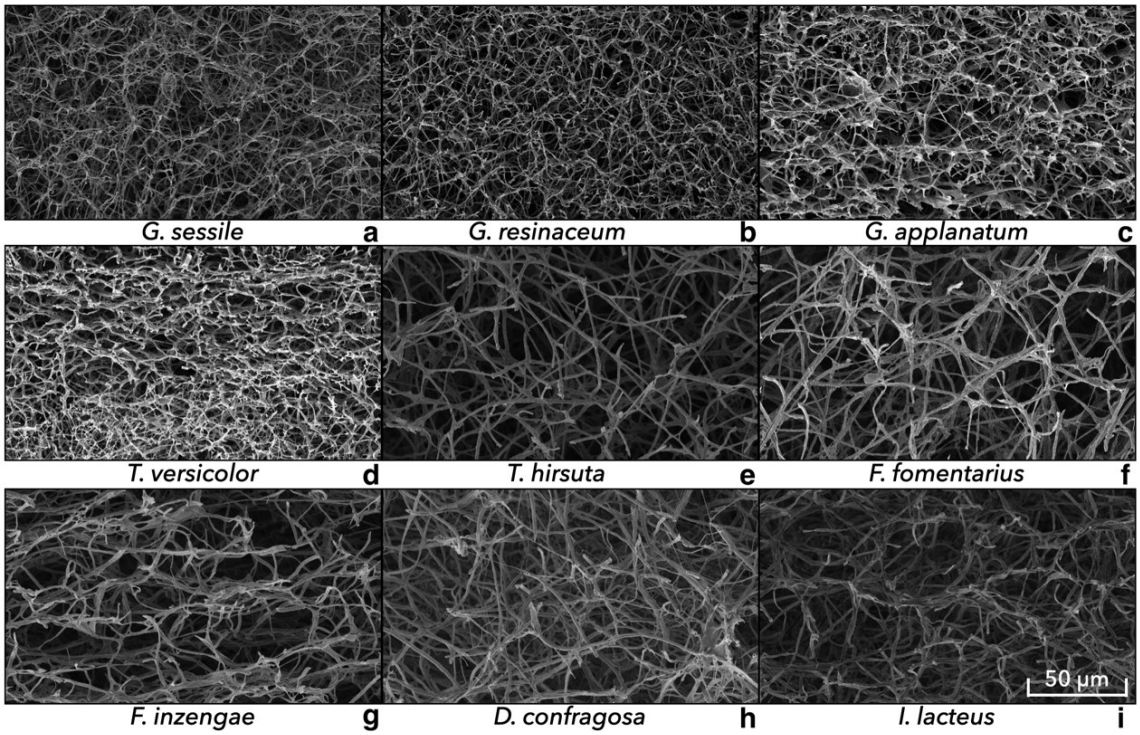

Fig. 7. SEM scans of the selected fungal species

As shown in Fig. 8a, the fungal species with the highest tensile strength was *D. confragosa*, with a median of 6.51 MPa (mean 8.68 MPa). This result was similar to the mechanical performance of *Schizophyllum commune* films from wild strains as reported by Appels *et al*. (2018), who found average tensile strengths between 4 and 9 MPa, depending on the incubation conditions. Although *D. confragosa* has been investigated as a potential raw material for nanopaper production (Nawawi *et al*. 2020), there are no publications investigating its potential as a fungal strain for MBC. The lowest tensile strength was observed in *G. resinaceum*, with a median of 0.96 MPa (mean 1.30 MPa). *I. lacteus*, the only dimitic species among tested fungi, showed a tensile strength similar to that of *G. sessile* and *G. resinaceum* (median 1.27 MPa; mean 1.40 MPa). When comparing all species to the fungus with the highest mycelium tensile strength, a statistically significant difference was found between *D. confragosa* and a group of four fungal species: *G. sessile, G. resinaceum, F. inzengae* and *I. lacteus* ($p < 0.05$).

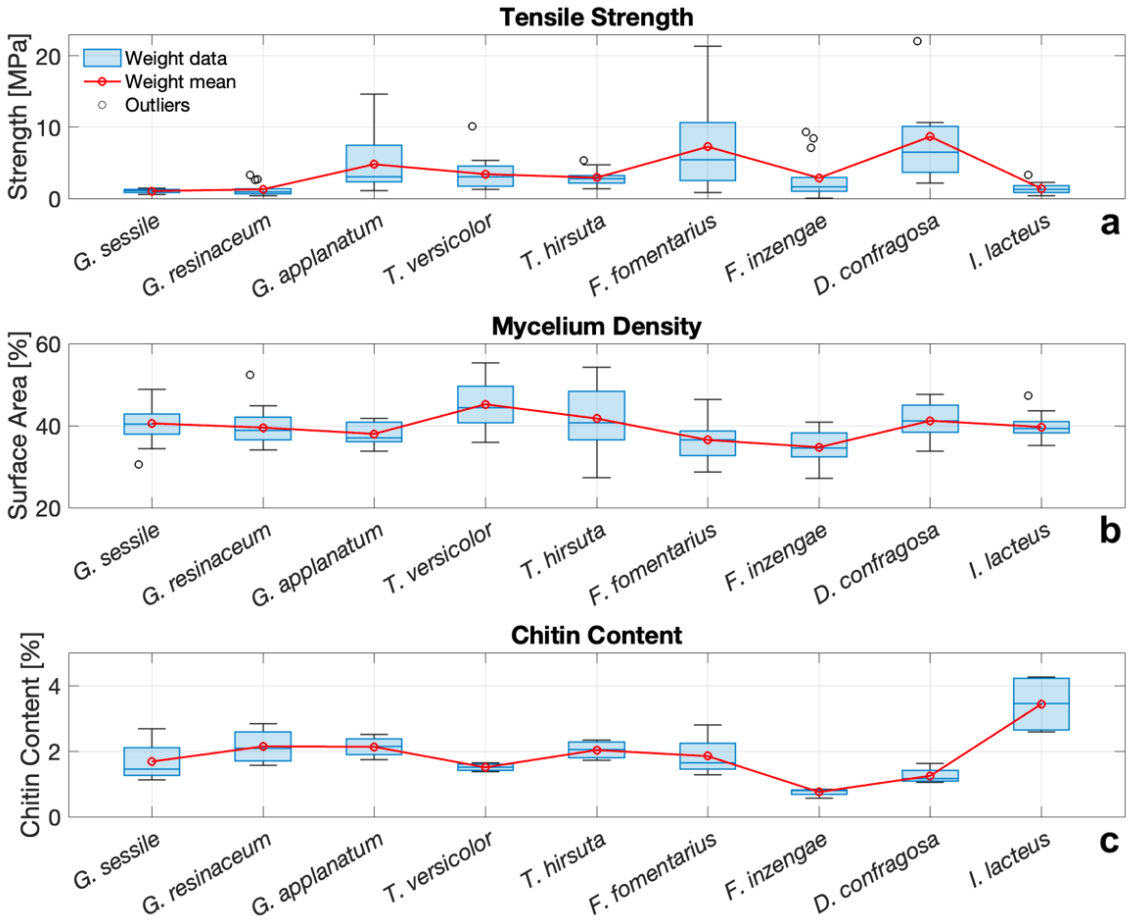

Fig. 8. Tensile strength (a) and mycelium density according to the image analysis of SEM scans (b) of the selected fungal species

In contrast to the results of preliminary testing presented in the methodology, Fig. 8a shows a remarkable difference in average tensile strength between *F. fomentarius* and *F. inzengae*. However, due to the high variability in the measured data for the two fungal species ($CV_{\text{fomentarius}}$ 77.9%; CV_{inzengae} 105.7%), no statistically significant difference between them was observed ($p > 0.05$). *F. fomentarius* exhibited the second highest mechanical performance of all tested species. The mechanical properties of MBC made

with *Fomes fomentarius* have been explored by Pohl *et al*. (2022), who identified it as a promising fungal species for the future development of MBC. However, it remains less extensively studied than other fungal species. According to our results, both *D. confragosa* and *F. fomentarius* warrant further investigation for their potential use in the fabrication of MBC. A notable difference was observed between *Ganoderma* spp.

While the average tensile strengths of *G. sessile* and G. *resinaceum* were relatively low, that measured for *G. applanatum* was significantly higher (median 3.05 MPa; mean 4.80 MPa), with a statistically significant difference from the other two *Ganoderma* spp. ($p < 0.05$). César *et al*. (2021) tested the tensile strength of two other *Ganoderma* spp. and reported an average tensile strength of 0.84 MPa for *G. curtisii* and 1.50 MPa for *G. mexicanum*. Haneef *et al*. (2017) tested *G. lucidum* films cultivated on a mixture of potato-dextrose agar and cellulose and reported an average strength of 1.1 MPa. These results are similar to those observed in this work for *G. sessile* and *G. resinaceum*, though still lower than the average strength measured in this work for *G. applanatum*. The differences in strength can be attributed to different incubation conditions (25 °C) and medium (liquid *vs.* solid, different components), which may influence the mycelium structure. *Trametes* spp. exhibited similar tensile strengths, with a median of 3.07 MPa for *T. versicolor* (mean 3.42 MPa) and 2.78 MPa for *T. hirsuta* (mean 2.93 MPa), with no statistically significant difference between them ($p > 0.05$). Despite the differences among the studied species, the high variability of the data (*CV* range 27.9 to 105.7%; mean 66.9%) should be considered. This variability can be attributed to factors such as the heterogeneity of mycelium films, the natural variability of mycelia as a biological material, and the physiological conditions of the selected strains during cultivation. The influence of these factors can also explain the differences between preliminary and final mechanical assessments (Figs. 3 and 9a). The sample thickness varied among species, ranging from 0.13 mm (CV = 41.6%) for *G. resinaceum* to 0.37 mm (CV = 27.4%) for *G. applanatum*. The variability (CV) in thickness ranged from 17.6% for *T. versicolor* to 41.6% for *G. applanatum*. A further study employing a larger sample of each fungal strain, and incorporating multiple different strains of each fungal species, would yield more informative results.

Comparing Fig. 8a with Fig. 8b, there was no visible relationship between tensile strength and mycelium density as determined by image analysis. However, a statistically significant difference in density was found between *D. confragosa* and *F. fomentarius*, although their mechanical performance was similar. *D. confragosa* and *G. resinaceum*, the strongest and weakest fungal species based on mechanical testing, respectively, exhibited similar hyphal densities in the central zone of the mycelium cross-section.

Another factor that may influence the mechanical performance of mycelium is its chitin content (Vadivel *et al*. 2024). The average chitin content (Fig. 8c) in mycelium films across the nine studied fungi varied by species—from 0.75% in *F. inzengae* to 3.44% in *I. lacteus*. These values were lower than those reported in the review by Nawawi *et al*. (2019), who documented chitin contents in various basidiomycetes such as *Agaricus bisporus* (3 to 19%) and *P. ostreatus* (2 to 15%). The discrepancy arises from differences in the fungal structures analysed (mycelium *vs*. fruiting bodies) and the age of the cultivated substrate, as younger cultures tend to have lower chitin levels (Cartabia *et al*. 2021).

Due to the limited number of mycelium samples used for chitin extraction (four specimens per species), statistical comparisons among species were not informative. Nevertheless, when comparing the results of mechanical testing with chitin content, no clear correlation emerged (see Fig. 8a). For instance, *I. lacteus*, despite having the highest chitin content, was among the weakest in terms of tensile strength. Similarly, *F.*

fomentarius and *T. hirsuta* had comparable chitin contents (2.03% and 1.85%, respectively), yet they showed a significant difference in average tensile strength (2.93 and 7.26 MPa, respectively). These findings suggest that chitin content alone does not determine the mechanical performance of the tested mycelium sheets. Other factors, such as the presence of glucans, which interact with chitin microfibrils to form a distinctive composite structure (Vadivel *et al*. 2024), as well as hyphal dimensions and cell wall thickness, may play a more substantial role in influencing the material's mechanical behaviour.

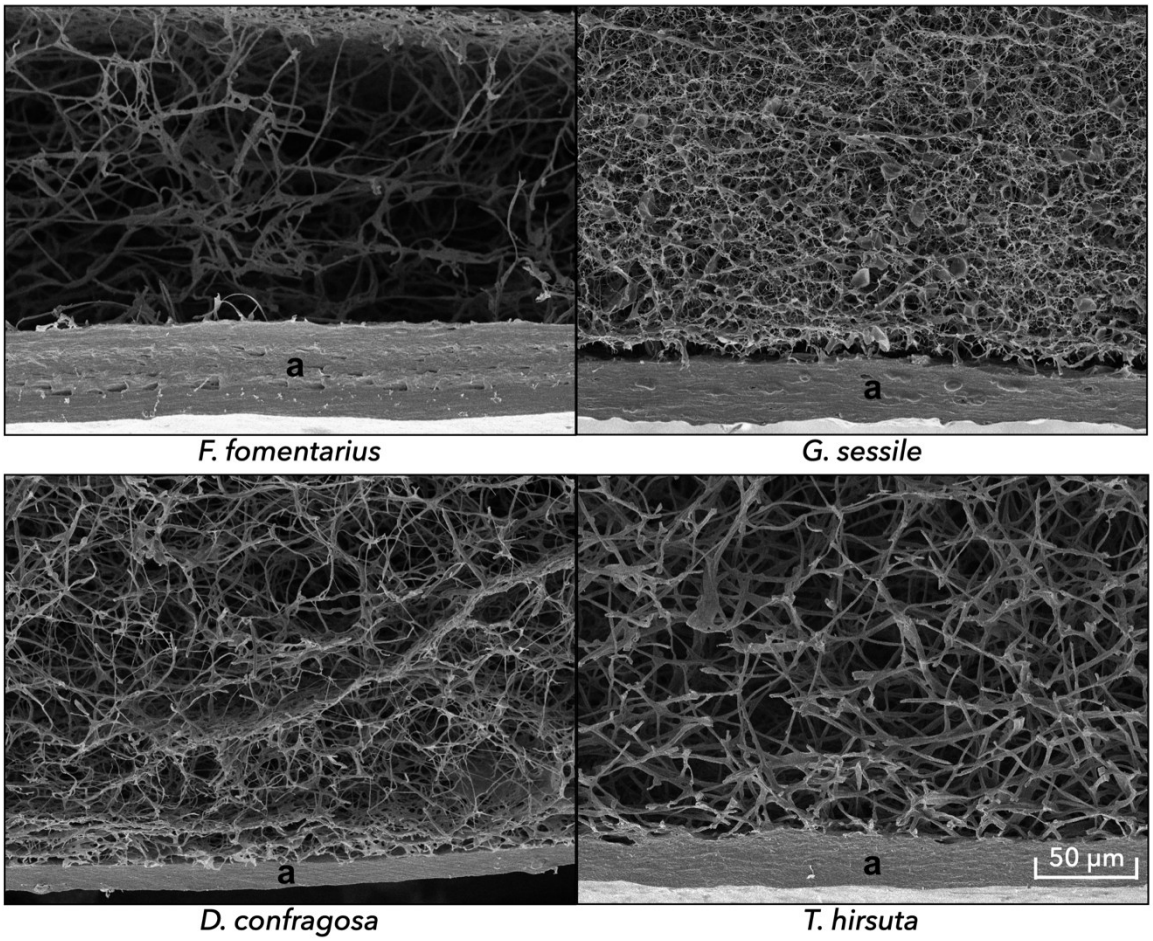

Fig. 9. SEM scans of selected fungal species showing the denser lower hyphal layer (a)

These inconsistences suggest that other morpho-chemical parameters, such as chitin or hyphal cell-wall thickness, may have a strong influence on the mechanical performance of mycelium films. Another possibility is that the tensile properties of mycelium films are primarily dependent on the thick hyphal layer often present on the lower side in contact with the growth media (Fig. 9). This laminar structure was observed by Appels *et al*. (2018) in *Schizophyllum commune* mycelium plates, where a genetically modified strain with tensile strength almost four times greater than wild strains developed a thick, dense layer on the side in contact with the growth medium. One factor that could potentially influence the mechanical test results was a malt-agar layer on the bottom of the mycelium film. According to experimental results, the average tensile strength of 12 clean, dried malt-agar plates (mixed as malt agar medium, then dried) was 6.60 MPa. All samples

were meticulously cleaned from all agar medium as much as possible. The presence of any remaining agar layer was also ruled out through SEM analysis.

Given the results, further research on promising species such as *D. confragosa* and *F. fomentarius* is essential for advancing the development of MBC. Optimising growing conditions, particularly temperature and media composition, could enhance the material properties of these fungi.

This investigation assessed the growth rates, morphological characteristics, chitin content and tensile strengths of various fungal species for potential application in MBC. The presented findings confirmed the diversity in both growth rates and mechanical performance among the investigated species, showing the potential of certain less studied fungi for MBC composite fabrication. The limitation of this study has to be acknowledged, including the high variability in the measured data, which can be attributed to the natural heterogeneity of mycelium as a biological material. The limited sample size also highlights the need to examine different strains of the same species to obtain more reliable and relevant results.

CONCLUSIONS

1. *I. lacteus* exhibited the fastest growth rate (8.0 mm/day), probably given by its specific metabolism.
2. *F. fomentarius* exhibited a moderate growth (5.7 mm/day), influenced by strain variability and environmental conditions (*e.g*. air humidity, temperature).
3. *D. confragosa* displayed the highest tensile strength (median 6.51 MPa), making it a promising candidate for use in MBC.
4. *F. fomentarius* gave the second-best mechanical performance, though with high data variability.
5. *Ganoderma* spp. and *Trametes* spp. demonstrated moderate to low tensile strengths, with significant variability between species and strains.
6. No consistent correlation was found between mycelium density and tensile strength. Similarly, despite some species having high chitin content, such as *I. lacteus*, this did not correspond to superior mechanical properties.
7. Chitin content alone is not a reliable predictor of mechanical strength of mycelium films. Other factors, such as glucan composition, hyphal diameter, and cell wall thickness, may play more influential roles in determining mechanical performance.
8. The mean surface area of the analyzed species ranged from 34.7% for *F. inzengae* to 45.2% for *T. versicolor*. Preliminary tests indicated no difference in mechanical performance based on the orientation of sample stamping relative to the growth direction of the mycelium. The mean thickness of the tested samples ranged from 0.13 mm for *G. applanatum* to 0.37 mm for *G. resinaceum*.

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Data Availability Statement

Data are available on request from the author.

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