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Assessing the Suitability of Two Tree Species for Mycelium-bound Composite Development Using Twelve White-Rot Fungi

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Abstract

Mycelium-bound composites are a promising alternative to conventional materials due to their biodegradability and sustainability. The fungal growth and colonization efficiency depend on substrates, which affects the feasibility of using different wood species as fungal growth media. This study investigated the growth performance of twelve fungal species (*Daedalea quercina, Trametes versicolor, Trametes suaveolens, Ganoderma sessile, Ganoderma adspersum, Ganoderma resinaceum, Ganoderma applanatum, Pycnoporus sanguineus, Pleurotus ostreatus, Fomes fomentarius, Stereum hirsutum, Fomitiporia robusta*) on European Beech (*Fagus sylvatica*) and Red Oak (*Quercus rubra*) substrates, using both falcon tube and bag incubation systems. Fungal growth was monitored over three weeks in falcon tubes to assess mycelial growth rates. Additionally, a bag incubation experiment was conducted. The final mycelium-bound materials were shaped using molds and then heat-treated.

The findings indicate that beech supports fungal growth more effectively than oak. Most species reach approximately 80 mm in three weeks. In contrast, oak exhibited limited fungal colonization. Only *Daedalea quercina* and *Stereum hirsutum* colonize successfully. Additionally, results from falcon tube experiments correlated well with bag incubation outcomes. It implies that small-scale screening can be used as a predictive tool for larger-scale composite production.

This study emphasizes the importance of substrate selection in mycelium-bound composite production. The superior fungal colonization on beech suggests that beech is a more ideal substrate for mycelium-bound composite applications. Future research should explore alternative hardwood substrates and mechanical properties of fungal composites to enhance their feasibility for sustainable material development.

Table of contents

List of Figures

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1. Introduction

Until now, we have been destroying our planet as if we had a spare one. UN Secretary-General António Guterres stressed the importance of curbing environmental degradation in his speech at the One Planet Summit 2021[1].

One of the biggest reasons for global warming is the growth of the global economy. Study shows that because of global population growth and rapid urbanization, global air pollution is expected to increase by 70%, from 2.1 billion tons in 2016 to 2.2 billion tons in 2025 and to 3.44 billion tons in 2050[2,3]. This waste mainly comes from the construction sector, agriculture, industry, and commercial centers[4].

Especially in the construction area, cement, concrete, metals, and polymers are usually used as conventional materials. These materials cost a lot of energy to produce, requiring complex equipment and manufacturing facilities, and processing costs are high. All these factors make it difficult to recycle and reuse the waste, which causes serious environmental pollution[5,6]. Moreover, conventional materials are not possible to degrade naturally; according to the report, it would take 100 years to fully degrade them[7].

Based on this situation, the EU has taken measures. Article 4 of the revised EU Waste Framework Directive (Directive 2008/98/EC) has defined five steps for waste management following a ranking based on the environmental impact, the “waste hierarchy.” The priority of the management is waste prevention. If pollution is produced, the first step is to reuse it, then recycling and recovery. Germany has already taken the “waste hierarchy” into its national law[8].

The conventional wood-based composite has been used widely in architectural and non-architectural areas, including exterior and interior siding and flooring. Composite furniture is usually made from wood or mixed materials, with thin reinforcements to enhance strength and durability. The selection of adhesive material plays an important role in binding wood-based composites[9].

Recent research is to integrate wood fibers into polymer composites because they have high mechanical properties and relatively low density. These fibers have a Young's modulus of 10-80 GPa and a tensile strength of 0.5-1.5 GPa, which make them a potential substitute for traditional fillers[10]. However, the hydroxyl groups in wood form strong bonds, bundling cellulose fibrils into larger fibers, which makes it difficult for

vegetable particles to be dispersed in polymeric matrices[10]. Further research into alternative bio-based materials, such as mycelium-bound composites, is needed.

The studies about the growth of white rot fungi in wood composites are still in their early stages. They mainly focused on comparing sawdust to other waste materials such as hemp, flax, flax waste, cork and straw, as well as different fiber processing methods, including loose, chopped, powdered, pre-compressed and bundled fibers[11].

Despite the limited research, some studies have demonstrated promising results. An experiment compared the growth of *Pleurotus ostreatus* and *Ganoderma lucidum* on beech and oak and concluded that the average compressive strength of the beech sawdust composite material was 2.49 MPa, and the longer the growth time of the fungus, the better the performance of the composite material. The composite material of *Pleurotus ostreatus* and *Ganoderma lucidum* has good waterproof performance, and there is no difference in water absorption between these two fungi and their combination of beech and oak[12]. However, the current study has not systematically measured white-rot fungal species on beech and oak. Whether there is a better combination of white rot fungi and sawdust is still unknown.

To address this challenge, this study investigates the growth of 12 different fungal species (*Daedalea quercina, Trametes versicolor, Trametes suaveolens, Ganoderma sessile, Ganoderma adspersum, Ganoderma resinaceum, Ganoderma applanatum, Pycnoporus sanguineus, Pleurotus ostreatus, Fomes fomentarius, Stereum hirsutum, Fomitiporia robusta*) on European Beech (*Fagus sylvatica*) and Red Oak (*Quercus rubra*) substrates, by systematically comparing their growth patterns on different wood types, this study evaluates the impact of substrate on fungal colonization and development.

2. Theoretical Framework

2.1. The Mechanism of Mycelial Network Formation

The term "Mycelium-bound Composite" derives from the mycelium, the filamentous network of the fungus that forms as it grow[7,13]. When fungi colonize a substrate rich in cellulose, hemicellulose, and lignin, they digest nutrients from the substrate and grow filamentous mycelium within it[11]. As the fungi expand, they form a dense, fluffy fungal layer inside and outside the substrate, called fungal skin[14]. After this substrate-fungus complex is heated at 65°C, the moisture is removed, and the organisms inside die[14,15]. At this stage, the mycelium is firmly bonded to the substrate, forming a lightweight and biodegradable material known as the mycelium-bound composite described above[16].

This property of fungi makes the mycelium a natural adhesive. Such materials with entangled lattices can be engineered to have desired properties such as hard, soft, porous, dense, corrosion-resistant, water-resistant, and gain mechanical strength[17].

2.2. Selection of Fungi

Fungi are mainly divided into three categories: white rot fungi, brown rot fungi, and soft rot fungi. Their classification depends on the type and amount of lignin-degrading enzymes they produce[18]. Among them, white rot fungi are known for their ability to completely degrade lignin and break down wood polysaccharides[19].

In addition, white rot fungi have a strong ability to colonize the substrate and have mycorrhizal, endophytic, and even pathogenic characteristics[20]. They can efficiently digest plant cell walls (lignocellulose) with their lignin-degrading enzymes, which also enables them to extract energy from the complex chemical structure of wood. This is why white rot fungi strains are the most used filamentous species in producing mycelium-bound composites[20]. Another key characteristic of white rot fungi is the white color of the fungal skin formed on the substrate, which can visually show their colonization and lignin degradation process[21].

Among white-rot fungi, *Ganoderma* spp. is widely used in mycelium-bound composites. They grow rapidly using organic waste as substrate. In addition, *Ganoderma* spp. can form dense, fibrous mycelial films, which can enhance the structural integrity of the composites[22].

However, using *Ganoderma* as composite fungi also faces some challenges. For example, it absorbs a large amount of water, has low tensile strength, and is easily contaminated, which limits its application. Most importantly, the potential molding risk of *Ganoderma* composites is high[22]. Because of these factors, researchers explored other fungal species that may provide better mechanical properties and environmental resistance.

According to current research, *Stereum hirsutum* grows well on wood substrates. It secretes a variety of glycoside hydrolases, including β-glucosidase, cellobiohydrolase, endoglucanase, endoxylanase, and laccase. These enzymes play a key role in lignocellulose degradation[23]. This suggests that *S. hirsutum* has a strong ability to degrade lignocellulose, which makes it a potential candidate for producing mycelium-bound composite materials.

Here are additional resources on various fungi. *Trametes versicolor* and *P. ostreatus* were successfully grown on beech (*Fagus orientalis*) blocks. Chemical analysis revealed that both fungi primarily degraded lignin, followed by cellulose and hemicellulose[24]. *Fomes fomentarius* is one of the most common white-rot fungi that colonize coarse wood and standing trees[25].

There is a lack of research on *Trametes suaveolens, Fomitiporia robusta, Pycnoporus sanguineus* and *Daedalea quercina*. This experiment provides valuable data on these fungi.

2.3. Selection of Feedstock

Fungi naturally grow on agricultural waste, such as straw, fibers, sawdust, and woodchips, and also on other inorganic substrates[10]. Appels et al.[26], Manan et al.[14], and Ghazvinian et al.[27] reported that the type of substrate affects the properties of the fungal skin formed after colonization of the substrate.

The interaction between white-rot fungi and different wood substrates is important to their decomposition efficiency and degradation patterns. A study shows that *Pleurotus ostreatus* and *Trametes versicolor* show different decay patterns on oak (*Quercus* spp.) and beech (*Fagus* spp.)[22]. *T. versicolor* followed a typical white-rot pattern on both kinds of wood and was able to effectively degrade the lignin. However, *P. ostreatus*

was more efficient in degrading lignin on beech, resulting in severe cell wall erosion, while on oak, it shows a soft-rot-like cavity formation instead of typical white-rot decay[22].

Both beech and oak have value as composite substrates. Both offer unique advantages: beech has a higher degradation efficiency, which can promote fungi to colonize the substrate more quickly, while oak provides a more durable structure because it is more resistant.

The reason why a cellulose-rich substrate is important is that the fungi colonize cellulose-rich environments quickly because cellulose is their main source of carbon and energy. Oak, with a cellulose content of 45% and beech, with 44-49% can serve as a good energy source for fungal growth[29,30]. Besides, higher cellulose content increases tensile strength, making the final material more durable[6,28].

To support the growth of mycelium, the substrate must provide the necessary nutrients, such as carbon, nitrogen, vitamins, minerals, and adequate moisture. Keeping mycelium in darkness can prevent fruiting bodies from forming[16].

3. Hypothesis:

Mycelium-bound composites have a wide range of uses in sustainable materials, but Fungal growth depends on the substrate, fungal species, and environmental conditions. Here are the proposed hypotheses based on past research:

Oak is known to have higher levels of tannins and lignin, with 25-30% compared to 22-24% in beech, which may inhibit fungal colonization[29,31,32]. On the other hand, beech is softer and has a lower chemical defense, which makes it easier for fungal growth.

If fungal species can effectively colonize a substrate under controlled conditions in a falcon tube under the same environmental conditions, it should also be able to expand at a larger volume, such as incubation in a bag.

Some fungi produce aggressive mycelial expansion, which may help them to outcompete pollutants, while others are more susceptible to microbial interference. Different resistance of fungal species to contamination affects their survival when incubated in bags.

4. Methods

4.1. Sample Preparation

4.1.1. Mycelium Cultivation

Initial fungal cultures were obtained from pre-existing agar plates available in the laboratory. These pre-cultures can be used as starting material for further fungal inoculations. Fresh fungal cultures were grown on potato dextrose agar (PDA) plates in a clean setup for bag inoculation and repeated experiments. Between these steps, flask cultivation was conducted.

To make the PDA plates, 39 g of PDA powder (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) was mixed with 1000 mL of deionized water, stirred well, and heated in an autoclave at 121°C for 20 minutes (VX-150, Systec GmbH, Germany). After sterilization, the liquid PDA was poured into 92 × 16 mm Petri dishes inside a clean airflow hood and left to harden.

After the PDA plates hardened, each one was inoculated in a clean setup using mycelium from existing agar cultures. A sterile scalpel or punch was used to cut a small piece of the colonized agar, which was placed in the center of a fresh PDA plate. The plates were sealed with parafilm to prevent contamination and kept at 90% relative humidity and 26°C in an HPP750eco climate chamber (Memmert, Germany). The plates were checked regularly to make sure the mycelium spread well.

Twelve fungal species were grown on two types of wood: beech (*Fagus* spp.) and oak (*Quercus* spp.). The experiment compared fungal growth on each wood type and examined differences in their ability to spread.

4.1.2. Substrate Preparation

Beech chips and oak chips are prepared to ensure a uniform size. They are processed into small 0.75mm chips that fit into the falcon tube. Each 15 ml falcon tube is loaded with either 5 grams of beech chips or 6 grams of oak. Oak is denser and has greater space between particles, while beech has more grain.

4.1.3. Autoclaving and Water Addition

To keep everything sterile, each falcon tube was weighed before sterilization. Water was added at a 1:1.2 ratio; 5 grams of beech were added to 6 ml of water, and 6 grams of oak were added to 7.2 ml of water. Water is added before autoclaving to prevent early contamination.

A VX-150 autoclave (Systec GmbH, Germany) was used to sterilize at 121°C for 20 min under liquid cycle conditions. After autoclaving, the tubes were placed in a sterile environment to cool before fungal inoculation. All steps were completed in a biosafety laminar flow room to maintain sterility. After cooling, the tubes were used for mycelial inoculation and growth measurements.

4.2. Inoculation of Falcon Tubes

To maintain sterility, all inoculation processes were performed under a laminar flow hood. Before starting, the sterile workbench was wiped with 80% ethanol. Tools such as punches and scalpels were dipped in ethanol and ignited to kill microorganisms. The process was repeated before processing each fungal sample to prevent cross-contamination.

Agar blocks were removed from the cultured fungi using a sterile punch. The agar blocks with hyphae were carefully transferred to a falcon tube using sterile forceps. After each use, the forceps were dipped in ethanol and flamed for sterilization. To ensure safety, only the lower two-thirds of the forceps were immersed in ethanol to prevent the flame from reaching the handler’s hands.

During placement, it was important to place the mycelium-covered side of the agar plug directly on the wood chips. This ensured even fungal growth and colonization. If the mycelium-free side faced the substrate, the fungi would take longer to establish, possibly slowing growth and causing inconsistencies between samples.

At the same time, fresh agar plates were inoculated with fungal cultures to maintain a steady supply of healthy mycelium for future liquid culture preparation. These plates were kept under controlled conditions to support optimal fungal growth before being used in later experiments, with a temperature of 26°C and relative humidity of 90%.

4.3. Growth Monitoring in Falcon Tubes

For the next three weeks, fungal growth was monitored regularly. Measure the distance from the mycelial tip to the lid at the same time each day (Figure 1). For each measurement, select four points and calculate their average to ensure accuracy. Growth rates on beech and oak were compared, and any issues like contamination or unusual colonization patterns were recorded.

Figure 1: Measuring the Daily Growth Length of Fungi

4.4. Bag Incubation Experiment

4.4.1. Preparation for Bag Incubation

Preparations for the bag culture experiment were carried out while measuring mycelial growth. This experiment was designed to study fungal colonization on larger substrates to simulate the practical application of biodegradable mycelium-bound composites. For consistency, all materials were accurately weighed and placed in sterilizable bags.

Each bag contained the following pre-measured materials:

- 150 g wood chips (Beech or Oak)
- 6 g gypsum ($CaSO_4 \cdot 2H_2O$) (to provide structural stability and regulate pH)
- 15 g potato dextrose broth (PDB) (to supply additional nutrients for fungal growth)

To avoid contamination, water was added, and autoclaving at 121°C for 20 minutes was done on the same day as inoculation. This kept the substrate sterile before adding the fungal culture. Adding water too early could cause bacteria or mold to grow, which might affect fungal colonization. Before inoculation, the bags were checked for contamination. Sawdust and additives were mixed well in each bag to help the fungus grow and spread during incubation.

4.4.2. Liquid Culture Preparation and Inoculation

A potato dextrose yeast (PDY) medium was prepared for liquid culture. A total of 1200 mL was needed to inoculate 12 fungal species, with two replicates for each species.

The medium was prepared in 50 mL Erlenmeyer flasks, using:

- 24 flasks × 50 mL ddH_2O = 1200 mL
- 24 g Potato dextrose broth (PDB)
- 5 g Yeast extract

Immediately after preparation, the medium was autoclaved at 121°C for 20 minutes. Flasks were left to cool overnight before inoculation.

Fungal mycelium was taken from the agar plates prepared on 4.1. (Sample Preparation). A sterile punch was used to extract five agar plugs for each Erlenmeyer flask. Each fungal species was inoculated into two flasks. The flasks were then incubated on a shaker at 26°C and 130 rpm for one week.

4.5. Bag Inoculation and Incubation

4.5.1. Final Autoclaving of Substrate Bags

Before the bag inoculation, water was added to the substrate bags. The bags were autoclaved at 121°C for 20 minutes to keep them clean and sterile.

4.5.2. Inoculation of Substrate Bags

The next day, liquid cultures were prepared for inoculation. Mycelium was blended and then added to the bags. The substrate was mixed well to spread the fungal culture evenly.

The bags were then placed in a climate chamber for incubation and further growth under controlled conditions: temperature 26°C and relative humidity 90%.

Figure 2: Mycelium Growth in Bags

4.6. Formation of the Mycelium-Wood Complex

4.6.1. Molding of the Complex

After the mycelium had grown inside and outside the substrate (Figure 2), the mycelium-wood complex was placed in a metal mold (15cm*5.2cm*2cm) to form the desired shape. Before this step, the plastic boxes (SAMLA 5L, IKEA, Delft, Netherlands) con-

taining the complex must be sealed and autoclaved at 121°C for 20 minutes. When placing the complex, contamination must be carefully avoided, as the mycelium is highly susceptible to contamination at this stage. The complex must be crushed into smaller pieces, as the clump complex can affect the molding process and final shape formation. Each complex must be kept separate to prevent cross-contamination between mycelium samples (Figure 3). The samples were incubated in a climate chamber under controlled conditions: temperature of 26°C and relative humidity of 90%.

The mycelium growth in the mold must be checked daily. Once the mycelium fully covers one side, the complex should be removed from the mold and flipped. It allows the other sides to be exposed to air, promoting the formation of fungi skin.

Figure 3: Complex Molding in Boxes

4.6.2. Drying and Hardening of the Mycelium Structure

After the fungi have grown on all six sides, they should be removed promptly and placed in an oven at a temperature of 65°C. This process removes moisture and kills the organisms, making it a harder structure that improves its tensile strength. The dried material should be taken out the next day on time.

5. Results and Discussion

5.1. Fungal Growth on Beech Substrate

5.1.1. Overall Growth of 12 Fungi on Beech Substrates

Fungal growth was checked daily to track colonization on different substrates. Observations started right after inoculation, focusing on early mycelial development. In the first few days, no visible changes were seen in the tubes.

By day 7, no significant visible growth was seen, but microscopic examination showed fine hyphae extending from the agar plugs into the substrate. This confirmed that fungal colonization had started, though slowly. To ensure accurate tracking, formal growth rate measurements began on day 7.

The tracking lasted for three weeks, with four measurements taken per tube each day. Figure 4 shows the growth data of 12 fungi on the beech substrate. It was generated by using Python. Each point represents the distance from the tube lid to the fungi tip, and the value is the average of four tubes from repeated experiments measured within a day.

Most fungal species grew well and consistently on the beech substrate (*Stereum hirsutum, Trametes versicolor, Pycnoporus sanguineus, Ganoderma applanatum, Ganoderma adspersum, Trametes suaveolens, Fomes fomentarius*), showing that beech substrate supported mycelial colonization. During the three-week incubation, fungi expanded steadily, with similar growth rates across samples, at a slope of 4.44. By the end of the experiment, the average mycelial growth for most species reached about 80 mm.

Ganoderma sessile and *Ganoderma resinaceum* showed the best growth among all tested fungi. Their mycelium spread quickly and covered most of the substrate within the first two weeks and nearly reached full growth by the final measurement. This rapid colonization suggests that these species may break down beech wood more efficiently, allowing them to absorb nutrients better.

However, some fungi (*Daedalea quercina, Pleurotus ostreatus, Fomitiporia robusta*) were not growing as well as the ones mentioned above. The growth pattern of the samples remained consistent, which means that factors such as moisture, nutrients,

and wood density had no significant effect on fungal development. This stability supports the reliability of beech as a material for producing fungal composites.

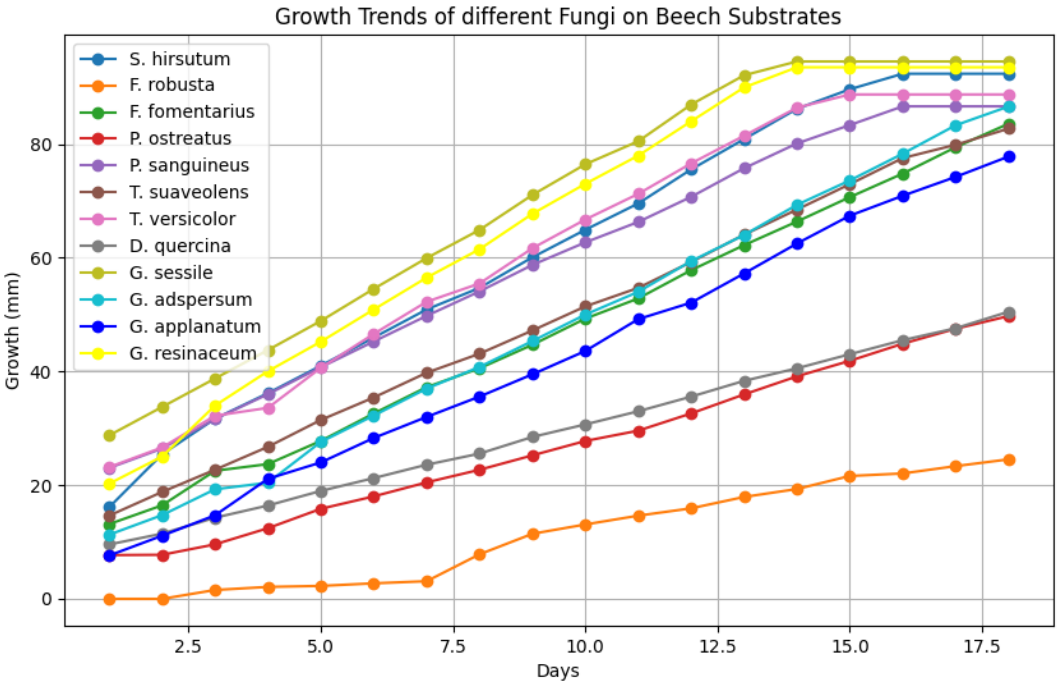

Figure 4: Growth Trends of Different Fungi on Beech Substrates

5.1.2. Variance Analysis for *T. versicolor*, *F. robusta*, *P. ostreatus* and *G. adpersum* on Beech

Figure 5 (left) displays the standard deviation (SD) for the growth of *T. versicolor*, *F. robusta*, *P. ostreatus,* and *G. adpersum* on beech substrates. The SD is presented in the form of error bars. The SD of *F. robusta* is large because there is only one tube showing fungal growth at the beginning. The SD decreased as the fungi in the other three tubes began to grow. By day 13, the fungi in all four tubes had stopped growing. For *P. ostreatus*, one tube had slower growth compared to the others, which increased the SD. The growth curve also shows the overall growth of *P. ostreatus*. was slower than other fungi. The SD of *G. adspersum* is also large because one of the four tubes showed slower growth. The best growth performance was observed in *T. versicolor*. These fungi grew quickly, and the variation between the four replicates was minimal.

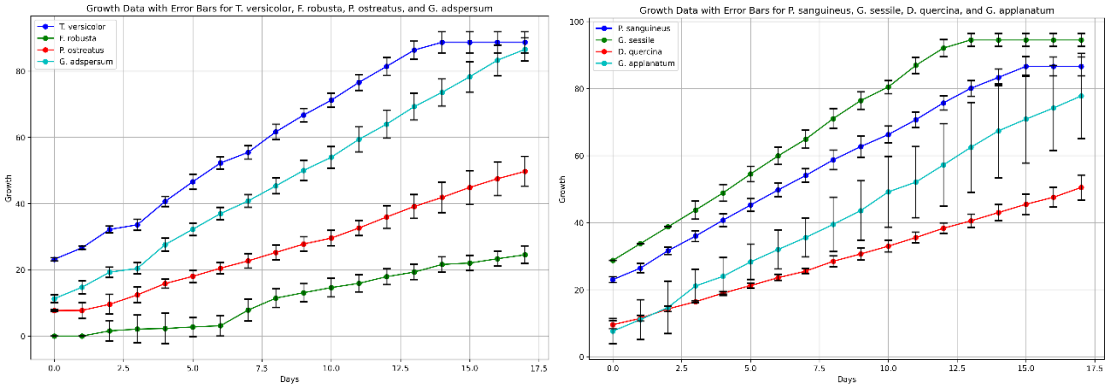

Figure 5: Growth Data with Error Bars for Different Fungi on Beech Substrates. (left) *T. versicolor, F. robusta, P. ostreatus*, and *G. adspersum* (right) *P. sanguineus, G. sessile, D. quercina,* and *G. applanatum*

5.1.3. Variance Analysis for *P. sanguineus, G. sessile, D. quercina* and *G. applanatum* on Beech

Figure 5 (right) displays the SD for the growth of *P. sanguineus, G. sessile, D. quercina* and *G. applanatum* on beech substrates. The SD of *G. applanatum* is particularly large because the fungus in one tube grew significantly faster than in the other three: it grew quickly beyond 40 mm, while the others remained around 30 mm. This may be due to a loosely distributed structure of wood chips in that tube, which provided a less resistant space for fungal growth. The SD of the other three species is within an acceptable range. This indicates that *P. sanguineus, G. sessile, and D. quercina* performed consistent growth under the same conditions.

5.1.4. Variance Analysis for *F. formentarius, G. resinaceum, T. suaveolens* and *S. hirsutum on Beech*

The SD for the growth of *F. formentarius, G. resinaceum, T. suaveolens* and *S. hirsutum* on beech substrates are displayed in Figure 6. The SD of *S. hirsutum* is large because one tube showed faster growth. The fungus in this tube had reached the bottom of the tube by day 12 and stopped growing. This made the SD decrease. The same

applies to m *G. resinaceum*, where one tube had already reached 95 mm, and the fungi in the other tubes had also grown to the end of the tubes, causing a decrease in SD.

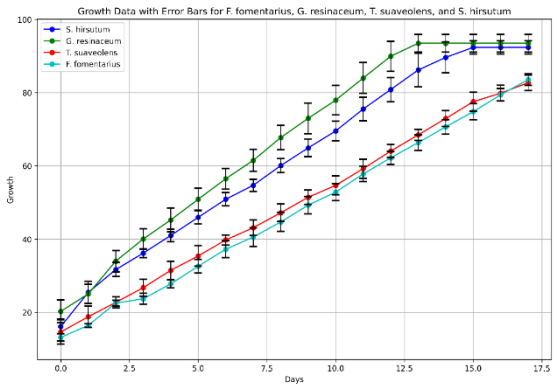

Figure 6: Growth Data with Error Bars for *F. formentarius, G. resinaceum, T. suaveolens,* and *S. hirsutum* on beech

5.2. Fungal Growth on Oak Substrate

5.2.1. Overall Growth of 12 Fungi on Oak Substrates

Unlike the beech, the oak substrates limited fungal growth. Several species, including *Fomitiporia robusta, Pleurotus ostreatus, Ganoderma adspersum, and Ganoderma applanatum,* showed no visible growth on oak substrate. However, there are two exceptions, *Daedalea quercina*, and *Stereum hirsutum*, which can adapt well to oak and successfully grow on sawdust. *Daedalea quercina* is a well-known white-rot fungus that breaks down hardwood. It grows steadily on the oak. Similarly, *Stereum hirsutum*, which naturally grows on decaying hardwood, was also able to colonize oak. It means that it can tolerate its chemical composition better than other tested fungi.

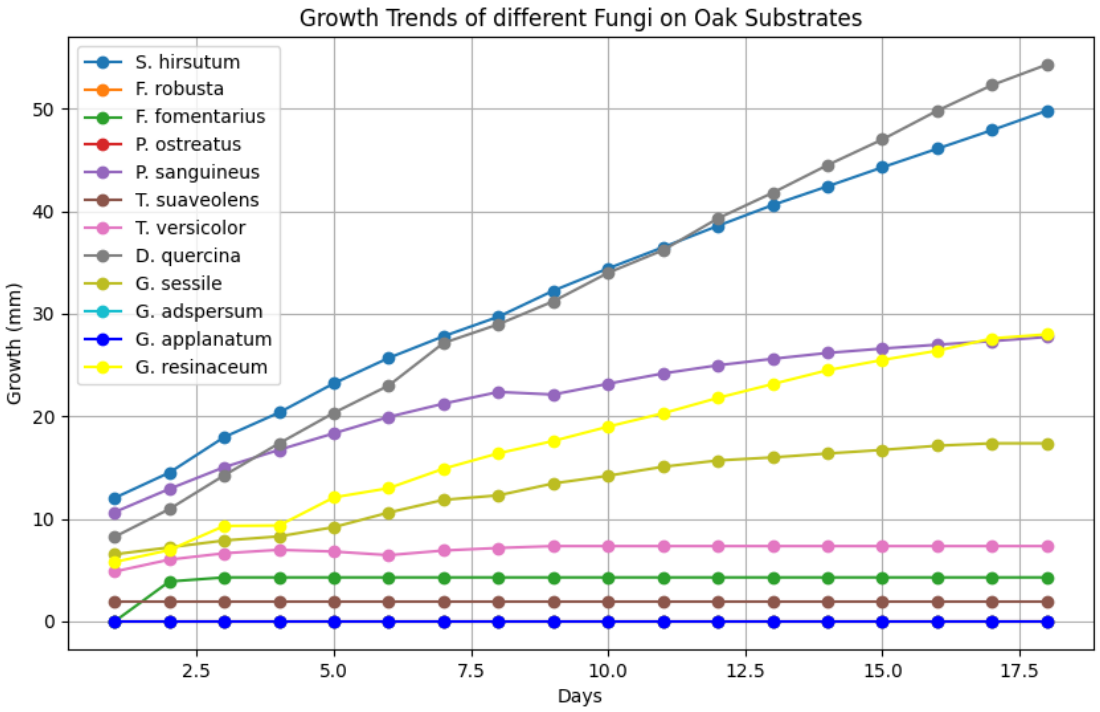

Figure 7: Growth Trends of Different Fungi on Oak Substrates

5.2.2. Variance Analysis for *P, sanguineus,T. versicolor, F. robusta, and P. ostreatus* on Oak

Figure 8 (left)displays the SD for the growth of *P. sanguineus, T. versicolor, F. robusta,* and *P. ostreatus* on oak substrates. The SD for the four tubes of *P. sanguineus* exhibits significant variation because the fungus in one tube grew slowly. By day 13, the growth rate of the fungus in all four tubes had slowed down. *T. versicolor* also shows considerable variation because one tube did not develop any fungal growth. However, since the growth length in the other tubes was relatively small, the error bars are not as pronounced as in *P. sanguineus*. And *F. robusta and P. ostreatus* showed no growth.

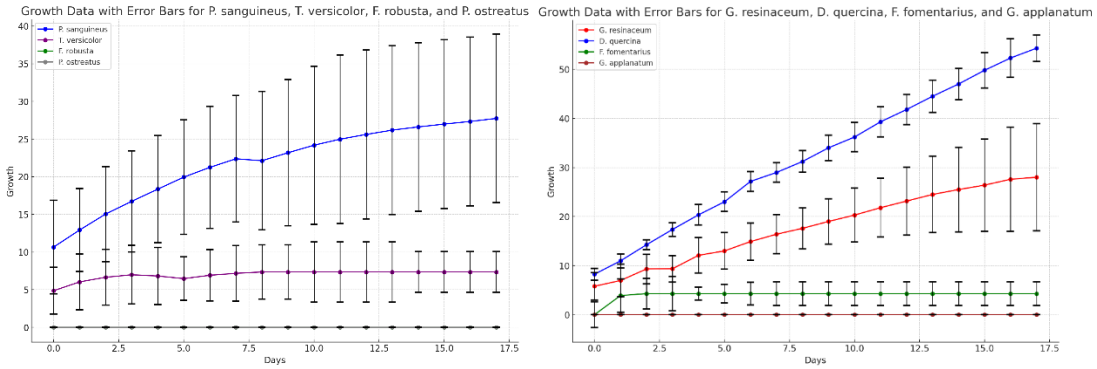

Figure 8: Growth Data with Error Bars for Different Fungi on Oak Substrate. (left) *P, sanguineus,T. versicolor, F. robusta, and P. ostreatus* (right) *G. resinaceum, D. quercina, F. formentarius*, and *G. applanatum*

5.2.3. Variance Analysis for *G. resinaceum, D. quercina, F. formentarius and G. applanatum* on Oak

The SD for the growth of *G. resinaceum, D. quercina, F. formentarius*, and *G. applanatum* on oak substrates are displayed in Figure 8 (right). The overall growth of *D. quercina* is fast, and there is significant variation among the four replicates. The SD for *G. resinaceum* is large because one tube showed no further growth after day 12, while the other three replicates continued growing, increasing SD. For *F. formentarius*, only one tube showed growth, while the other three showed no growth over the three-week period. *G. applanatum* showed no growth at all.

5.2.4. Variance Analysis for *S. hirsutum, G. adspersum, T. suaveolens, and G. sessile* on Oak

Figure 9 diaplays the SD for the growth of *S. hirsutum, G. adspersum, T. suaveolens, and G. sessile* on oak substrates. For *S. hirsutum* one tube grew faster than the others, resulting in an increased SD. Similarly, for *G. sessile*, one tube initially showed little growth, which caused a larger SD. As it started to thrive, the SD decreased, but by day 12, this tube stopped growing at 12.3 mm and reduced the SD. In the case of *T. suaveolens*, only one tube showed growth, while *G. adspersum* exhibited no growth at

all throughout the three-week period. Despite these variations, the SD values remained within an acceptable range.

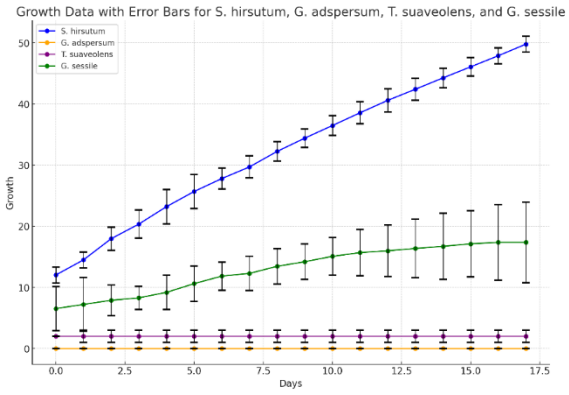

Figure 9: Growth Data with Error Bars for *S. hirsutum, G. adspersum, T. suaveolens and G. sessile* on Oak

5.3. Comparison Between Beech and Oak

The result above indicates that compared to oak, beech is a more suitable substrate for fungi growth. Most fungi grew steadily and consistently on beech, while the fungi struggled to grow on oak. This situation may be due to oak's high lignin and tannin content[29,31,32]. These substances have antifungal properties that can block the decomposition action of enzymes, affecting fungal colonization and growth. Only fungi with specialized enzyme systems, such as *Daedalea quercina* and *Stereum hirsutum*, can overcome these limitations and successfully colonize.

Based on the SD analysis, the main reason for the high SD in fungal growth on beech is that one of the four tubes grew significantly faster than the others. This suggests that minor variations in microenvironmental conditions (e.g., moisture distribution, aeration, or fungal inoculum density) could influence fungal expansion rates. In contrast, the high SD on oak is mainly due to one tube showing no growth or growing much slower than the others. And for some fungi, only one falcon tube exhibited growth. This result gives a more detailed analysis of the growth on beech and oak, indicating that beech substrates can provide a more ideal condition for fungi growth than oak substrates.

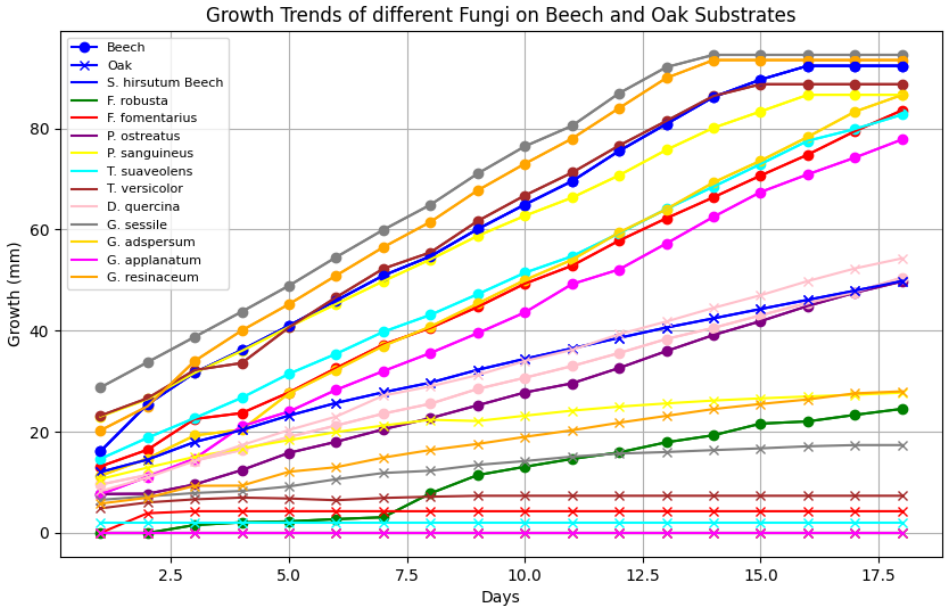

Figure 10: Growth Trends of Different Fungi on Beech and Oak Substrates

5.4. Performance in Bag Incubation Experiment

The bag incubation experiment provided a visual representation of fungal growth on different substrates and supported the results of the tube experiment. A key observation was that fungi that grew well in tubes also thrived in bags. It shows a clear link between fungal growth in tubes and bags, which means that if the fungi can grow in a smaller volume with a controlled setup, they can also grow in a larger system.

Ganoderma sessile and *Ganoderma resinaceum* grew the fastest on beech in tube experiments, also performed well in the bags, and later formed a stable shape (Figure 11). This result supports previous research findings[22]. And even though *Ganoderma* spp. has consistently shown susceptibility to contamination[22], no mold was observed in the bags of *G. resinaceum* and *G. sessile*. This may be because these two fungi grew rapidly, suppressing mold growth. Similarly, *Stereum hirsutum*, which grew best on oak in the tube, also showed strong colonization in bags (Figure 11). Moreover, *T. suaveolens* and *T. versicolor* also performed well in both experiments. The results of *S. hirsutum* and *T. versicolor* are consistent with previous research findings[23,24]. This shows that fungi adapt to specific wood types maintain similar growth patterns in different en-

vironments. It makes *G. sessile*, *G. resinaceum,* and *T. versicolor* on beech and *S. hirsutum* on oak potential use for developing mycelium-bound composites.

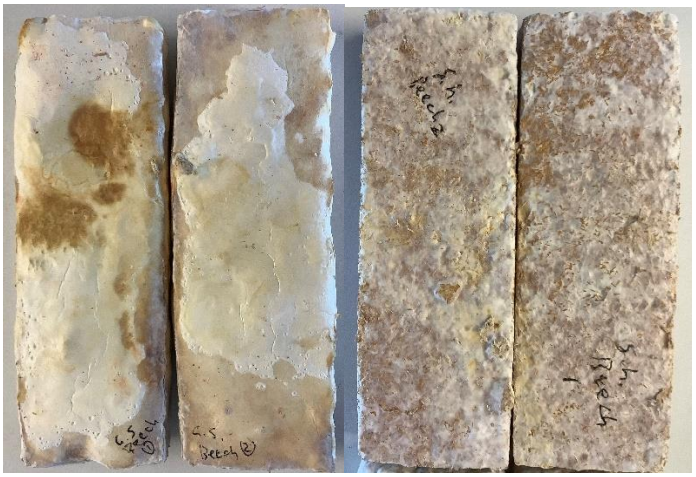

Figure 11: (left): Dried *G. sessile* on Beech Substrate (right): Dried *S.hirsutum* on Oak Substrate

G. adspersum on oak and *G. applanatum* on beech exhibited mold problems. Based on previous experimental results, *Ganoderma* spp. has been susceptible to contamination[22]. Since *G. adspersum* and *G. applanatum* did not grow as quickly in the tube experiments as *G. sessile* and *G. resinaceum*, this may have provided space for mold to develop. This indicates that these two fungi are not suitable for composite production unless the contamination issue can be resolved.

Some fungal species that showed little or no growth in tube incubation also failed to colonize the substrates in bags; for example, *Fomitiporia robusta* and *Pleurotus ostreatus* on both substrates showed little growth in tubes also struggled to grow in bags. This result of *Pleurotus ostreatus* differs from previous research findings[26]. Perhaps *P. ostreatus* requires a longer time to grow properly, as the bag inoculation lasted only two weeks. The limited growth of these species suggests that beech and oak may not be ideal substrates for these fungi. They might lack the necessary chemical composition or structure for mycelial expansion.

Interestingly, *Ganoderma applanatum* struggled on oak in tube incubation grew better in bags. The larger surface area and better aeration in bags may have created a more ideal environment, which provided the fungi with better access to air and nutrients and formed a stronger structure. This result suggests that it may be better for bulk cultivation rather than in a limited space.

However, contamination was severe, especially in the bags, where it was more visible. For example, *Daedalea quercina* showed small green spots in the tubes, but due to poor airflow, the mold did not spread to the mycelium. In contrast, in the bags, mold almost completely suppresses fungal growth. Even after redoing the bag inoculation with fresh agar plates, contamination persisted.

After molding and baking, most of the fungi (*Trametes suaveolens, Trametes versicolor, Ganoderma sessile, Ganoderma resinaceum, Stereum hirsutum, Pycnoporus sanguineus*) on beech and oak; *G. adspersum* on beech, *G. applanatum* on oak successfully formed mycelium-bound complex (Figure 11). These findings confirm that certain fungi have the ability to form cohesive mycelial networks, which can be used for biodegradable material production. After drying, the length was 14.1 ± 0.1 cm, the width was 4.9 ± 0.1 cm, and the thickness was 2 cm. Compared to the pre-heating size (15 cm × 5.2 cm × 2 cm), this indicates that longer samples are affected by dehydration. The degree of shrinkage was independent of the fungal-wood combination.

However, some fungi (*Pleurotus ostreatus, Daedalea quercina, Fomitiporia robusta, and Fomes fomentarius*) did not form composites on either beech or oak due to unsuccessful colonization and mold issues. *Ganoderma adspersum* failed on Oak, *Ganoderma applanatum* failed on beech due to mold contamination. This can further confirm that these wood types are not suitable for the growth of these fungi.

Figure 12: (left): Dried *Trametes suaveolens* on oak (right): Dried *Pycnoporus sanguineus* on oak

5.5. Limitations and Future Research

Considering the contamination problem of *D. quercina*, it is necessary to maintain a strict sterile environment during culture preparation and inoculation. For example, ensure that the fungi stain is not contaminated or implement a grain spawn method instead of direct inoculation. Future work could also explore mechanical testing of the composites to evaluate how fungal colonization influences material strength, durability, and water resistance.

6. Conclusion

This study investigated the growth of the twelve fungal species on beech and oak substrates, their performance in falcon tubes as well as bag incubation. The result implies that beech is generally more ideal for fungal colonization than oak, as most fungi species showed higher and consistent growth rates on beech and successfully formed a stable structure after molding and heating. On the contrary, oak restricted fungal growth. *Trametes suaveolens, Trametes versicolor, Ganoderma sessile, Ganoderma resinaceum, Stereum hirsutum, Pycnoporus sanguineus* on beech and oak substrate; *G. adspersum* on beech, *G. applanatum* on oak successfully formed mycelium-bound complex. Their combinations show a potential use for developing mycelium-bound composites. This work contributes to the knowledge about growth data of twelve white rot fungi on beech and oak substrate and confirms the importance of substrate species selection for mycelium-bound composites production.