Comparison of Fungi with Sclerotia as Sustainable Materials for Product Design

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Abstract. Due to the increasing problems related to environmental pollution, sustainable and biodegradable materials gained importance. Fungi are among the most promising sources as biomaterials and have recently been used in product design. Sclerotia are compact hyphal masses produced by fungi under certain conditions, especially for survival. In this study four sclerotia forming fungi; *Botrytis cinerea*, *Laetisaria arvalis*, *Macrophomina phaseolina* and *Rhizoctonia solani* were compared in terms of features of sclerotia and possible usage as sustainable materials. Fungi were grown on a solid medium (potato dextrose agar) for two weeks and sclerotia formations were compared by daily observations. *L. arvalis* and *M. phaseolina* had smaller sclerotia homogeneously scattered all over the medium surface, while *B. cinerea* and *R. solani* formed sparse and bigger sclerotia. Shapes, colors and hardness of the sclerotia were also different from each other. Like fungal mycelia, sclerotia with more resistant structures may also be used as biomaterials in product design.

1 Introduction

Environmental problems caused by plastic waste, mostly from packaging, are becoming huge worldwide. These problems led to an increase in research and public interest in developing sustainable and biodegradable materials due to the opportunities that biomaterials provided for renewability within the past few years. Different polymers derived from different sources, such as alginate, carrageenan, cellulose, chitosan, collagen, fungi, and various starches, can be given as examples of the materials that get more attention with the advances in biotechnology and the need to develop more sustainable alternatives for a better future [1-3].

To address environmental problems, designers collaborate with scientists to work on biofabrication and *'grow'* design products, to change the reliance of traditional production methods to exhaustable natural sources [4]. "Growing Materials" produced with living organisms are a topic emerged to support this stance to use more sustainable materials and production methods. According to Karana and colleagues [5], the "Growing Design" concept involves creating materials and products derived from living organisms. The opportunity of novel aesthetics, biodegradability, and the experience of co-creating with nature makes growing materials crucial for the future [6]. While various organisms can be utilized in "Growing Design", such as chitin and protocells, mycelium (fungal materials), bacteria, and algae are particularly favored and commonly employed by designers in recent years. In "Growing Design", designers generally guide the organisms to grow and create a material, and in some other cases, designers experiment with different shape possibilities by directly growing materials into a desired product [2].

Fungi are the second species-richest group after insects exist in various habitats and have many functions important for the continuity of ecosystems and the food chain [7]. They are eukaryotic organisms that have various relationships with other living organisms. Some of them are mutualists and live with other organisms. Mycorrhiza is a kind of mutualistic relationship between fungi and plant roots, and fungi help plants to obtain nutrients and water from poor soils, also to tolerate biotic or abiotic stress factors. Lichens are another examples of mutualism between fungi and algae, which cause the formation of soil from rocks. Some fungi are parasites feeding on living plants or animals and causing diseases on them, while a large group of fungi are saprobes obtaining nutrients from dead organisms. Especially saprotrophic fungi are important decomposers responsible for the carbon cycle and continuation of the life on Earth. They are also beneficial for humans with their usage in agriculture as biocontrol agents, food and chemical industry as producers of some essential chemicals like enzymes, hormones, antibiotics or vaccines [8].

With their unique properties and abilities, fungi have garnered considerable interest in various fields, including biotechnology, architecture, and environmental sustainability. Fungi are one of the most promising sources of such materials due to their ability to grow on various substrates and produce a wide range of compounds with functional properties. Fungi-based

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materials can be used in product design to create eco-friendly and innovative solutions to various problems. For example, mycelium-based materials have been used to create biodegradable packaging, furniture, and building materials. These materials have unique properties such as high strength-to-weight ratios, good insulation properties, and the ability to grow into complex shapes [9].

1.1 Use of fungi in product design

Mycelium, a densely interconnected network of thread-like hyphae, serves as the vegetative component of fungi and plays a crucial role in the creation of fungal materials, which exhibit unique properties [10]. These mycelial materials can be utilized in two primary forms: as composites or as pure mycelium. In the case of mycelium composites, fungi are cultivated in conjunction with other substances, resulting in the formation of bulk materials. On the other hand, pure mycelium is grown in a liquid culture [6].

For mycelium composites, a wide range of organic elements can be employed as substrates, providing necessary nutrients for mycelium growth. It is important to note that the choice of substrate significantly impacts the final material, influencing both its technical properties and experiential qualities [6]. By carefully selecting the substrate, researchers and designers can tailor the mycelium composite to achieve desired characteristics and functionalities, opening up new possibilities in material development.

Both pure mycelium and composite are affected by factors like strain type, substrate type, mycelium growth conditions, incubation time, additives used, fabrication methods, and types of inoculum used. Change in these conditions can differ the strength, elasticity, density, intensity, and color of the fungi [10]. According to these factors and the nature of the fungi, mycelium-based products have been used for a decade as synthetic leather, kitchen utensils, packaging items, furniture, building materials as composites molded into different shapes or as pure mycelium according to their hydrophobic and thermal resistance properties [11].

1.2 Fungi with sclerotia

Fungi that show promise for materials science are those that produce sclerotia. Sclerotia are compact masses of hyphae that various fungi produce under certain environmental conditions. They contain some storage materials such as glycogen, protein and lipid, that fungi can use to regrow. Only a small group of fungi belonging to Ascomycota and Basidiomycota phyla can produce sclerotia. Types, colors, shapes and dimensions of sclerotia varies depending on the species and growth conditions. At the beginning they are light colored, but they become darker in time. Some fungi like *Rhizoctonia* species form loose type sclerotia on the surface of the growth substrate, with loose hyphae on the outer layer and more complex hyphal strands in the inner part. Another type of sclerotia formed by gray mold pathogen of agricultural crops, *Botrytis cinerea* are made by dichotomous branched hyphae with cross-wall formation. It has a rind with several dark colored rounded cells and center part with loose hyphal filaments. Sclerotia have been shown to possess unique mechanical, thermal, and chemical properties [12]. These properties make them a promising candidate for developing new materials with various applications in product design. This study seeks to contribute to the growing knowledge of new biological materials and their use in product design.

2 Material and Method

In the study, isolates of four sclerotia forming fungi, kept in the culture collection of the Mycology laboratory of Plant Protection Department of the Faculty of Agriculture, Isparta Applied Sciences University, Isparta, Türkiye, were used; *Botrytis cinerea* Pers., *Laetisaria arvalis* Burdsall, *Macrophomina phaseolina* (Tassi) Goid and *Rhizoctonia solani* Kühn. *B. cinerea* is known as the agent of gray mold disease, affecting more than 1400 plant species and causing economically important yield losses [13]. *L. arvalis* is a soil-inhabiting fungus known as a hyperparasite of *R. solani* and some other plant pathogens [14]. *M. phaseolina* is a common plant pathogen causing charcoal rot on more than 500 plant species all over the world, especially under high temperatures and low soil moisture [15]. *R. solani* is also a common and destructive plant pathogen causing diseases on many agricultural crops [16].

Fungi were grown on solid medium (Potato dextrose agar-Merck) according to the instructions given by the manufacturer. The medium was sterilized for 20 minutes in an autoclave, and poured into 9-cm-diameter Petri dishes. Fungal cultures were incubated at 22±2 ºC in the dark for four weeks and observed periodically and photographed, to understand the sclerotia formation process. Three replicate Petri dishes were used for each species.

The formation of sclerotia was a focal point of the study, as it is a difference in the texture of the fungi, which can make it a potential material. During the study, four species of fungi were compared for their respective abilities to form sclerotia. Each species was meticulously monitored and evaluated for intensity, size, sclerotia formation velocity, and patterns. Through record-keeping and photograph comparisons, it is aimed to uncover patterns and variations in sclerotia development among the species. After four weeks of observation, one replicate of each species was dried at 60 ºC in an oven for two hours and checked for the change of their structures. In addition to the determination of the formation and intensity of sclerotia, the study also aimed to explore potential design ideas that could incorporate these fungi. The innovative concepts and potential applications that could leverage the unique features of fungi with sclerotia are considered, and design concepts that use these fungi are prepared.

3 Results and Discussion

All four fungi used in the study covered the agar surface with mycelia in the first week and formed sclerotia in a few weeks. Their sclerotia had different shape, colour, texture and distribution patterns. *B. cinerea* formed black, irregularly shaped sclerotia about 2-8 mm long. Bigger ones were in the middle of the medium and the others were scattered around mainly on the border of the medium (Figure 1). They were soft and elastic, but became very hard when dried, like small stones. It is known that melanin pigment, responsible for the black outer colour of the sclerotia, had a stress protectant function and ensured the resistance of the fungus against extreme temperatures, desiccation, UV irradiation and toxic chemicals [13]. This feature may allow the material made from *B. cinerea* sclerotia to withstand similar stress conditions.

Figure 1. Sclerotia of *Botrytis cinerea* formed on potato dextrose agar.

L. arvalis formed small sclerotia of about 0.4-0.6 mm in two weeks. Their colour was lighter at the beginning, became reddish brown in two replicates, and dark brown in the other (Figure 2). They were scattered on agar surface but more intense at the edges of the dishes. When dried they turned into a leather-like structure together with the agar.

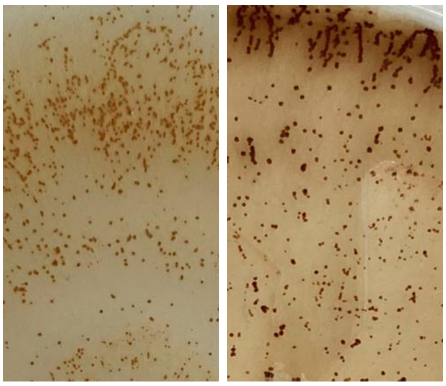

Figure 2. Two different colored sclerotia of *Laetisaria arvalis* formed on potato dextrose agar.

M. phaseolina sclerotia were dark brown when young, became black with age, spherical or oblong, very intense and homogeneously scattered on the agar surface (Figure 3). During drying process, these sclerotia lost their moisture rapidly and became a paper-like unflexible material. It was mentioned that the sclerotia allowed this plant pathogen to survive up to 15 years in soil [17].

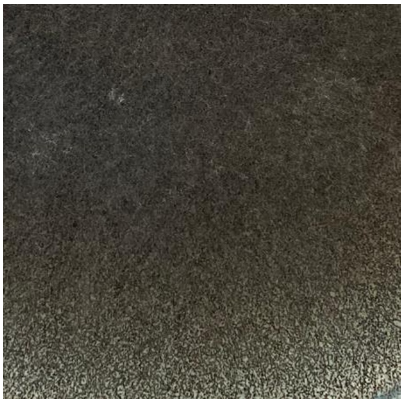

Figure 3. Sclerotia of *Macrophomina phaseolina* formed on potato dextrose agar.

R. solani produced tan to dark brown, irregular shaped sclerotia, some too small to see, some others about 6 mm diameter and they coalesced into crusts (Figure 4). They were known to be formed from barrel-shaped cells that are thicker than the ordinary mycelium cells. It was determined that this fungus formed larger and intense sclerotia on glucose-rich media [18]. For some *R. solani* isolates phosphorus and magnesium amounts of the medium changed the number and weight of sclerotia [19].

Figure 4. Crusts of *Rhizoctonia solani* sclerotia formed on potato dextrose agar.

These four fungi species had different growth speeds. *R. solani* grew faster than the others and spread all over the agar surface in four days, but *M. phaseolina* started sclerotia formation earlier than the others. However, in all fungal colonies sclerotia initials formed after one week incubation and in three weeks all sclerotia matured (Figure 5). Since the sclerotia of *B. cinerea* and *R. solani* were sparse, they may better be picked and used together with some other materials like plant waste to form mycelium composite. *M. phaseolina* and *L. arvalis* sclerotia were small and intense, so they can be used as a leather-like material as pure mycelium.

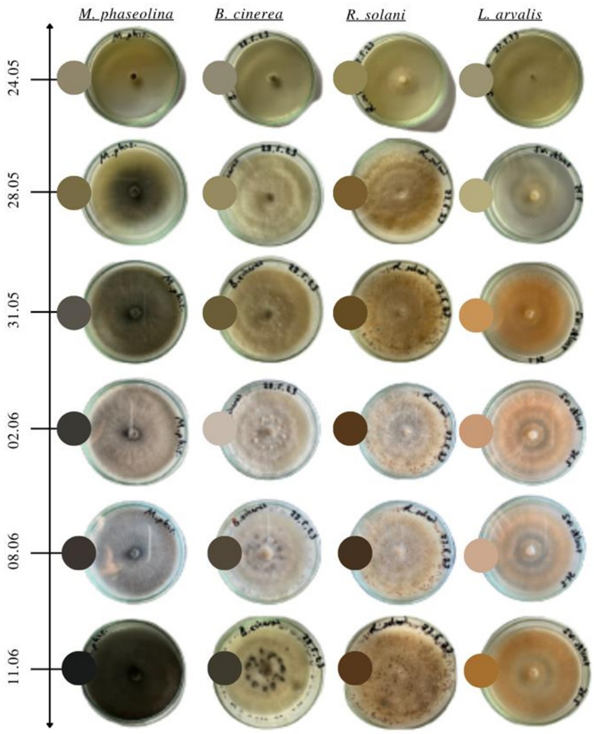

Figure 5. Growth and sclerotia formation of *Macrophomina phaseolina*, *Botrytis cinerea*, *Rhizoctonia solani* and *Laetisaria arvalis* on potato dextrose agar in three weeks.

The growth speed and sclerotia formation varied among the fungal species. Based on the characteristics of the sclerotia, different design concepts were proposed as disposable tableware, composite wall panels, and decorative blinds. Some examples of the usage of such fungi are illustrated in Figure 6 and Figure 7. In Figure 6, *M. phaseolina* or *L. arvalis* are thought to be used as an alternative to disposable plastic tableware. The design concept is to grow fungi in an incubation machine where they will be inoculated into the mold, grow into that shape, and be ready to use after it is dried. In Figure 7, the composite fungi material as wall panel to insulate heat and sound and as a decorative blind is shown. *B. cinerea* and *R. solani* are thought to be used in the wall panel to enhance the insulation properties of the material.

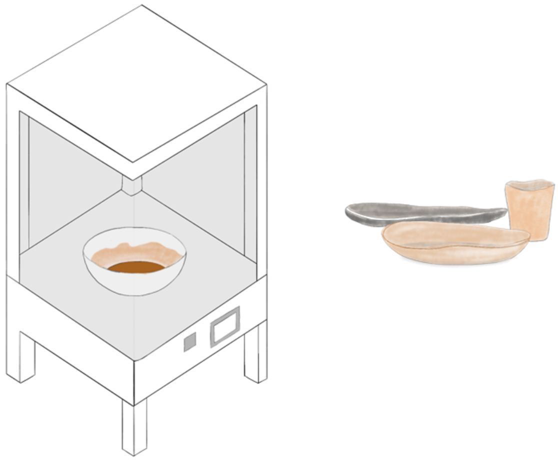

Figure 6. Concept design for possible application of fungi with sclerotia as disposable tableware.

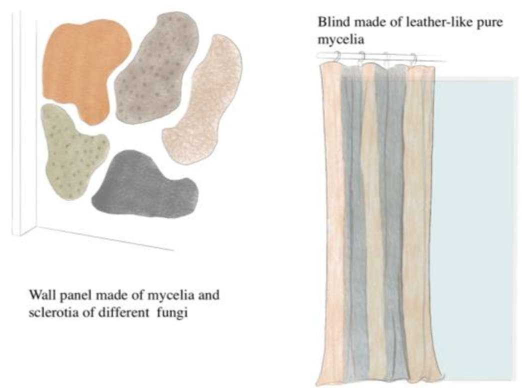

Figure 7. Concept design for possible application of fungi with sclerotia as wall panel and blind.

Up today, research and applications have mostly focused on mycelium or macro fungi. One of the first examples are composite mycelium materials (CMM) developed mostly with saprotrophic species, inoculated into waste materials originated from agriculture or forestry. In these types of materials, fungi act as a glue, binding the loose substrate particles. The resulted foam-like material is used in packaging, insulation and lightweight furniture applications [20]. Recently, new methods have emerged that involve cultivating the fungi as a flexible layer over a liquid or solid surface. After undergoing post-production treatments, these materials consist of fungal tissue and exhibit properties similar to textiles, leather or foam. They are suitable for various consumer applications, such as clothing, bags, furniture, and more [21]. With recent advancements in technology, 3D printing has emerged as an alternative approach for biofabrication which offers novel form and shapes, making mass production economically feasible. Even early prototypes were not very adaptable to everyday life, with further research and development 3D printing can be used as a production method for fungi based materials [4].

The utilization of fungi in design applications have been relatively limited thus far. However, the inclusion of sclerotia presents a novel approach with the potential to significantly enhance material durability and offer positive attributes to sustainable product design. Sclerotia contain storage materials like glycogen, protein, and lipid, which can contribute to the mechanical, thermal, and chemical properties of the materials [22]. These unique characteristics may result in increased resistance against stress conditions, leading to longer material lifespans and opening up new possibilities for sustainable design solutions. By harnessing the resilience and beneficial properties of fungi with sclerotia, innovative and eco-friendly materials can be developed to address current environmental challenges.

4 Conclusion

The growing global concern over plastic waste and its environmental impact has led to a surge in research and public interest in developing sustainable and biodegradable materials. Biomaterials derived from various sources, such as fungi, have emerged as promising alternatives to traditional materials in product design. Fungi-based materials offer unique properties that make them attractive for various applications, including packaging, furniture, and building materials. This study focused on fungi that produce sclerotia, compact mass of hyphae containing food reserves, as potential materials for product design. Four sclerotia-forming fungi, *B. cinerea*, *L. arvalis*, *M. phaseolina*, and *R. solani*, were examined for their ability to form sclerotia and their characteristics.

Fungi with sclerotia show great potential for developing innovative and sustainable materials in product design. By harnessing the unique properties of these fungi, designers can create eco-friendly and functional products that contribute to a more sustainable future. Further research and exploration in this field, together with mycology and biotechnology, can uncover new possibilities and applications for fungi-based materials, paving the way for a greener and more responsible approach to product design and manufacturing.

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