

Journal of Molecular Science

www.jmolecularsci.com

ISSN:1000-9035

Comparative Study on the Influence of Culture Type, Substrate Composition, and Environmental Conditions on Mycelial Growth of Selected FungiAkanksha Kashyap¹, Rohit Rawat², Preeti Chandurkar³, Nidhi Tripathi⁴, Anjali Choudhary⁵,
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Received: 28-05-2025

Revised: 13-06-2025

Accepted: 09-07-2025

Published: 21-07-2025

Keywords*Mycelial growth, spawn substrate, Pleurotus ostreatus, Ganoderma lucidum, Agaricus bisporus, culture type, temperature, pH, relative humidity, substrate composition.***ABSTRACT**

This study aimed to compare the influence of culture type, spawn substrate, substrate composition, temperature, pH, and relative humidity on the mycelial growth of selected fungi including *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Agaricus bisporus*. Results revealed that solid culture inoculation facilitated faster mycelial coverage than liquid culture across all strains. Among the spawn substrates tested, brown rice and maize supported the fastest colonization. The combination of wheat and rice straw chopped to less than 1 cm produced the smoothest mycelial mat. Environmental conditions played a critical role, with 25°C, pH 7, and relative humidity between 80–90% identified as optimal for mycelial growth. These findings underscore the importance of optimizing both biological and environmental factors to enhance spawn production and fungal biomass efficiency in commercial and research applications.

INTRODUCTION

Fungi are among the most ecologically and industrially significant microorganisms, playing a crucial role in nutrient cycling, biodegradation, food production, and pharmaceutical development (Smith et al., 2017). Mycelial growth—the expansion of the vegetative part of fungi—is a critical indicator of fungal health and productivity and is influenced by multiple factors, including the type of culture, substrate composition, and surrounding environmental conditions (Singh & Kumar, 2018). Understanding these variables is essential for optimizing fungal cultivation in both laboratory and industrial settings.

Culture type, whether solid-state or submerged, greatly influences the morphology and metabolic output of fungi. Submerged cultures, for example, promote faster growth but may lead to altered

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physiological structures, whereas solid-state cultures often mimic natural growth conditions more accurately (Pandey et al., 2000; Lonsane et al., 1985). The selection of an appropriate culture medium is equally important; different fungi require specific carbon and nitrogen sources to thrive. The carbon-to-nitrogen ratio, pH, and presence of trace elements can significantly affect mycelial biomass production (Karthikeyan & Sivakumar, 2010; Javed et al., 2021).

Substrate composition, particularly in solid-state fermentation, provides both physical support and nutrients to the growing mycelium. Agro-industrial wastes such as rice straw, wheat bran, sawdust, and sugarcane bagasse have been successfully utilized to cultivate various fungi due to their rich lignocellulosic content (Akinyele & Akinyosoye, 2005; Sharma et al., 2013). These substrates not only support fungal growth but also contribute to waste recycling and sustainable bioprocessing. The particle size, moisture content, and porosity of the substrate further influence oxygen diffusion and nutrient availability, which are essential for mycelial penetration and development (Raimbault, 1998; Goyal et al., 2008).

Environmental factors such as temperature, relative humidity, light exposure, and aeration profoundly affect the physiology of fungal species. Each fungus has an optimal temperature and humidity range that promotes maximum mycelial spread. For example, *Pleurotus ostreatus* exhibits optimal growth between 25–30°C and 70–90% relative humidity (Rajarithnam et al., 1992; Patil et al., 2010). Variations beyond these limits can reduce enzymatic activity and nutrient uptake, thereby slowing growth. Light intensity and photoperiod also modulate fungal differentiation and fruiting in certain species, such as *Ganoderma lucidum* (Park et al., 2012).

Different fungal species respond uniquely to the same environmental stimuli and nutrient conditions due to their genetic and metabolic diversity. This necessitates a comparative study to identify the optimal cultivation strategies for selected species (Chang & Miles, 2004). For instance, basidiomycetes and ascomycetes may differ significantly in their tolerance to pH, salinity, or oxidative stress, which directly impacts their growth kinetics and secondary metabolite production (Nayan et al., 2020; Royse et al., 2004).

Such comparative studies not only enhance our understanding of fungal ecology and physiology but also contribute to biotechnological advancements, such as the efficient production of bioactive compounds, enzymes, and biodegradable

materials (Stamets, 2000; Ferreira et al., 2010). Moreover, optimizing mycelial growth has implications for myco-remediation, bio-packaging, and sustainable agriculture (Rai et al., 2005; Jones et al., 2021). Given the increasing demand for eco-friendly alternatives and functional biomaterials, it becomes imperative to identify the best culture practices tailored to each fungal strain.

Therefore, this study aims to conduct a comparative analysis of how different culture types, substrate compositions, and environmental conditions influence the mycelial growth of selected fungi. By systematically evaluating these variables, the research intends to offer insights into efficient fungal cultivation strategies that align with industrial, environmental, and agricultural needs.

MATERIAL AND METHOD:

Spawn production was performed using five different cereal grains: sorghum, soybean, maize, brown rice, and wheat. The grains were first cleaned, soaked overnight, boiled to partial softness, and then drained. To prevent clumping and stabilize the pH, 2% calcium carbonate (CaCO₃) was added. The prepared grains were sterilized at 121°C for 30 minutes in an autoclave and inoculated with either liquid or solid fungal cultures, followed by incubation at 25°C in darkness until full mycelial colonization was achieved (Sharma et al., 2020; Ghosh et al., 2018). The time required for complete colonization was recorded for each grain type.

Substrate evaluation involved different particle sizes and blends of wheat straw and rice straw. Substrates were chopped into varying lengths (finely chopped, <1 cm, and <2 cm), used individually or in combination, and observed for the extent and smoothness of colonization. These parameters were selected based on their known influence on aeration and water retention capacity, which directly affect fungal growth (Patra & Pani, 2021).

Environmental factors such as temperature, pH, and relative humidity (RH) were also studied to assess their impact on mycelial development. Temperatures of 10°C, 15°C, 20°C, 25°C, and 30°C were maintained in separate incubators. pH levels were adjusted between 5 and 9 using dilute HCl or NaOH before substrate sterilization. Relative humidity was regulated within growth chambers using saturated salt solutions to maintain specific humidity ranges: 50–60%, 60–70%, 70–80%, 80–90%, and 90–100% (Atri et al., 2016; Yang et al., 2020).

Daily observations were recorded for both

quantitative (time to full colonization) and qualitative (density, texture, smoothness) parameters of mycelial growth. These findings were compared across different variables to determine the most effective combination of culture type, grain substrate, straw mixture, and environmental conditions for optimal mycelial development and spawn production.

RESULTS AND DISCUSSION:

Results of Days Taken for Mycelium Coverage in Spawn Jars When Inoculated with Liquid And Solid Culture

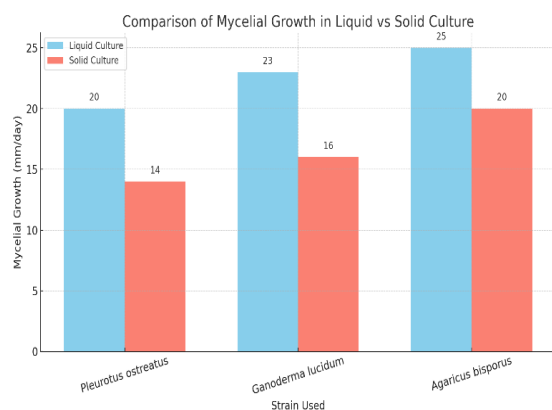


Figure 1- Graph showing results of days taken for mycelium coverage in spawn jars when inoculated with liquid and solid culture

The bar graph presents a comparative analysis of the mycelial growth rates of three fungal species—*Pleurotus ostreatus*, *Ganoderma lucidum*, and *Agaricus bisporus*—under two distinct cultivation systems: liquid culture and solid culture. The data clearly indicate that all three species exhibit enhanced mycelial proliferation in liquid media relative to solid media, suggesting that liquid cultivation conditions are more favorable for accelerated hyphal extension and biomass accumulation.

Among the species studied, *Agaricus bisporus* demonstrated the highest growth velocity, with a mean radial extension of 25 mm/day in liquid culture and 20 mm/day in solid culture. This reflects its robust adaptability and efficient nutrient assimilation capabilities across both media types, with superior performance in liquid culture. *Ganoderma lucidum* followed with a growth rate of 23 mm/day in liquid medium and 16 mm/day in solid medium, indicating a moderate but consistent growth response. *Pleurotus ostreatus* exhibited the lowest mycelial expansion, achieving 20 mm/day in liquid and 14 mm/day in solid medium; however, it maintained the general trend of enhanced growth under liquid culture conditions.

The comparative results underscore the efficacy of

liquid culture systems in promoting rapid mycelial development, which is of significant relevance to commercial mushroom production and biotechnological applications. These findings may contribute to the optimization of cultivation protocols, particularly in the context of spawn generation, large-scale biomass production, and secondary metabolite extraction.

Results of Days Taken for Mycelium Coverage in Spawn When Inoculated with *Pleurotus Ostreatus*:

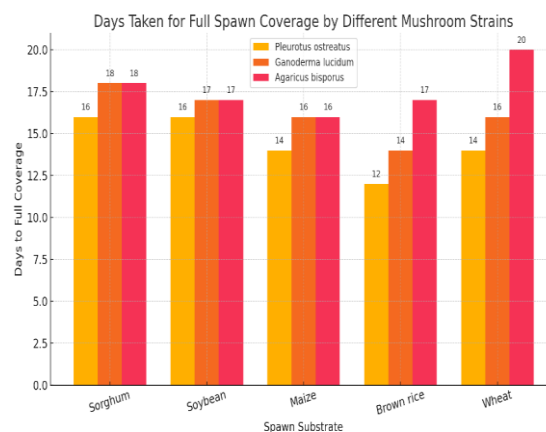


Figure 2- Graph showing results of days taken for mycelium coverage in spawn when inoculated with different fungus

The bar graph provides a comparative assessment of the duration required for complete mycelial colonization on five distinct spawn substrates—sorghum, soybean, maize, brown rice, and wheat—by three mushroom species: *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Agaricus bisporus*. The results demonstrate significant interspecies and substrate-specific variability in colonization efficiency. *Pleurotus ostreatus* exhibited the most rapid and consistent mycelial expansion, achieving full substrate colonization within a range of 12 to 16 days. The shortest colonization period was observed on brown rice (12 days), indicating its superior compatibility and nutrient availability for *Pleurotus* growth. In contrast, colonization on sorghum and soybean required up to 16 days, though still within an efficient range, reflecting the strain's broad substrate adaptability.

Ganoderma lucidum displayed comparatively slower mycelial proliferation, requiring 14 to 18 days for full colonization across the tested substrates. Brown rice again supported the most efficient growth (14 days), while sorghum resulted in the longest colonization period (18 days). This pattern aligns with the known physiological characteristics of *Ganoderma*, which typically produces denser, more slowly extending mycelium. *Agaricus bisporus* demonstrated the slowest and most variable growth among the three species, with

colonization times ranging from 17 to 20 days. The longest duration was recorded on wheat (20 days), whereas brown rice and soybean supported relatively faster colonization at 17 days. Maize served as a moderately suitable substrate for all three species, supporting colonization within 14 to 16 days.

The critical influence of substrate composition on the kinetics of mycelial development is seen. Brown rice emerged as the most conducive substrate, particularly for *Pleurotus ostreatus*, likely due to its favorable physicochemical properties and readily accessible nutrients. These insights hold practical significance for commercial mushroom cultivation, as optimizing substrate selection can enhance spawn quality, reduce incubation periods, and improve the overall efficiency and cost-effectiveness of production systems.

Result of Using Substrates at Different Size And Combinations:

Table 1- Table showing result of using substrates at different size and combinations

S.no.	Substrate	Result
1	Wheat straw finely chopped	Smoothness not up to the mark
2	Rice straw finely chopped	No mycelium run
3	Wheat straw less than 1 cm (approx.)	Smoothness not up to the mark
4	Rice straw less than 1 cm (approx.)	Smoothness not up to the mark
5	Wheat straw less than 2 cm (approx.)	Smoothness not up to the mark
6	Rice straw less than 2 cm (approx.)	Smoothness not up to the mark
7	Finely chopped wheat straw+ finely chopped rice straw	Smoothness not up to the mark
8	Wheat straw less than 1 cm (approx.) +rice straw less the 1 cm (approx.)	Maximum smoothness
9	Wheat straw less than 2 cm (approx.) +rice straw less the 2 cm (approx.)	Smooth but not more than wheat straw less than 1 cm (approx.) +rice straw less the 1 cm (approx.)

Table presents a systematic evaluation of substrate particle size and composition on the physical quality of the final product, particularly focusing on surface smoothness—an important parameter for mushroom-based myco-material or packaging applications. The experiment involved various particle sizes of wheat straw and rice straw, used both individually and in combination. Finely chopped wheat straw alone, as well as wheat straw cut to lengths less than 1 cm and less than 2 cm, failed to achieve satisfactory surface smoothness. Similar results were observed with rice straw at all tested sizes, including finely chopped, <1 cm, and

<2 cm, with the added observation that finely chopped rice straw did not support any visible mycelial colonization—indicating it may lack sufficient porosity or structural integrity to facilitate hyphal penetration and nutrient exchange.

Combining finely chopped wheat and rice straw also did not yield improved results, as the final product's surface remained uneven and lacked the desired texture uniformity. However, the combination of wheat and rice straw cut to lengths of less than 1 cm produced a significantly smoother surface texture compared to all other treatments. This suggests that smaller, but not excessively fine, substrate particles of both materials in combination provide an optimal balance of aeration, compaction, and mycelial adherence. When the particle size of both substrates was increased to less than 2 cm, the resulting material still exhibited acceptable smoothness, although it was inferior to the <1 cm combination. These results conclude that substrate particle size plays a crucial role in determining not only mycelial colonization dynamics but also the final structural and surface properties of the mycelium-based composite. The combination of wheat straw and rice straw at a particle size of less than 1 cm appears to be the most effective formulation for achieving maximum surface smoothness, potentially due to enhanced substrate homogeneity, improved binding, and optimized porosity for fungal growth.

Result of Effect of Temperature on Mycelial Growth on Substrate:

Table 2- Table showing result of effect of temperature on mycelial growth on substrate

S.no.	Temperature	Result
1	10°C	Minimum run
2	15°C	Less run than 20°C
3	20°C	Less run than 25°C
4	25°C	Maximum run
5	30°C	Less run than 25°C

Table provides a comparative analysis of the effect of incubation temperature on the mycelial growth of the fungal strain cultivated on substrate. The results clearly indicate a temperature-dependent variation in mycelial colonization, with optimal growth observed at 25°C. At 10°C, mycelial expansion was minimal, likely due to reduced metabolic activity and enzymatic function at low temperatures. A modest increase in growth was recorded at 15°C, although it remained inferior to higher temperature conditions. At 20°C, mycelial growth improved further but did not reach the optimal levels achieved at 25°C, where the mycelium exhibited the most vigorous and extensive colonization of the substrate.

When the incubation temperature was increased to

30°C, a decline in growth was observed compared to 25°C, suggesting that temperatures above the optimal threshold may negatively affect enzymatic activity or induce thermal stress, thereby inhibiting hyphal expansion. Overall, the findings demonstrate that 25°C is the most favorable temperature for promoting mycelial growth on the given substrate, whereas both sub-optimal (10–20°C) and supra-optimal (30°C) conditions result in reduced colonization efficiency. This temperature sensitivity is critical for optimizing incubation protocols in both research and commercial mycelium cultivation, ensuring rapid substrate colonization and improved production outcomes.

Result of Effect of Ph on Mycelial Growth on Substrate:

Table 3- Table showing result of effect of pH on mycelial growth on substrate

S.NO.	PH	RESULT
1	5	Minimum run
2	6	Less run than 7 pH
3	7	Maximum run
4	8	Less run than 7 pH
5	9	Less run than 8 pH

Table illustrates the effect of substrate pH on mycelial growth, demonstrating that pH plays a significant role in influencing fungal colonization and biomass expansion. The data indicate a distinct pH-dependent response in mycelial development, with optimal growth occurring at neutral pH (7.0). At pH 5, mycelial growth was minimal, likely due to increased acidity inhibiting enzymatic function and impairing nutrient availability. Growth improved at pH 6, though it remained suboptimal compared to pH 7. The highest rate of mycelial colonization was observed at pH 7, suggesting that a neutral pH provides the most favorable conditions for enzymatic activity, nutrient absorption, and hyphal expansion.

At alkaline pH levels (8 and 9), a gradual decline in growth was recorded. While pH 8 supported more growth than pH 6, it was still inferior to that observed at pH 7. At pH 9, mycelial development was further reduced, indicating that higher alkalinity adversely affects the metabolic efficiency of the fungal strain. The neutral pH (7.0) is optimal for mycelial growth on the substrate, while both acidic and alkaline deviations result in progressively reduced colonization. These results are important for substrate preparation and environmental control in mushroom cultivation and myco-material production, ensuring maximum yield and quality by maintaining optimal pH conditions.

Result of Effect of Relative Humidity on Mycelial Growth on Substrate:

Table 4- Table showing result of effect of relative humidity on mycelial growth on substrate.

S.no.	Relative humidity	Result
1	50-60 %	Minimum run
2	60-70 %	Less run than 70- 80%
3	70- 80%	Less run than 80- 90%
4	80- 90%	Maximum run
5	90- 100%	Less run than 80- 90%

Table presents the influence of relative humidity (RH) levels on the mycelial growth of fungi on substrate. The data reveal a clear relationship between RH and the extent of mycelial colonization, with optimal growth occurring under moderately high humidity conditions. At 50–60% RH, mycelial expansion was minimal, likely due to insufficient moisture availability leading to desiccation stress and reduced enzymatic activity. Growth improved at 60–70% RH but remained significantly lower than at higher humidity levels. Further enhancement in mycelial growth was observed at 70–80% RH, indicating a positive correlation between RH and fungal development up to a certain threshold.

The highest level of colonization was recorded at 80–90% RH, suggesting that this range provides the most conducive microenvironment for fungal metabolism, enzymatic function, and hyphal extension. However, increasing the humidity beyond this level to 90–100% resulted in a decline in mycelial growth. This reduction may be attributed to excessive moisture leading to poor aeration, accumulation of metabolic byproducts, or increased risk of contamination, all of which can inhibit growth. The experiment highlights that maintaining relative humidity within the range of 80–90% is optimal for promoting maximum mycelial growth on substrate. Deviations below or above this range result in suboptimal development, emphasizing the importance of precise environmental control during the incubation phase in fungal cultivation and myco-material processing.

CONCLUSION

The study clearly demonstrates that the type of culture, spawn grain, substrate composition, and environmental conditions significantly influence the rate and quality of mycelial growth in selected fungi. Solid culture inoculation was found to be more effective than liquid culture across all tested strains, resulting in faster and more consistent mycelial coverage. Among the spawn grains tested, brown rice and maize proved to be the most suitable for rapid colonization, particularly for *Pleurotus ostreatus* and *Ganoderma lucidum*. The

combination of wheat and rice straw chopped to less than 1 cm yielded the smoothest mycelial mat, indicating that fine particle size and mixed fibrous substrates enhance substrate colonization. Environmental factors played a crucial role, with optimal mycelial growth observed at 25°C, pH 7, and 80–90% relative humidity. Deviations from these optimal conditions resulted in slower or suboptimal growth. These results show the importance of selecting appropriate biological and environmental parameters to maximize efficiency in spawn production and mushroom cultivation.

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