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Chemical composition of substrates and their influence on mycelium growth: A review

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Abstract Mycelium growth, the vegetative colonization phase that precedes fruiting in mushroom-forming fungi, is governed largely by the chemical composition of the cultivation substrate. This review synthesized 34 verified sources on how carbon source composition, nitrogen content and carbon-to-nitrogen (C/N) ratio, nitrogen source type, mineral and trace-element content, and pH affect the rate, density, and pattern of mycelial growth. In a seven-species race-tube comparison, extension rates were highest on cotton gin-trash, peanut shells, and poplar sawdust and depended on species-specific temperature optima (20–35 °C). Cotton-fiber-rich pine bark blends gave the fastest radial growth in three species (up to 5.4 mm/day), although the faster-growing species formed thinner mycelium than *Ganoderma lucidum*. A C/N ratio near 27:1 balanced colonization and yield in *Flammulina velutipes*, and organic nitrogen sources, particularly yeast extract, generally outperformed inorganic salts, although this preference was species- and strain-dependent. Moderate liming (2% $CaCO_3$, pH 7.8) completed *Pleurotus* colonization fastest (18 days), outperforming both lower and higher lime levels. In mycelium-based composites, the fungus-substrate mixing method also affected material performance: pre-cultured, re-molded substrate produced the densest mycelial network and the lowest water absorption (25% after 60 min), whereas secondary inoculation produced weaker and more absorbent materials; spawn loading, particle size, and pre-compression further altered compressive strength and stiffness. Inconsistent growth metrics, species- and strain-specific optima, and confounding between chemical and physical substrate properties limit generalization. Standardized multi-metric growth reporting, factorial designs separating chemical and physical effects, and screening of mixing methods across substrates of defined chemistry are recommended.

Keywords: Carbon-to-nitrogen ratio, Mineral nutrition, Mycelial colonization, Nitrogen source

Introduction

Before a mushroom-forming fungus can fruit, its mycelium must first colonize the substrate. During this vegetative growth phase, hyphae extend

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through the material, secrete enzymes, and begin breaking down and absorbing nutrients. This colonization phase determines the pace and success of the entire crop: substrates that support fast, dense mycelial growth reduce the window during which competing molds and bacteria can establish, while substrates that support slow or sparse growth increase contamination risk and delay fruiting (Zervakis *et al.*, 2001; Schoder *et al.*, 2024). The chemical composition of the substrate, including its carbon source structure, nitrogen content, nitrogen source identity, mineral and trace-element profile, and pH, is one of the most important determinants of how well and how quickly this colonization proceeds (Suwannarach *et al.*, 2022).

Commercial mushroom production relies on a wide range of lignocellulosic agro-industrial residues, including cereal straws, sawdust, corn cobs, bagasse, cotton waste, and rice husk, whose cellulose, hemicellulose, and lignin contents differ considerably (Liew *et al.*, 2012; Song and Zhang, 2015; Mustafa *et al.*, 2016; Zhang *et al.*, 2018). White-rot and litter-decomposing fungi depolymerize these fractions using extracellular enzymes such as laccase and manganese peroxidase, whose production is itself regulated by the availability of carbon and nitrogen in the substrate (Elisashvili and Kachlishvili, 2009). Substrates are therefore commonly supplemented with nitrogen-rich materials such as wheat or rice bran and amended with lime or gypsum to adjust pH, and the resulting C/N ratio, nitrogen form, mineral profile, and pH together define the chemical environment in which colonization occurs (Khan *et al.*, 2013; Jahedi *et al.*, 2023; Han *et al.*, 2024). Even within one material category, chemical composition varies with plant species and processing: sawdust from different tree species differs in pH and hemicellulose content (Boadu *et al.*, 2023), and composting can remove substantial fractions of substrate carbon and nitrogen before spawning (Geng *et al.*, 2024).

Despite extensive experimentation, evidence on substrate chemistry and mycelium growth remains difficult to compare. Studies differ in the species and strains tested, the growth metric reported (linear extension rate, days to full colonization, colony diameter, or biomass), the incubation temperature, and whether chemical and physical substrate properties were varied independently (Zervakis *et al.*, 2001; Philippoussis *et al.*, 2003; Schoder *et al.*, 2024). Many studies also measure mycelium growth only as a step toward fruiting-body yield, so growth responses are reported incompletely or combined with yield outcomes (Girmay *et al.*, 2016; Costa *et al.*, 2023; Samarasiri and Chen, 2025). In addition, fast extension does not necessarily indicate dense or robust colonization, as shown by the thinner mycelium of fast-growing *Pleurotus ostreatus* and *Trametes versicolor* compared with the slower-growing *Ganoderma lucidum* on the same substrates (Schoder *et al.*, 2024). A synthesis organized by chemical

parameter, with attention to these methodological differences, can help growers and researchers select and adjust substrates and identify where further controlled studies are needed.

This review focuses specifically on mycelium growth as a distinct outcome, separate from (though related to) fruiting-body yield and quality, which have been reviewed extensively elsewhere. Because different chemical parameters can act on growth through different mechanisms (carbon source structure governs enzymatic accessibility, nitrogen source identity affects assimilation pathways, pH governs enzyme activity, and mineral content affects specific metabolic cofactors), the evidence is organized parameter by parameter rather than substrate by substrate.

Colonized substrates are also increasingly used outside food production, as mycelium-based composites for packaging, insulation, and construction, in which the hyphae act as a natural binder for lignocellulosic particles (Haneef *et al.*, 2017; Appels *et al.*, 2019). In these materials, the way the fungus and substrate are combined adds a fabrication variable to the substrate chemistry that governs colonization. The objectives of this review were therefore to synthesize evidence on how substrate chemical composition influences mycelium growth, and to examine how fungus-substrate mixing methods influence the mechanical and physicochemical properties of mycelium composites. The Literature search and study selection section describes source identification; the subsequent sections review the evidence for each chemical parameter and for mixing methods; and the Discussion addresses challenges and future research directions.

Literature search and study selection

Literature was identified through targeted searches of Scopus, PubMed, and Google Scholar (1985–2026) using combinations of the terms “mycelial growth”, “substrate chemical composition”, “C/N ratio”, “nitrogen source”, “pH”, “mineral nutrition”, “colonization”, “mixing method”, “mycelium composite”, and named genera (*Agaricus*, *Auricularia*, *Flammulina*, *Ganoderma*, *Lentinula*, and *Pleurotus*). Priority was given to studies reporting quantitative growth-rate, biomass, or material-property data linked to a defined, manipulated chemical or fabrication parameter.

Source identification and screening are reported in a PRISMA-informed format (Figure 1). Because this review builds on source-verification work completed for earlier reviews in this series, its evidence base combines newly identified sources with sources carried forward from that prior work and re-screened for relevance to the scope of this review. Twelve topic-specific search queries, including a dedicated evidence-strengthening pass, were executed using

a multi-engine literature search (results indexed from PubMed, PMC, Scopus-listed publisher platforms, and Google Scholar) rather than a single indexed database with a fixed export count. These queries identified seven new candidate sources; one was excluded because a candidate source on the mineral composition of six *Pleurotus* strains under differing nitrogen forms could not be traced to a verifiable author list or publication record, and six were verified and included. A further 14 sources were carried forward from prior reviews in this series and re-screened for relevance to chemical composition and mycelium growth. Seven sources were included to supply cellulose, hemicellulose, and lignin data for commonly used substrates (Table 1), and seven sources on fungus-substrate mixing methods and the properties of mycelium composites were added during revision in response to peer review. In total, 34 sources were included, each verified against a publisher or indexing record.

From each included study, the fungal species and strain, substrate and supplements, chemical or fabrication parameter manipulated, growth or material metric and its reported value, incubation temperature, and optimal condition were extracted. Because growth metrics, species, and culture systems varied widely, quantitative pooling was not attempted. Studies were instead grouped by chemical parameter (carbon source composition; nitrogen content and C/N ratio; nitrogen source type; minerals, trace elements, and pH) and, for mycelium composites, by mixing or fabrication method, and representative findings were tabulated (Tables 2 and 3). Where a study reported several outcomes, growth-related outcomes were prioritized over yield and quality outcomes.

Screening and verification were performed by a single reviewer; dual independent screening, which is required for a fully PRISMA-compliant systematic review, was not performed. The review therefore follows a narrative rather than a fully systematic methodology, includes no meta-analysis, and does not claim exhaustive coverage of the primary literature.

Chemical composition of cultivation substrates: An overview

Carbon source composition

Lignocellulosic substrates supply carbon primarily as cellulose, hemicellulose, and lignin, whose relative proportions and physical arrangement determine how readily the enzymatic machinery of a fungus can access fermentable sugars (Philippoussis *et al.*, 2003). Substrate chemistry also varies by wood or residue source even within a single material category: sawdust pH and hemicellulose content, for example, differ substantially across tree species and affect downstream mycelial and mushroom composition (Boadu *et al.*, 2023).

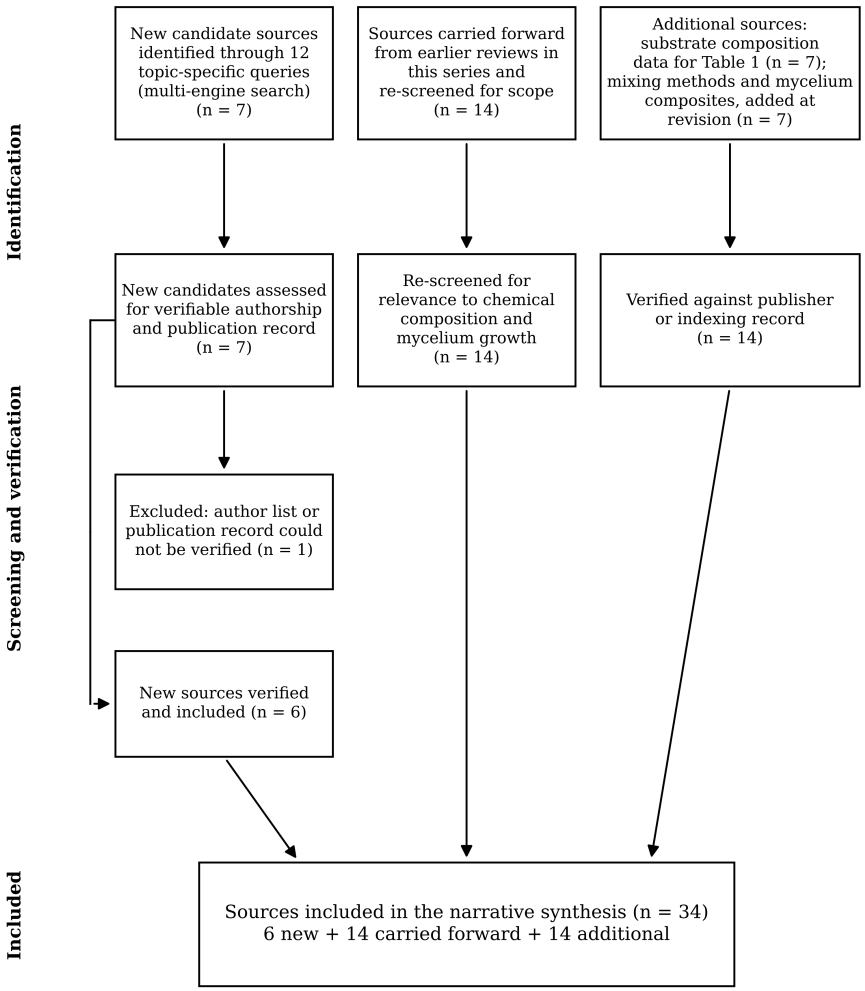


Figure 1. PRISMA-informed flow diagram of source identification, screening, and inclusion for this review

Large quantities of agro-industrial wastes are generated from various agricultural activities, and many of these materials have been used as substrates for the cultivation of commercially important edible and medicinal mushrooms. The suitability of agro-industrial lignocellulosic by-products as substrates for mushroom production largely depends on their chemical composition and nutrient availability. The cellulose, hemicellulose, and lignin contents of commonly used cultivation substrates are summarized in Table 1. Most of these substrates have relatively high cellulose contents, particularly cotton stalk, wheat straw, and corn cob, and comparatively low lignin levels, which makes them

suitable for mushroom cultivation (Liew *et al.*, 2012; Karthikeyan and Visvanathan, 2013; Li *et al.*, 2013; Song and Zhang, 2015; Muktham *et al.*, 2016; Mustafa *et al.*, 2016; Zhang *et al.*, 2018).

Table 1. Cellulose, hemicellulose, and lignin contents of agro-industrial substrates commonly used for mushroom cultivation

Substrate	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reference
Rice straw	37.8	29.6	14.8	Mustafa *et al.* (2016)
Rice husk	41.4	18.0	20.4	Li *et al.* (2013)
Wheat straw	48.6	29.4	7.3	Song and Zhang (2015)
Bagasse	38.2	27.1	20.2	Karthikeyan and Visvanathan (2013)
Corn cob	45.0	35.0	1.05	Muktham *et al.* (2016)
Corn stover	33.7	19.1	15.2	Liew *et al.* (2012)
Cotton stalk	50.4	15.6	16.3	Zhang *et al.* (2018)

Nitrogen content and carbon-to-nitrogen ratio

The C/N ratio is widely regarded as one of the most influential chemical parameters in mushroom cultivation, because nitrogen availability is closely tied to the enzymatic machinery, including laccase and manganese peroxidase, that fungi use to depolymerize substrate carbon (Elisashvili and Kachlishvili, 2009). Because these enzymes are themselves nitrogen-containing proteins, substrate nitrogen availability directly constrains how quickly a fungus can build the enzymatic capacity needed to access substrate carbon during colonization (Elisashvili and Kachlishvili, 2009; Han *et al.*, 2024).

Nitrogen source type: Organic versus inorganic

Independent of total nitrogen content or C/N ratio, the chemical form in which nitrogen is supplied (complex organic compounds such as yeast extract, peptone, and amino acids, versus simple inorganic salts such as ammonium sulfate, ammonium nitrate, or potassium nitrate) affects how efficiently a fungus can assimilate it, with substantial species- and strain-level variation (Manu-Tawiah and Martin, 1988; Kirsch *et al.*, 2016; Maseko *et al.*, 2024).

Mineral and trace-element composition

Substrates supply macrominerals (potassium, calcium, magnesium, and phosphorus) and trace elements (iron, zinc, manganese, and copper) that function as enzyme cofactors and structural components during hyphal growth. Elemental composition varies substantially even among substrate blends built from similar

raw materials, and can shift with the ratio of component materials used (Schoder *et al.*, 2024).

Substrate pH

Substrate pH governs the activity of the extracellular enzymes that fungi use to digest lignocellulosic material, and is commonly adjusted through the addition of lime ($CaCO_3$) or, less often, mild acids, with optimal ranges varying by species (Khan *et al.*, 2013).

Effects of carbon source composition on mycelium growth

The most direct multi-species evidence on how substrate chemistry affects mycelium growth rate comes from a race-tube comparison of seven species (*Pleurotus ostreatus*, *P. eryngii*, *P. pulmonarius*, *Agrocybe aegerita*, *Lentinula edodes*, *Volvariella volvacea*, and *Auricularia auricula-judae*) across seven substrates: wheat straw, cotton gin-trash, peanut shells, poplar sawdust, oak sawdust, corn cobs, and olive press-cake (Zervakis *et al.*, 2001). Extension rates were consistently highest on cotton gin-trash, peanut shells, and poplar sawdust, while supplemented oak sawdust and olive press-cake performed poorly for most species, and nearly all strains grew adequately on corn cobs (Zervakis *et al.*, 2001). The same study established that growth-rate comparisons across substrates are only meaningful when temperature is controlled, since each species had a distinct thermal optimum for colonization (20–35 ℃ depending on species) (Zervakis *et al.*, 2001).

A controlled comparison isolating substrate fiber structure independently of species identity found that cotton-fiber-rich substrate mixtures (pine bark blended with cellulose-rich cotton fiber) consistently produced the highest radial growth rates across three structurally and taxonomically distinct fungi: *Pleurotus ostreatus*, *Trametes versicolor*, and the medicinal species *Ganoderma lucidum*. *P. ostreatus* and *T. versicolor* reached up to 5.4 and 5.3 mm/day, respectively, although their mycelium remained thin and transparent, while *G. lucidum* grew more slowly (3.3 mm/day) but produced a visibly denser mat (Schoder *et al.*, 2024). This finding highlights that fiber content and water-holding capacity, not just the gross lignin-to-cellulose ratio, shape colonization: fiber-poor substrate mixtures dried out during incubation and stopped supporting growth altogether (Schoder *et al.*, 2024). For *Lentinula edodes* specifically, mycelial extension was faster on oak-wood sawdust and wheat straw than on corn cobs, with lag-phase length and colonization rate correlated with strain identity on each substrate (Philippoussis *et al.*, 2003).

Effects of nitrogen content, C/N ratio, and nitrogen source type on mycelium growth

Carbon-to-nitrogen ratio

The C/N ratio shapes the balance between vegetative growth and the subsequent transition to fruiting: substrates with higher C/N ratios generally favor robust mycelial colonization but can delay fruiting, while lower C/N ratios promote earlier fruiting, sometimes at the expense of colonization vigor. In *Flammulina velutipes*, an optimal C/N ratio near 27:1 in composted soybean-straw substrate supported the best-balanced combination of growth and subsequent yield (Han *et al.*, 2024). In *Hypsizygus ulmarius*, hemicellulose, a major carbon fraction, was consumed predominantly during the spawn-running (active mycelial growth) period, indicating that carbon-fraction availability is most consequential for growth during this early colonization window rather than later in the cropping cycle (Ozturk and Atila, 2021).

Organic versus inorganic nitrogen sources

A substantial body of evidence, largely from submerged and solid-substrate culture studies, indicates that organic nitrogen sources generally support better mycelial growth than inorganic sources, although the magnitude and even the direction of this preference vary by species and strain. In an early controlled comparison for *Pleurotus ostreatus*, organic nitrogen sources (yeast extract, peptone, and urea) and inorganic sources (ammonia solution, ammonium phosphate dibasic, ammonium citrate, ammonium nitrate, and potassium nitrate) were each supplied at equivalent nitrogen concentration to synthetic and peat-extract media; the response depended on both nitrogen source and base medium, but yeast extract and ammonium phosphate dibasic emerged as the most effective additions to the peat-extract medium (Manu-Tawiah and Martin, 1988). For *Pleurotus albidus* grown in submerged fermentation, inorganic nitrogen sources did not stimulate growth at all, while yeast extract produced significantly higher mycelial biomass (7.98 g/L), and the overall optimum (30 g/L saccharose with 2.5 g/L yeast extract at pH 7.0) reached 9.81 g/L biomass (Kirsch *et al.*, 2016). For *P. ostreatus* cultured on solid and liquid media, glucose as the primary carbon source combined with an initial pH of 6.0 gave optimal mycelial development, and protein-rich plant flours (amaranth seed and Bambara groundnut) as nitrogen sources, further supplemented with 0.01% urea, maximized mycelial protein content, raising it from 31.4% to 38.7% on a dry-weight basis (Maseko *et al.*, 2024).

Species- and strain-level variability in nitrogen preference

The organic-versus-inorganic nitrogen preference is not universal across species or even across strains of the same species, so nitrogen-source recommendations optimized for one species or strain do not necessarily transfer to another (Manu-Tawiah and Martin, 1988; Kirsch *et al.*, 2016). This is consistent with the broader pattern seen for *Lentinula edodes*, where substituting sawdust with rice straw at different ratios shifted the proximate composition and taste-compound profiles of the resulting mushrooms even at substitution levels that did not markedly change yield, illustrating that nitrogen and carbon source changes propagate through the whole cultivation cycle, not just the colonization phase (Gao *et al.*, 2020).

Effects of minerals, trace elements, and pH on mycelium growth

Minerals and trace elements

Substrate mineral and trace-element content shifts substantially even among substrates built from broadly similar raw materials: in the pine-bark/cotton-fiber comparison, potassium, calcium, magnesium, phosphorus, iron, and several trace elements varied several-fold across substrate blends differing only in their pine-bark-to-cotton-fiber ratio, and this compositional shift accompanied the observed differences in growth rate and mycelium density across the three species tested (Schoder *et al.*, 2024). Beyond growth rate, the substrate also determines the ergosterol pool available for postharvest vitamin D2 conversion in *Lentinula edodes*, with ergosterol content varying significantly by wood substrate species even among substrates that supported comparable colonization (Thakur *et al.*, 2024).

Substrate pH and lime amendment

Because pH governs the activity of the extracellular enzymes that fungi use to digest lignocellulosic material, substrate pH adjustment is a common cultivation practice, most often achieved by adding lime ($CaCO_3$). In a controlled trial on *Pleurotus* spp. grown on cotton waste, lime was added at 0%, 2%, 4%, and 6% of substrate weight, corresponding to substrate pH values of 7.2, 7.8, 8.2, and 8.7; the 2% lime treatment (pH 7.8) completed mycelial growth fastest, in 18 days, and subsequent primordia initiation and yield also favored this treatment over both lower and higher lime levels (Khan *et al.*, 2013). This non-monotonic response, in which a moderate pH adjustment outperforms both no adjustment and heavier adjustment, shows that pH optimization requires empirical testing rather than a simple “more lime is better” assumption.

Interactions between nitrogen management and substrate processing

For composted substrates, nitrogen and carbon losses during substrate preparation itself affect the chemical composition ultimately available to the mycelium. Optimizing the traditional wheat-straw/cow-manure composting process for *Agaricus bisporus* to reduce nutrient losses (carbon loss reduced from 43.55% to 37.75%, and nitrogen loss from 22.01% to 18.49%) improved the nutrient pool retained for subsequent mycelial growth and fruiting-body development (Geng *et al.*, 2024). Substrate chemical composition at spawning is thus itself the outcome of an upstream processing step that can be optimized independently of the raw ingredient list.

Interactions with physical structure and colonization pattern

Chemical composition does not act on mycelium growth in isolation from substrate physical structure. Fiber-rich substrates support better water retention, which sustains active hyphal extension over the multi-day colonization period, whereas substrates with low water-holding capacity can dry out mid-colonization and halt growth even if their chemical composition is otherwise favorable (Schoder *et al.*, 2024). This physical dimension may help account for why cotton-based materials (cotton gin-trash, cotton seed, and cotton fiber) recur across several independent studies as substrates supporting fast colonization, although none of these studies directly tested water-holding capacity as the causal mechanism, so the connection should be read as a pattern worth further investigation rather than an established explanation. Cotton seed and paper waste supported markedly faster *Pleurotus ostreatus* colonization (14 days) than wheat straw (15.7 days) or sawdust (19.7 days) in one four-material trial (Girmay *et al.*, 2016), echoing the cotton gin-trash advantage seen in the seven-species race-tube study (Zervakis *et al.*, 2001) and the cotton-fiber advantage seen in the three-species comparison (Schoder *et al.*, 2024). Independent support for the water-holding-capacity mechanism comes from a seven-substrate trial in which cotton waste produced the fastest oyster mushroom spawn run (18.2 days) of all materials tested, with the authors explicitly attributing this to the water-holding capacity of the substrate rather than to its C/N ratio alone (Muswati *et al.*, 2021).

For *Auricularia cornea*, optimizing both substrate composition (70% beech sawdust + 30% wheat bran) and temperature (28 °C) together produced the fastest mycelial growth rate (9.3 mm/day) and the shortest spawn-run period (9.0 days) among sixteen tested formulations, illustrating that chemical and environmental optimization are most effective when addressed jointly rather than separately (Jahedi *et al.*, 2023). Species-specific responses recur throughout: rice

husk incorporated into a base substrate increased *P. ostreatus* production (Costa *et al.*, 2023), and okara supplementation of sawdust nearly doubled first-flush yield for the same species (Samarasiri and Chen, 2025), but neither study found these chemical changes to have uniform effects across the growth, yield, and quality outcomes measured, reinforcing that substrate chemistry optimized for one outcome is not automatically optimal for another. Representative effects of substrate chemical composition on mycelium growth are summarized in Table 2.

Table 2. Representative effects of substrate chemical composition on mycelium growth across mushroom species

Species	Chemical parameter varied	Effect on mycelium growth	Optimal condition identified	Source
7 species (*Pleurotus*, *Lentinula*, *Agrocybe*, *Volvariella*, *Auricularia*)	Substrate type across 7 lignocellulosic materials	Extension rate highest on cotton gin-trash, peanut shells, and poplar sawdust; poor on oak sawdust and olive press-cake	Species-specific temperature optima (20–35 °C) interact with substrate	Zervakis *et al.* (2001)
Lentinula edodes	Sawdust vs. wheat straw vs. corn cobs	Faster extension on sawdust and wheat straw than on corn cobs (strain-dependent)	Substrate ranking strain-dependent	Philippoussis *et al.* (2003)
Pleurotus spp.	Substrate pH via lime ($CaCO_3$) addition (0–6%)	Growth and fructification improved with moderate liming; too little or too much lime reduced performance	2% lime (pH ≈ 7.8) on cotton waste	Khan *et al.* (2013)
Pleurotus ostreatus	Organic (yeast extract, peptone, urea) vs. inorganic (ammonium salts, nitrate) N sources	Response depended on N source and base medium	Yeast extract or ammonium phosphate dibasic in peat-extract medium	Manu-Tawiah and Martin (1988)
Pleurotus albidus	Carbon source (saccharose, fructose, maltose) and N source (yeast extract vs. inorganic)	Inorganic N did not stimulate growth; yeast extract gave the highest biomass (7.98 g/L)	30 g/L saccharose + 2.5 g/L yeast extract, pH 7.0, 180 rpm	Kirsch *et al.* (2016)

Species	Chemical parameter varied	Effect on mycelium growth	Optimal condition identified	Source
Pleurotus ostreatus	Cotton seed vs. paper waste vs. wheat straw vs. sawdust	Fastest colonization on cotton seed and paper waste (14 days); slowest on sawdust (19.7 days)	Cotton seed for both speed and yield	Girmay *et al.* (2016)
Hypsizygus ulmarius	Lignin, cellulose, and hemicellulose loss over the cultivation cycle	Hemicellulose consumed mainly during spawn run (active mycelium growth phase)	Substrate-dependent; lower-lignin substrates favor faster colonization	Ozturk and Atila (2021)
Auricularia cornea	Beech/hornbeam sawdust + wheat/rice bran; sugar supplementation; temperature	Highest growth rate (9.3 mm/day) and shortest spawn run (9.0 days) on a specific blend	70% beech sawdust + 30% wheat bran, 28 °C	Jahedi *et al.* (2023)
Ganoderma lucidum, *Pleurotus ostreatus*, *Trametes versicolor*	Pine bark : cotton fiber ratio; $CaCO_3$ (0–5 wt.%); CO_2 (0–10 vol.%)	Cotton-fiber-rich substrate gave the highest radial growth for all three species (up to 5.4 mm/day)	25% pine bark + 75% cotton fiber; *G. lucidum* best with 5% CO_2 and no $CaCO_3$	Schoder *et al.* (2024)
Flammulina velutipes	C/N ratio of composted soybean-straw substrate	Growth rate and vigor shifted with C/N; excess N caused fast but weak colonization	C/N ≈ 27:1 for balanced growth and yield	Han *et al.* (2024)
Pleurotus ostreatus	Carbon source, pH, and protein-flour-based N sources	Glucose as carbon source and pH 6.0 gave optimal mycelial development	Amaranth or Bambara groundnut flour + 0.01% urea maximized protein content	Maseko *et al.* (2024)

Fungus-substrate mixing methods and properties of mycelium composites

When colonized substrates are used as mycelium-based composites, the method used to combine the fungus with the substrate is an additional fabrication variable that shapes how evenly hyphae are distributed and, through this, the mechanical and physicochemical properties of the final material (Appels *et al.*, 2019; Dong *et al.*, 2025). Dong *et al.* (2025) compared three mixing methods for

Ganoderma sichuanense grown on a soybean straw-wheat bran substrate (78:20, with 1% lime and 1% gypsum, at about 60% moisture): direct inoculation of agar-grown mycelium into sterilized, molded substrate; pre-culture, in which the substrate was fully colonized in spawn bags, broken into granules, and re-molded; and secondary inoculation, in which 10–32% of a pre-cultured mixture was blended into fresh sterilized substrate before molding. Directly inoculated and pre-cultured materials both reached compressive strengths of at least 0.08 MPa, whereas secondary inoculation produced weaker materials, with a mean flexural breaking load of 7.94 N. After 60 min of immersion, water absorption was lowest for pre-cultured material (25%), intermediate for direct inoculation (35%), and highest for secondary inoculation (42%). Scanning electron microscopy showed that the pre-cultured material had the densest mycelial network enveloping the substrate particles, whereas secondary inoculation yielded mostly aerial hyphae without a continuous surface skin. Thermal behavior was similar across methods, with degradation beginning near 170 ℃ and peaking near 350 ℃ (Dong *et al.*, 2025), indicating that the mixing method mainly affected mycelial binding and surface properties rather than the thermal stability of the substrate-derived matrix.

Inoculum quantity and substrate preparation are closely related variables. In *Pleurotus ostreatus* biofoam grown on rice husk supplemented with 2% $CaCO_3$ and 10% rice bran, spawn loading was varied from 5% to 50% (w/w), and the highest compressive strength (1.35 MPa) was obtained at 40% spawn loading (Nashiruddin *et al.*, 2022). Yang *et al.* (2017) tested three mixing protocols and two packing conditions for combining fungal inoculum with wood pulp, millet grain, wheat bran, natural fiber, and calcium sulfate, and found that denser packing yielded higher compressive strength and elastic moduli, while longer cultivation increased compressive strength but lowered elastic moduli. Physical processing of the substrate before mixing can matter as much as substrate type: in composites of *Trametes versicolor* on hemp, flax, flax waste, wood, and straw, fiber size and processing (loose, chopped, or pre-compressed) influenced compressive stiffness more than fiber chemistry; chopping and pre-compression increased stiffness largely by raising density, and dust-sized particles supported poor growth and became contaminated (Elsacker *et al.*, 2019). Similarly, in *P. ostreatus* composites grown on oak sawdust and wheat straw at sawdust-to-straw ratios from 1:0 to 1:7, denser, sawdust-rich mixtures were stronger; strength declined with longer total cultivation time and was higher when more of the growth period took place in the final formwork rather than in the spawn bag (Ghazvinian and Gürsoy, 2022).

Substrate chemistry nonetheless remains linked to material properties. *G. lucidum* and *P. ostreatus* mycelium grown on pure cellulose had a greater chitin

share and greater stiffness (a larger Young's modulus with reduced elongation at break) compared with mycelium fed cellulose plus potato dextrose broth, a more easily digested carbon source; every material in that study repelled water, with contact angles exceeding 120° (Haneef *et al.*, 2017). In composites, substrate type (straw, sawdust, or cotton), fungal species (*P. ostreatus* or *Trametes multicolor*), colonization level, and outer skin thickness jointly determined stiffness and water resistance: composites made from straw were stiffer, whereas those made from cotton resisted moisture better, and heat pressing improved homogeneity, strength, and stiffness (Appels *et al.*, 2019). Taken together, these studies indicate that the factors governing mycelial colonization reviewed above (carbon source accessibility, supplementation, moisture, particle size, and aeration) also govern composite performance, and that the mixing method determines how uniformly that colonization is achieved throughout the material (Table 3).

Table 3. Effects of fungus-substrate mixing and fabrication methods on the mechanical and physicochemical properties of mycelium composites

Fungus	Substrate	Method or factor varied	Main effects on composite properties	Source
Ganoderma lucidum, *Pleurotus ostreatus*	Cellulose vs. cellulose + potato dextrose broth	Nutrient (carbon source) composition	Cellulose-grown mycelium richer in chitin and stiffer; all materials hydrophobic (contact angle > 120°)	Haneef *et al.* (2017)
Not specified	Wood pulp, millet grain, wheat bran, natural fiber, calcium sulfate	Three mixing protocols; two packing conditions; cultivation time	Denser packing raised compressive strength and elastic moduli; longer cultivation raised strength but lowered moduli	Yang *et al.* (2017)
Pleurotus ostreatus, *Trametes multicolor*	Straw, sawdust, cotton	Substrate, species, colonization level, heat pressing	Straw composites stiffer but less moisture-resistant than cotton composites; heat pressing improved homogeneity, strength, and stiffness	Appels *et al.* (2019)

Fungus	Substrate	Method or factor varied	Main effects on composite properties	Source
Trametes versicolor	Hemp, flax, flax waste, wood, straw	Fiber size and processing (loose, chopped, pre-compressed)	Processing and size influenced stiffness more than fiber type; dust particles grew poorly	Elsacker *et al.* (2019)
Pleurotus ostreatus	Oak sawdust and wheat straw (1:0 to 1:7)	Substrate mixture; time in bag vs. formwork	Denser sawdust-rich mixtures stronger; more growth time in formwork increased strength	Ghazvinian and Gürsoy (2022)
Pleurotus ostreatus	Rice husk + 2% $CaCO_3$ + 10% rice bran	Spawn loading (5–50% w/w); moisture; temperature	Highest compressive strength (1.35 MPa) at 40% spawn loading	Nashiruddin *et al.* (2022)
Ganoderma sichuanense	Soybean straw + wheat bran (78:20) + lime and gypsum	Direct inoculation vs. pre-culture vs. secondary inoculation	Pre-culture gave the densest mycelium and lowest water absorption (25% at 60 min); secondary inoculation weakest (7.94 N) and most absorbent (42%)	Dong *et al.* (2025)

Discussion

Inconsistent growth metrics and reporting

Mycelium growth is measured inconsistently across the literature as linear extension rate (mm/day), days to full colonization, colony diameter on agar, or biomass yield in submerged culture, and these metrics are not always interconvertible. A substrate or nitrogen source that produces the fastest linear extension does not necessarily produce the densest or most robust mycelium, as shown directly in the three-species pine-bark/cotton-fiber comparison, where the fastest-growing species produced visibly thinner mycelium than the slower-growing *Ganoderma lucidum* (Schoder *et al.*, 2024). Studies on mycelium composites add a further set of metrics (density, compressive and flexural strength, water absorption, and thermal stability) that are rarely reported alongside growth kinetics, so links between colonization chemistry and material performance must currently be inferred across studies (Elsacker *et al.*, 2019; Dong *et al.*, 2025). A related limitation concerns the search process of this review

(see Literature search and study selection): source identification relied on a multi-engine literature search rather than a single indexed database, and screening was performed by a single reviewer rather than two independent reviewers.

Species- and strain-specific optima limit generalization

Because nitrogen-source preference, C/N optimum, and pH optimum all vary by species and often by strain within a species (Manu-Tawiah and Martin, 1988; Zervakis *et al.*, 2001; Khan *et al.*, 2013; Kirsch *et al.*, 2016), chemical-composition recommendations derived from one species or strain cannot be assumed to transfer to another without direct testing. The same caution applies to mixing methods, which have so far been compared mainly within single fungus-substrate combinations (Dong *et al.*, 2025).

Confounding between chemical composition and physical structure

Because fiber content, particle size, and water-holding capacity co-vary with chemical composition in most real substrates, it is often difficult to determine whether an observed growth effect is chemical (nutrient availability) or physical (moisture retention or porosity) in origin (Schoder *et al.*, 2024). Few studies manipulate chemical and physical substrate properties independently, although composite studies show that particle size and pre-compression can outweigh fiber chemistry for stiffness (Elsacker *et al.*, 2019).

Limited coverage of medicinal and emerging species

Much of the chemical-composition literature focuses on *Pleurotus* and *Lentinula*. Medicinal species such as *Ganoderma lucidum*, in which mycelium growth rate and density both affect downstream bioactive-compound yield, remain comparatively understudied for basic chemical-composition optimization (Schoder *et al.*, 2024; Thakur *et al.*, 2024).

Future directions

Future studies would benefit from reporting linear extension rate, mycelial density or morphology, and time to full colonization together, at controlled and stated temperatures, to allow direct comparison across studies and species along the lines established by existing multi-species comparisons (Zervakis *et al.*, 2001; Schoder *et al.*, 2024). Factorial designs that vary chemical composition (C/N ratio, nitrogen source, mineral content, and pH) and physical structure

(fiber content, particle size, and water-holding capacity) independently, following the approach used in the three-species pine-bark/cotton-fiber study (Schoder *et al.*, 2024), would help disentangle which growth effects are truly chemical and which are structural in origin. Given the demonstrated species- and strain-level variability in organic-versus-inorganic nitrogen preference (Manu-Tawiah and Martin, 1988; Kirsch *et al.*, 2016; Maseko *et al.*, 2024), systematic nitrogen-source screening across a wider range of commercially important species and strains would provide more actionable guidance than the current patchwork of single-species studies.

Because the mixing method determined mycelial density and water resistance in *Ganoderma* composites (Dong *et al.*, 2025), and spawn loading, particle size, and pre-compression altered strength in other systems (Elsacker *et al.*, 2019; Nashiruddin *et al.*, 2022), future studies should compare direct inoculation, pre-culture, and secondary inoculation across substrates of defined C/N ratio, lignocellulose composition, and moisture content, reporting growth kinetics together with density, compressive and flexural strength, and water absorption. Because mycelium density and growth rate can trade off against each other, as shown for *Ganoderma lucidum* relative to faster-growing species (Schoder *et al.*, 2024), and because medicinal species are often valued for mycelial bioactive-compound content rather than growth speed alone, systematic chemical-composition screening should also be extended to *Ganoderma* and other medicinal or emerging genera with this dual objective in mind.

In summary, substrate chemical composition is a primary determinant of mycelium growth, acting through at least four largely distinct mechanisms: carbon source structure, which governs enzymatic accessibility and is most effectively supplied by fiber-rich materials such as cotton gin-trash, cotton seed, and cotton fiber blends; nitrogen content and C/N ratio, which govern the balance between vegetative colonization and the subsequent transition to fruiting; nitrogen source identity, for which organic sources such as yeast extract and peptone generally, though not universally, outperform inorganic salts; and mineral content and pH, which govern enzyme activity and cofactor availability, with lime amendment as the most common pH-management tool. These mechanisms interact with each other and with substrate physical structure, particularly water-holding capacity, and, in mycelium composites, with the method used to combine fungus and substrate, which influences mycelial density, mechanical strength, and water absorption. Because species- and strain-specific optima recur throughout this literature, chemical-composition recommendations should be treated as starting points for empirical testing rather than universal prescriptions. Addressing inconsistent growth-rate reporting, disentangling chemical from physical substrate effects, linking colonization chemistry and

mixing methods to composite performance, and extending systematic screening to medicinal and underexplored species are the most promising directions for future research.

Conflicts of interest

The authors declare no conflict of interest.

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