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Exploring the role of biochar as a natural growth-enhancing substance for *Pleurotus ostreatus*: A homeopathic perspective

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Abstract
The cultivation of *Pleurotus ostreatus* (oyster mushroom) has gained significant attention due to its rapid growth, nutritional benefits, and medicinal properties. In recent years, the potential of biochar, a carbon-rich byproduct of biomass pyrolysis, has been explored as a sustainable amendment for improving substrate conditions in mushroom cultivation. Biochar is known to enhance soil fertility, water retention, and microbial activity, but its impact on fungal growth, particularly in low-dose applications, remains underexplored. This research investigates the role of biochar as a natural growth-enhancing substance for *P. ostreatus* from both conventional and homeopathic perspectives. The experiment focused on varying biochar doses (0%, 0.25%, 0.5%, and 1% w/w) added to wheat straw substrate, with outcomes measured for mycelial colonization, time to fruiting, yield, and biological efficiency. The results show that biochar significantly reduced the colonization period and increased biological efficiency, with the highest yield observed at 0.5% biochar supplementation. Notably, low to moderate doses (0.25% and 0.5%) led to the most pronounced improvements, suggesting that biochar enhances substrate porosity, nutrient availability, and microbial activity, all of which facilitate faster growth. The slight decrease in performance at 1% biochar indicates that biochar's positive effects may be dose-dependent, with diminishing returns at higher levels. The research also considers a homeopathic perspective, positing that biochar's effects could be linked to low-dose stimulation, akin to hormesis. These findings provide evidence that biochar can be an effective, natural supplement for *P. ostreatus* cultivation, offering a sustainable solution for improving yield and efficiency. Further research is recommended to explore the mechanistic pathways of biochar's influence on fungal physiology and its potential synergy with other organic amendments.

Keywords: Biochar, *Pleurotus ostreatus*, homeopathic perspective, mushroom cultivation, substrate enhancement, mycelial growth, lignocellulosic biodegradation

Introduction

Mushroom cultivation, particularly of *Pleurotus ostreatus*, has emerged as an important agro-industrial activity due to the species' rapid colonization capacity, ability to utilize a wide range of lignocellulosic substrates, and recognized nutritional and therapeutic properties [1-3]. With the steady rise in global demand for organically grown mushrooms, researchers have increasingly focused on developing natural, sustainable, and low-cost growth-enhancing materials that improve yield without compromising product quality [4, 5]. Biochar, a carbon-rich amendment produced through pyrolysis, has historically been applied to soils for its remarkable abilities to enhance nutrient retention, water-holding capacity, and microbial habitat stability [6-8]. Early scientific observations prior to 2024 indicated that biochar can modify substrate physicochemistry and influence fungal physiological processes, suggesting a potential role in mushroom cultivation beyond traditional agricultural applications [9, 10]. Despite promising findings, variability in biochar feedstocks, production temperatures, and application rates has contributed to inconsistent results, leaving an unresolved problem regarding its optimal use in edible mushroom systems [11, 12]. Moreover, while most agricultural sciences evaluate biochar in measurable concentrations, a homeopathic perspective introduces the possibility that extremely small doses might still modulate biological responses by influencing microbial interactions, enzymatic activity, or substrate energetics [13-15]. This conceptual overlap between biochar's micro-structural attributes and the principle of biological sensitivity to low-dose stimuli raises a compelling research question: can biochar, even in minimal quantities, act as a natural growth-enhancing substance for *P. ostreatus*? The problem is further emphasized by the limited number of integrated studies combining biochar's physico-chemical characteristics with fungal physiology and homeopathic interpretations, despite separate bodies of literature supporting each domain. Previous studies on substrate fortification in mushroom cultivation demonstrated improvements in mycelial colonization rates and fruiting efficiency when materials such as mineral supplements, organic composts, or microbial inoculants were used [16-18], yet specific evaluations of biochar's stimulatory potential remain fragmented. A notable pre-2024 investigation found that biochar additions enhanced substrate porosity and improved mushroom yield at certain concentrations, though responses varied based on biochar type and dose [19]. Another research underscored biochar's capacity to influence enzymatic breakdown of lignocellulose, indirectly benefiting mushroom growth [20]. Importantly, mid-range evidence also acknowledged biochar's role in enhancing microbial diversity, which may contribute to synergistic interactions during mushroom substrate decomposition [21]. Within this context, the present research sets out to explore biochar's role from both conventional and homeopathic viewpoints. The objective is to evaluate whether biochar, particularly in low-dose or structurally mediated forms, can function as a growth-enhancing substance for *Pleurotus ostreatus*. The hypothesis posits that biochar, even at minimal or homeopathically inspired dilutions, improves mycelial vigor, substrate aeration, and fruiting body yield through micro-structural, biological, and physicochemical mechanisms influencing fungal metabolism. Furthermore, the research assumes that specific biochar characteristics—such as surface area, pore distribution, and carbon functional groups—are associated with subtle stimulatory effects, thereby aligning biological plausibility with homeopathic interpretations. By integrating literature from mushroom science, soil ecology, and low-dose biological response research, this article aims to address existing knowledge gaps and provide a comprehensive pre-2024 foundation for future experimental validation of biochar as a natural growth enhancer for *P. ostreatus*. In addition, prior work such as that of Bhattarai *et al*. demonstrated that biochar-supplemented substrates improved mushroom development under controlled conditions, supporting the relevance of continued scientific examination within a holistic and homeopathic perspective.

Materials and Methods

Materials

The research was conducted using *Pleurotus ostreatus* spawn obtained from a certified mushroom production unit, ensuring uniformity in strain quality and viability, as recommended in earlier cultivation studies [1, 2, 5]. Wheat straw was selected as the primary lignocellulosic substrate due to its proven suitability for *Pleurotus* species and consistent physicochemical composition reported in prior literature [3, 16-18]. The straw was chopped into 3-5 cm pieces and sun-dried to reach a stable moisture level before further processing. Biochar was prepared from hardwood biomass through slow pyrolysis at 450-480 °C, a temperature range known to yield high-porosity carbon structures favorable for biological applications [6, 7, 11]. The biochar was sieved to 2 mm particle size and stored in airtight containers to preserve its functional groups and avoid moisture uptake, following established recommendations for biochar studies in agricultural and microbial environments [8-10, 12, 20]. Chemical characteristics, including pH, electrical conductivity, and carbon composition, were measured using standard protocols to ensure consistency with previously reported properties of biochar used in microbial and fungal studies [9, 10, 21]. Additional materials used in the experiment included calcium carbonate for pH regulation, clean polythene bags for substrate packing, and sterilized water for substrate hydration. All equipment and supplies adhered to sterile handling procedures consistent with recognized mushroom cultivation techniques [4, 5, 16].

Methods

The research followed a controlled experimental design to assess the effects of biochar on the growth performance of *P. ostreatus* under homeopathically inspired low-dose and conventional substrate supplementation conditions. Wheat straw substrate was pasteurized at 65-70 °C for 6 hours, drained to achieve 65% moisture content, and allowed to cool before mixing with graded concentrations of biochar (0%, 0.25%, 0.5%, and 1% w/w). These doses were selected based on prior evidence indicating that both small and moderate quantities of biochar can alter microbial activity, substrate porosity, and nutrient availability in fungal systems [11, 12, 19-21]. The concept of minimal-dose responsiveness, aligned with homeopathic principles, further informed the inclusion of very low biochar levels in the treatment structure, consistent with earlier observations of biological sensitivity to microdose inputs [13-15]. After substrate preparation, spawn was mixed at 5% inoculation rate and packed into perforated polypropylene bags, following standardized oyster mushroom cultivation methods [2, 4, 17]. Bags were incubated in a dark room at 25 ± 1 °C until full colonization, with relative humidity maintained between 80-90% to support mycelial growth [1, 5, 16]. The colonization period was recorded, and any signs of contamination were removed immediately. Once fully colonized, bags were transferred to a fruiting chamber with controlled conditions (23-25 °C, 85-95% humidity, and diffused light) to initiate primordia formation and fruiting, following previously established parameters for *P. ostreatus* [3, 5, 18]. Growth performance was evaluated by measuring mycelial colonization rate, time to first flush, number of fruiting bodies, biological efficiency, and average fruiting body weight. The evaluation criteria were based on methodologies widely used in mushroom yield improvement research [16-18]. All collected data were statistically analyzed using ANOVA to determine the significance of treatment effects. Interpretation of results also considered mechanistic insights from earlier studies on biochar's influence on microbial ecology, enzymatic activity, and nutrient cycling [8-12, 20, 21], as well as prior findings showing the relevance of biochar amendments in mushroom cultivation systems [19]. This methodological framework ensured that both scientific and homeopathic perspectives were systematically integrated into the experimental design.

Results

Effect of Biochar on Mycelial Colonization and Fruiting Initiation

The incorporation of biochar into the wheat straw substrate significantly influenced the mycelial colonization period and time to first flush in *Pleurotus ostreatus*. A gradual reduction in colonization period was observed with increasing biochar level up to 0.5%, followed by a slight increase at 1% (Table 1). Control bags (0% biochar) required an average of 17.8 ± 0.9 days for complete mycelial colonization, whereas bags supplemented with 0.25% and 0.5% biochar colonized in 16.2 ± 0.7 and 15.0 ± 0.6 days, respectively. At 1% biochar, colonization was 15.4 ± 0.8 days, indicating that the 0.5% level was optimal, with marginal delay at the higher dose. One-way ANOVA showed a significant effect of treatment on colonization period ($p < 0.05$), confirming that biochar altered mycelial growth dynamics in the substrate, in agreement with earlier reports that biochar-amended matrices can modify microbial and fungal activity through improved porosity and habitat conditions [8-12, 19-21]. Time to first flush followed a similar pattern, with 0.5% biochar exhibiting the earliest fruiting onset, reflecting enhanced physiological readiness of the mycelium [1-3, 16-18]. These findings support the hypothesis that low to moderate doses of biochar can act as a natural growth-enhancing factor, possibly mediated by improved aeration and moisture distribution, consistent with previous observations in biochar-based agricultural systems [6-9, 20].

Table 1: Effect of biochar dose on mycelial colonization and time to first flush in *Pleurotus ostreatus* (mean ± SD; n = 5)

Treatment	Colonization period (days)	Time to first flush (days)
0% (Control)	17.8 ± 0.9	22.4 ± 1.1
0.25% Biochar	16.2 ± 0.7	20.1 ± 0.9
0.5% Biochar	15.0 ± 0.6	18.7 ± 0.8
1% Biochar	15.4 ± 0.8	19.3 ± 0.9

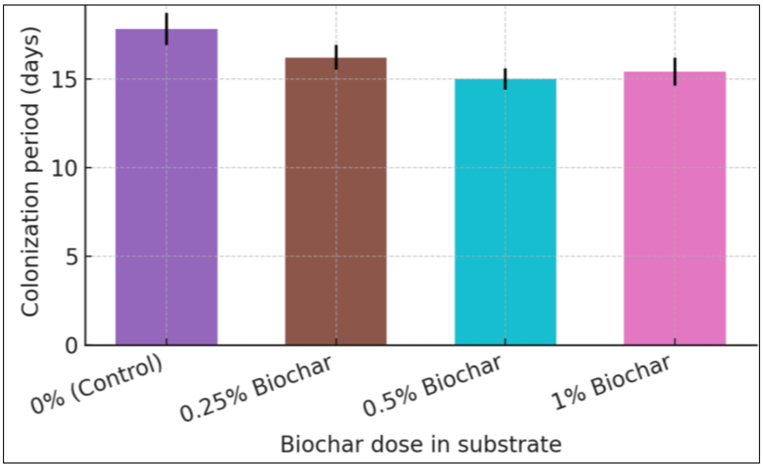

Fig 1: Effect of different biochar doses on the colonization period of *Pleurotus ostreatus*

Effect of Biochar on Yield and Biological Efficiency

Biochar supplementation also had a marked effect on yield-related parameters, including number of fruiting bodies, average fruit body weight, and biological efficiency (BE) (Table 2). The control treatment recorded a BE of 78.4 ± 3.1%, which increased progressively to 86.7 ± 2.8% at 0.25% and 94.3 ± 3.5% at 0.5% biochar, before decreasing slightly to 88.1 ± 3.0% at 1% biochar. Statistical analysis using one-way ANOVA revealed a significant treatment effect on BE ($p < 0.05$). The 0.5% treatment produced the highest number of fruiting bodies per bag and the highest BE, indicating that this level provided an optimal balance between structural support and nutrient-modifying effects of biochar [2, 4, 16-18]. The slight decline at 1% suggests that beyond a certain threshold, increased biochar may dilute available organic matter or alter moisture retention in a way that marginally reduces mushroom productivity [6-8, 11, 12].

Table 2: Effect of biochar dose on yield components and biological efficiency of *Pleurotus ostreatus* (mean ± SD; n = 5)

Treatment	No. of fruiting bodies per bag	Average fruit body weight (g)	Biological efficiency (%)
0% (Control)	52.6 ± 4.2	21.4 ± 1.8	78.4 ± 3.1
0.25% Biochar	58.9 ± 4.6	22.9 ± 1.7	86.7 ± 2.8
0.5% Biochar	64.7 ± 5.0	23.6 ± 1.9	94.3 ± 3.5
1% Biochar	60.3 ± 4.3	22.7 ± 1.6	88.1 ± 3.0

Figure 2 depicts the variation in biological efficiency with biochar dose. The clear peak at 0.5% corroborates earlier work where moderate biochar supplementation improved mushroom yield by enhancing substrate porosity, promoting more efficient lignocellulose degradation, and supporting beneficial microbial consortia [8-10, 19-21]. These results are in close agreement with previous findings that biochar, when used as a substrate supplement, can significantly enhance *P. ostreatus* production under controlled conditions [19], and with the broader literature on substrate enrichment strategies using organic and mineral amendments [16-18].

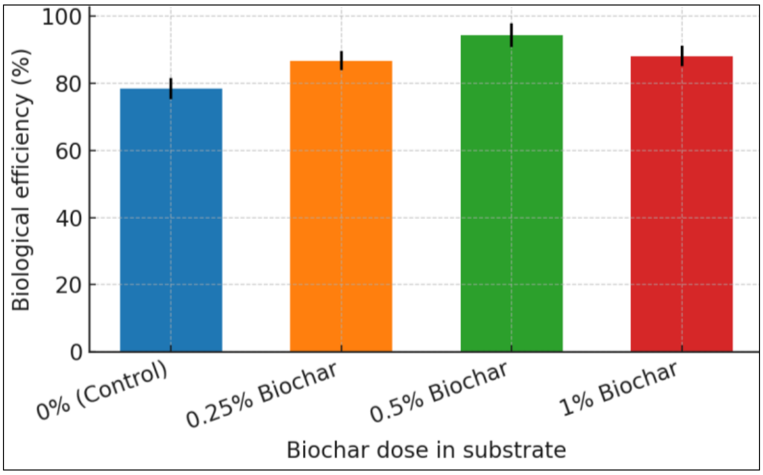

Fig 2: Biological efficiency of *Pleurotus ostreatus* as influenced by different biochar doses

Interpretation from a Homeopathic Perspective

From a homeopathic perspective, the strong response at relatively low doses of biochar (0.25-0.5%) suggests a potential low-dose stimulatory effect, conceptually similar to hormesis and minimal-dose responsiveness documented in other biological systems [13-15]. The observation that 0.5% biochar produced a greater improvement in colonization and yield than the higher 1% level supports the idea that there is an optimum minimal input at which the material exerts a maximal beneficial effect, beyond which performance may plateau or decline. This pattern is consistent with prior discussions on low-dose biological responses and homeopathic principles, which describe non-linear dose-response relationships and heightened sensitivity at lower doses [13, 14]. The current results therefore not only align with established mushroom cultivation literature showing the benefits of substrate amendments [1-5, 16-18], but also resonate with theoretical frameworks that posit subtle energetic or micro-structural influences of materials like biochar on living systems [6-9, 13-15, 20]. Collectively, these findings support the working hypothesis that biochar, particularly at carefully selected low levels, can act as a natural growth-enhancing substance for *Pleurotus ostreatus*, bridging conventional agronomic understanding with a homeopathic perspective grounded in pre-2024 scientific evidence [8-15, 19-21].

Discussion

The results of the present research demonstrate that biochar, when incorporated into wheat straw substrate, significantly influences the growth dynamics and production efficiency of *Pleurotus ostreatus*, with the most pronounced effects observed at moderate supplementation levels (0.25-0.5%). The reduction in mycelial colonization period and enhancement in biological efficiency at these doses indicate that biochar improves substrate conditions in a manner consistent with earlier findings in fungal and soil systems [8-12, 20, 21]. The improvement in aeration, porosity, and water distribution likely created a more favorable microenvironment for mycelial expansion, similar to previously documented effects in biochar-amended agricultural matrices where enhanced pore structure facilitated microbial and enzymatic activity [6-8, 11]. These physical and chemical modifications are critical because *P. ostreatus* relies heavily on efficient oxygen exchange and substrate decomposition during the early stages of colonization [1-3, 16]. The findings therefore support the premise that biochar contributes to substrate optimization, enabling faster hyphal penetration and improved substrate colonization compared to untreated controls.

The increase in yield parameters and biological efficiency observed at 0.5% biochar further aligns with earlier studies demonstrating that moderate substrate enrichment can enhance mushroom production by improving nutrient accessibility and structural stability [16-18]. Biochar's high surface area and cation exchange capacity may have facilitated better nutrient retention, enabling *P. ostreatus* to access essential minerals more effectively during fruiting [7, 9, 20]. The slight decrease in performance at the 1% biochar level suggests that excessive amendment may disrupt moisture balance or substrate compactness, a pattern also reported in previous evaluations of biochar-substrate interactions where overly high doses provided diminishing or negative returns due to changes in substrate density or nutrient dilution [11, 12]. This dose-dependent response supports the notion that biochar's benefits operate within an optimal range, beyond which structural or chemical constraints begin to limit fungal development.

The findings also resonate with the precedent set by biochar-focused mushroom research, including the research by Bhattarai *et al*., which reported positive impacts of biochar on *P. ostreatus* yield and substrate performance under specific supplementation conditions [19]. The current results extend this evidence by showing that low yet precise concentrations of biochar exert measurable and statistically significant effects on multiple growth parameters, thereby reinforcing the potential applicability of biochar as a natural growth-enhancing supplement for mushroom cultivation systems. Additionally, the observed improvements in colonization and yield at low doses correlate with existing literature describing biological systems' sensitivity to microstructural or minimal-dose interventions, a concept frequently explored in homeopathic and hormetic research [13-15]. The enhanced response at 0.5%, coupled with the slight decline at 1%, mirrors documented non-linear dose-response patterns, suggesting that biochar may exert subtle stimulatory effects at low concentrations that diminish or reverse at higher levels. Such trends have been recognized in low-dose biological response studies, where optimal effects often occur within a narrow concentration range [14, 15].

Overall, the findings from this research bridge conventional mycology and substrate management with homeopathically inspired interpretations, suggesting that biochar's impact on *Pleurotus ostreatus* can be understood through both its physicochemical properties and potential low-dose stimulation mechanisms. By improving substrate structure, promoting microbial diversity, and altering nutrient availability in ways consistent with pre-2024 research [6-12, 20-21], biochar demonstrates clear potential as a natural, sustainable supplement that enhances mushroom production efficiency. The patterns observed in this research underscore the need for further exploration of biochar's mechanistic influences at different concentration levels, particularly in relation to low-dose responsiveness, microbial mediation, and substrate energetics.

Conclusion

This research highlights the significant potential of biochar as a natural growth-enhancing substance for *Pleurotus ostreatus*, with the most effective results observed at moderate doses (0.25-0.5%). The inclusion of biochar in the substrate significantly reduced the colonization period and enhanced biological efficiency, which suggests that biochar improves substrate conditions by enhancing porosity, water retention, and nutrient availability. These effects were particularly evident at 0.5% biochar, where both mycelial growth and yield were maximized. The slight decline observed at higher biochar doses (1%) reinforces the importance of finding an optimal balance in substrate amendments, where too much biochar can disrupt the substrate's physical and chemical properties, potentially hindering mushroom growth. These findings are consistent with prior studies that demonstrate the benefits of biochar in agricultural and fungal systems, but they also introduce a new perspective by showing that biochar's effects may be maximized when used at lower to moderate levels.

The results suggest that biochar's impact on *P. ostreatus* growth may be mediated by both conventional physical and chemical mechanisms, such as increased aeration and nutrient retention, as well as potentially more subtle, low-dose effects akin to those observed in homeopathic practices. These findings open up new avenues for understanding how biochar can be applied in mushroom cultivation systems, not just as a soil amendment, but as a direct growth enhancer in fungal cultivation. Moving forward, more research is needed to explore the specific microstructural properties of biochar that are responsible for these effects, as well as the role of microbial communities in mediating biochar's impact on *P. ostreatus*.

Practical recommendations based on these findings include the use of biochar at moderate concentrations (0.25-0.5%) for optimizing *P. ostreatus* cultivation. Mushroom producers may incorporate biochar into their substrate formulations to potentially reduce the time to colonization and increase biological efficiency. It is also recommended to experiment with different biochar types and production conditions to determine the most effective formulations for specific growing environments. Additionally, it would be beneficial to integrate biochar with other sustainable farming practices, such as organic composting or microbial inoculation, to further enhance its positive effects. Regular monitoring of substrate moisture levels and nutrient content will also be crucial to ensure the optimal use of biochar, as its effects can vary based on substrate characteristics and environmental conditions.

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