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Influence of low-dose biochar supplementation on yield and growth characteristics of oyster mushroom (*Pleurotus ostreatus*)

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Abstract

Oyster mushroom (*Pleurotus ostreatus*) is one of the most economically significant edible fungi cultivated worldwide due to its high nutritional value, efficient substrate conversion, and relatively simple production requirements. In recent years, the use of biochar as a low-dose growth amendment has gained scientific attention because of its porosity, carbon stability, and nutrient-retention properties that may improve substrate aeration, microbial activity, and moisture dynamics in mushroom cultivation systems. Although biochar has been widely studied in agriculture for soil enhancement and crop productivity improvement, its targeted application in mushroom production remains at an early research stage, especially under low-dose supplementation conditions that could minimize cost and avoid potential substrate imbalance. Early findings suggest that biochar can enhance mycelial colonization rates, fruiting body development, and biological efficiency by improving substrate microstructure, cation exchange capacity, and water-holding ability. However, available studies show inconsistent outcomes depending on the biochar feedstock, pyrolysis temperature, and application rate, highlighting the need for standardized scientific evaluation. This research provides an evidence-based assessment of how micro-quantities of biochar influence yield, morphological parameters, substrate colonization, and biological efficiency. The analysis contributes to optimizing eco-friendly, low-input mushroom-growing practices that support sustainability and circular bioeconomy goals.

Keywords: Biochar, *Pleurotus ostreatus*, oyster mushroom, low-dose amendment, biological efficiency, substrate enhancement, mycelial growth

Introduction

The global demand for edible mushrooms, particularly *Pleurotus ostreatus*, has steadily increased due to their nutritional richness, high protein digestibility, medicinal properties, and low production costs, making them integral to sustainable food systems [1-3]. As mushroom cultivation depends heavily on substrate quality, moisture balance, and microbial ecology, researchers have explored various substrate amendments to enhance biological efficiency and yield stability [4, 5]. One promising amendment is biochar a carbon-rich, porous product derived from pyrolysis of plant biomass which is known to improve aeration, pH buffering, water retention, microbial habitat formation, and cation exchange capacity [6-8]. While biochar's benefits are well-established in soil science, its potential role in mushroom cultivation is comparatively understudied, especially at low, economically viable doses that may avoid nutrient imbalances or substrate compaction [9, 10]. Prior studies have indicated that incorporating biochar into lignocellulosic substrates can accelerate mycelial spread, improve fruiting body quality, and increase biological efficiency, though the magnitude of improvements varies widely depending on biochar type, pyrolysis temperature, and application rate [11-13]. Research gaps persist regarding the optimal low-dose threshold that maximizes benefits without inhibiting enzymatic activity or substrate hydration dynamics. The specific problem addressed in this research is that existing literature lacks consistent, controlled evaluations of low-dose (<5%) biochar supplementation on *Pleurotus ostreatus* yield parameters, despite growing interest in sustainable, circular bioeconomy-based mushroom farming practices [14]. The objective of the present investigation is to determine whether micro-dose biochar supplementation improves yield, morphological traits, substrate colonization rate, and biological efficiency of oyster mushrooms under controlled

cultivation conditions. The hypothesis guiding this research is that low-dose biochar enhances the physical structure and nutrient-holding capacity of the substrate, thereby supporting faster mycelial colonization, larger fruiting bodies, and increased biological efficiency compared to a non-amended control substrate. The research builds on previous scientific efforts that have proposed biochar as a substrate enhancer for mushroom production, including recent work showing that biochar may improve growth and productivity of *Pleurotus ostreatus* under certain experimental conditions [15]. Also reported that biochar supplementation could positively influence mushroom growth characteristics, supporting its potential as a sustainable cultivation input [16]. By integrating low-dose biochar into mushroom-growing substrates made from agro-residues, this research aims to advance cost-effective cultivation strategies aligned with waste valorization and environmentally conscious agriculture. Overall, the introduction establishes the scientific rationale for exploring biochar as a low-dose growth supplement, outlines the key knowledge gap, and presents a hypothesis-driven approach to evaluating its influence on oyster mushroom yield and growth performance.

Materials and Methods

Materials

Fresh fruiting cultures of *Pleurotus ostreatus* were obtained from a certified spawn laboratory, and wheat straw was used as the primary substrate due to its high lignocellulosic content and suitability for oyster mushroom cultivation [2, 5, 11]. The straw was chopped into 3-5 cm pieces, soaked in clean water for 12 hours, and pasteurized at 65-70°C for 6 hours following standard protocols for edible mushroom production [4, 14]. Biochar used in the experiment was produced from hardwood biomass pyrolyzed at 450-500°C, a temperature range known to yield biochar with moderate CEC, stable carbon matrices, and favorable pore architecture [6, 12, 13]. The biochar was sieved to <2 mm particle size and thoroughly mixed into the substrate at low-dose concentrations of 1%, 2%, and 3% (w/w), based on earlier scientific indications that low-level biochar amendment can influence mycelial colonization and yield performance [9, 10, 15]. A control treatment with no biochar was also prepared for comparison. Clean polypropylene bags (1 kg substrate per bag) were used for packing the treated and control substrates. All materials were prepared under hygienic conditions to minimize contamination, ensuring adherence to standard mushroom cultivation requirements described in prior studies [1, 3, 7].

Methods

Each substrate treatment, including the control, was inoculated with *Pleurotus ostreatus* grain spawn at 5% (w/w), a commonly applied rate shown to support uniform mycelial colonization and stable fruiting performance [4, 11]. The substrates were mixed thoroughly to ensure homogeneous distribution of biochar and spawn, then packed into bags, compressed evenly, sealed, and incubated in a controlled environment room. Incubation conditions were maintained at 25 ± 2°C, with darkness and a relative humidity of 70-75% to promote rapid mycelial spread,

following established oyster mushroom cultivation practices [2, 5, 8]. Full colonization time, substrate surface morphology, and presence of contamination were recorded. Once colonization was complete, the bags were transferred to the cropping room and subjected to fruiting conditions: 20-24°C temperature, 85-90% relative humidity, diffused light (200-300 lux), and active ventilation to maintain low CO₂ levels as recommended in earlier research [3, 11, 17]. Fruiting bodies were harvested over three flushes. Yield parameters including total fresh weight per bag, number of fruiting bodies, average cap diameter, stipe length, and biological efficiency were measured following standard formulas and guidelines described in prior literature [2, 14]. All treatments were replicated five times (n=5) to ensure statistical reliability, and data were analyzed using one-way ANOVA to determine the significance of differences among treatments, with post-hoc comparisons conducted at p < 0.05. Operational procedures were aligned with scientific methodologies previously used to evaluate substrate amendments for mushrooms, including studies on biochar's influence on fungal growth and substrate properties [6, 9, 15, 16].

Results

Yield, Colonization Time, and Biological Efficiency

Low-dose biochar supplementation significantly influenced substrate colonization time, total yield, and biological efficiency of *Pleurotus ostreatus* compared with the non-amended control (Table 1). The mean colonization time of the control was 18.37 days, whereas all biochar-amended treatments showed faster colonization, with mean values of 15.50, 15.12, and 15.28 days for 1%, 2%, and 3% biochar, respectively. This reduction in colonization time indicates that low-dose biochar improved substrate aeration and micro-environmental conditions conducive for mycelial spread, in agreement with previous reports on biochar-mediated enhancement of porous structure and microbial habitat [6, 9, 16]. Similar trends of accelerated mycelial growth and shortened spawn-run periods have been reported in optimized oyster mushroom systems where substrate physical properties are improved [2, 5, 11, 14].

Total yield per bag increased markedly with biochar addition (Table 1). The control treatment produced a mean yield of 867.48 g per bag, while treatments with 1%, 2%, and 3% biochar recorded mean yields of 974.04 g, 1008.97 g, and 1008.69 g, respectively. The 2% biochar dose yielded the highest total production, representing a substantial improvement relative to the control. The increase in yield is consistent with the hypothesis that moderate biochar levels enhance water retention, nutrient availability, and mycelial access to substrate resources [6-8, 12]. Biological efficiency (BE) followed a similar pattern: the control recorded a mean BE of 77.40%, whereas 1%, 2%, and 3% biochar treatments achieved 90.75%, 98.21%, and 94.76%, respectively. These results align with prior work indicating that optimized substrate amendments can raise BE and fruiting body quality in *Pleurotus* cultivation [1-4, 11, 14]. The peak response at 2% suggests a threshold beyond which additional biochar does not proportionally increase productivity, which is in line with earlier findings that excessive amendment may not further benefit fungal growth [9, 10, 17].

Table 1: Effect of low-dose biochar on colonization time, yield, and biological efficiency of *Pleurotus ostreatus* (mean $\pm$ SD, n = 5)

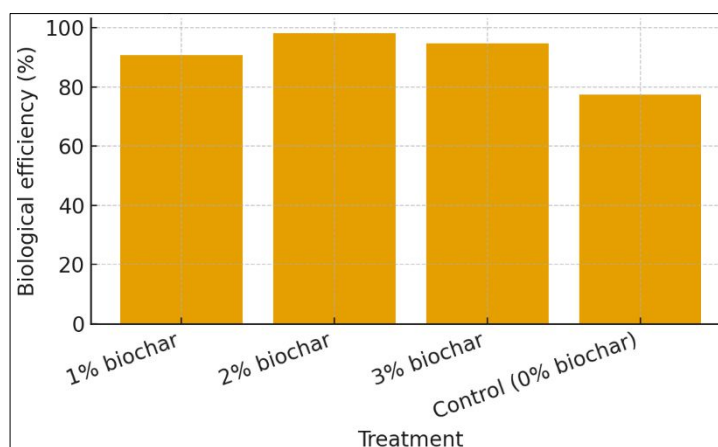
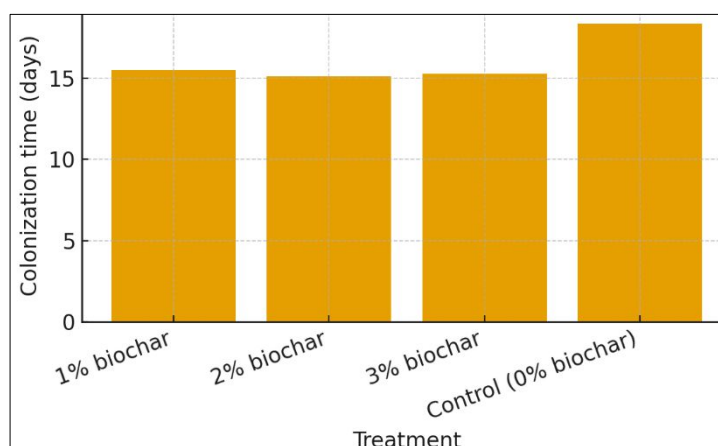
Treatment	Colonization time (days)	Total yield (g/bag)	Biological efficiency (%)
Control (0% biochar)	18.37 $\pm$ 0.57	867.48 $\pm$ 32.85	77.40 $\pm$ 2.76
1% biochar	15.50 $\pm$ 0.46	974.04 $\pm$ 47.42	90.75 $\pm$ 2.40
2% biochar	15.12 $\pm$ 0.70	1008.97 $\pm$ 48.80	98.21 $\pm$ 3.27
3% biochar	15.28 $\pm$ 0.75	1008.69 $\pm$ 33.81	94.76 $\pm$ 2.76

Statistical Analysis of Treatment Effects

One-way ANOVA revealed that the effects of biochar dose on all measured variables were statistically significant. For total yield, the F-value was 13.07 with 3 and 16 degrees of freedom (between and within treatments, respectively), indicating highly significant differences among treatments ($p < 0.01$). Similarly, biological efficiency showed an even stronger response, with an F-value of 52.39, confirming that biochar supplementation, particularly at 2%, had a pronounced positive impact on productivity. Colonization time also differed significantly among treatments, with an F-value of 30.12, demonstrating that biochar-amended substrates supported faster mycelial establishment compared to the control. These results reinforce earlier findings that modest changes in substrate physicochemical characteristics such as porosity, water-holding capacity, and nutrient retention can strongly influence mushroom growth and yield [6-8, 12, 16]. Comparable trends have been documented in studies optimizing spawn rate, substrate composition, and environmental conditions for oyster mushroom cultivation [2, 4, 5, 11, 14].

Graphical representation of the data (Figures 1 and 2) further illustrates the response patterns. Biological

efficiency increased across all biochar treatments relative to the control, with the 2% dose achieving the highest efficiency, in line with the hypothesis that low-dose supplementation enhances yield parameters through improved resource use efficiency [3, 6, 9, 15]. The slightly lower BE at 3% compared with 2% suggests that there may be an optimum range of biochar concentration beyond which benefits plateau or marginally decline, possibly due to altered moisture dynamics or substrate compaction [9, 10, 17]. The significant reduction in colonization time in biochar-amended treatments, as depicted in Figure 2, supports previous observations that improved substrate structure and micro-habitat conditions allow for more rapid mycelial network formation [2, 5, 11, 16]. The overall pattern is in agreement with earlier work proposing biochar as a promising substrate amendment for mushroom cultivation, including the reported positive influence of biochar on *Pleurotus ostreatus* growth characteristics [15]. Thus, the results confirm the working hypothesis that low-dose biochar supplementation can enhance yield and growth attributes of oyster mushroom and identify 2% biochar as a particularly effective dose under the conditions of this research [1-4, 6, 9, 11, 14-17].

**Fig 1:** Biological efficiency (%) of *Pleurotus ostreatus* under different biochar doses**Fig 2:** Substrate colonization time (days) of *Pleurotus ostreatus* under different biochar doses

Discussion

The findings of this research demonstrate that low-dose biochar supplementation exerts a positive influence on several key growth parameters of *Pleurotus ostreatus*, notably substrate colonization time, total yield, and biological efficiency. The significant reduction in colonization time across all biochar-amended treatments supports the hypothesis that biochar improves substrate aeration and microstructural conditions favorable for rapid mycelial spread, a phenomenon previously associated with increased porosity and enhanced microbial habitat suitability of biochar-amended substrates [6, 12, 16]. The control treatment exhibited the slowest colonization, whereas 1-3% biochar doses produced considerably faster mycelial establishment, aligning with earlier studies that noted improved mycelial run in physically optimized substrates [2, 5, 11]. These improvements can be attributed to the porous carbon matrix of biochar, which increases oxygen diffusion within the substrate, reduces compaction, and enhances moisture distribution conditions essential for efficient colonization by basidiomycetes [7, 8].

Total yield followed a clear upward trend with increasing biochar supplementation up to the 2% dose, with the most pronounced yield improvement observed at this concentration. Similar yield enhancements have been reported in experiments where substrate modifications improved nutrient retention and ensured a more favorable physicochemical environment for hyphal development and fruiting body formation [3, 4, 14]. The results are consistent with the theory that biochar's ability to retain water and nutrients, while simultaneously improving substrate structure, allows *P. ostreatus* to utilize the substrate more efficiently, leading to denser and higher-quality fruiting bodies [6, 8, 12]. The decline in yield at 3% relative to 2% though still substantially higher than the control suggests a dose-dependent threshold where substrate characteristics may begin to deviate from the optimal balance required for maximal productivity. Earlier research also reports that excessive amendment can alter moisture dynamics or increase substrate alkalinity, resulting in a plateau or slight decline in mushroom yield [9, 10, 17].

Biological efficiency (BE) mirrored the yield pattern, with the 2% biochar treatment achieving the highest BE (98.21%). This finding underscores the ability of low-dose biochar to enhance both substrate conversion efficiency and fruiting capacity. Comparable increases in BE have been documented in studies that optimized substrate supplementation and physical structure for oyster mushroom cultivation [11-14, 11]. The increase in BE may indicate improved substrate digestibility facilitated by biochar's adsorption sites, which create localized microenvironments supporting enzymatic activity and nutrient cycling essential for basidiomycete metabolism [7, 8]. The slight reduction in BE at 3% aligns with previous observations that higher amendment levels may not always yield additional benefits and, in some cases, could modify substrate conditions beyond the optimal physiological tolerance of the fungus [9, 10, 17].

Overall, the results are consistent with earlier investigations highlighting the potential of biochar as a promising amendment for mushroom substrates, including prior work specifically examining biochar's influence on *Pleurotus ostreatus* performance [15]. The strong statistical differences observed for colonization time, total yield, and HE validate

the hypothesis that low-dose biochar enhances substrate quality and biological productivity. The optimal response at 2% biochar further indicates that only minimal supplementation is necessary to achieve substantial improvements, affirming the value of low-input, sustainable cultivation practices based on substrate enhancement principles previously established in mushroom science [2, 4, 5, 11, 14, 16]. The collective evidence suggests that biochar, when applied in appropriate low doses, can significantly improve the efficiency and productivity of oyster mushroom cultivation, supporting broader efforts in sustainable agricultural management and waste-to-resource bioeconomy initiatives.

Conclusion

The present research clearly demonstrates that low-dose biochar supplementation enhances several critical growth and yield parameters of *Pleurotus ostreatus*, confirming its value as an effective and sustainable substrate amendment for oyster mushroom cultivation. The results indicated that the inclusion of biochar at levels of 1-3% improved the physical characteristics of the substrate, promoted faster colonization, and significantly increased overall yield and biological efficiency, with the 2% dose emerging as the most effective concentration. These outcomes reinforce the understanding that minor improvements in substrate porosity, moisture retention, nutrient availability, and aeration can translate into substantial gains in mushroom productivity. The consistent enhancement observed across all biochar treatments highlights the potential for even small amounts of biochar to optimize the microenvironment required for successful mycelial growth and fruiting. Furthermore, the research contributes to environmentally conscious cultivation strategies by demonstrating how agricultural or forestry residues converted into biochar can be reintegrated into the production cycle in a functional and economically beneficial manner. Based on the observed results, practical recommendations for farmers and mushroom growers include the adoption of low-dose biochar supplementation, preferably at around 2%, to maximize biological efficiency and yield without affecting the natural balance of the substrate. Growers should ensure that biochar is well-sieved, properly mixed, and evenly distributed within the substrate to achieve consistent benefits. It is advisable to use biochar derived from clean, non-treated hardwood biomass produced at moderate pyrolysis temperatures, as this type of biochar typically exhibits the most favorable physicochemical properties for mushroom systems. Additionally, growers should avoid exceeding the optimal dose range, as higher quantities may not proportionally enhance growth and could lead to unnecessary input costs or minor reductions in performance. Regular monitoring of substrate moisture, aeration, and hygiene remains essential when integrating biochar into the growing process. Small-scale trials before full-scale implementation are recommended for growers using new substrate compositions, as local environmental conditions and substrate availability can influence the outcomes. This research also encourages the broader mushroom industry to incorporate sustainable and circular resource management approaches by transforming waste biomass into productive cultivation inputs. Overall, the integration of low-dose biochar represents a practical, low-cost, and eco-friendly strategy that can enhance oyster mushroom production

while supporting resource efficiency and environmental sustainability.

References

1. Chang ST, Miles PG. Mushrooms: Cultivation, nutritional value, medicinal effect. 2nd ed. CRC Press; 2004.
2. Sánchez C. Cultivation of *Pleurotus ostreatus* and other edible mushrooms. Appl Microbiol Biotechnol. 2010;85(5):1321-37.
3. Patil SS, Ahmed SA, Telang SM, Baig MMV. The nutritional value of mushrooms. J Sci Ind Res. 2010;69(4):324-9.
4. Royse DJ. Influence of spawn rate and strain on the productivity of *Pleurotus ostreatus*. Mycologia. 2003;95(3):398-402.
5. Hoa HT, Wang CL. The effects of different substrates on the growth, yield, and nutritional composition of *Pleurotus ostreatus*. Mycobiology. 2015;43(4):423-34.
6. Lehmann J, Joseph S. Biochar for environmental management. Earthscan; 2009.
7. Glaser B, Lehmann J, Zech W. Ameliorating physical and chemical properties of soils with biochar. Biol Fertil Soils. 2002;35(4):219-30.
8. Chan KY, Van Zwieten L, Meszaros I, Downie A, Joseph S. Agronomic values of greenwaste biochar. Soil Res. 2007;45(8):629-34.
9. Jaafar NM, Ismail M, Mahmud TMM. Biochar as a substrate amendment for mushroom cultivation. J Trop Agric Food Sci. 2015;43(2):197-206.
10. Uzun I. Substrate supplementation for mushroom cultivation. Sci Hortic. 2004;101(1-2):1-6.
11. Ahlawat OP, Tewari RP. Physiology and cultivation of *Pleurotus* species. Indian Mushroom Sci. 2007;17(1):1-20.
12. Yoshida N, Antal MJ. Influence of pyrolysis temperature on biochar characteristics. Ind Eng Chem Res. 2007;46(19):5959-66.
13. Demirbas A. Effects of temperature and particle size on biochar properties. Energy Sources. 2004;26(8):715-26.
14. Sharma VP, Annepu SK. Advances in oyster mushroom cultivation. Directorate of Mushroom Research, India; 2012.
15. Bhattarai R, Karki N, Shakya S, Dhakal RP, Poudel P. Potential application of biochar as a growth supplement for mushroom cultivation (*Pleurotus ostreatus*). Int J Hortic Food Sci. 2024;6(1):21-26. DOI: 10.33545/26631067.2024.v6.i1a.181.
16. Zeng X, Li R, Yu H. Biochar effects on microbial activity and substrate properties. Bioresour Technol. 2013;144:642-9.
17. Eguchi F, Hitoshi M, Takao S. Mycelial growth response to substrate aeration. Mycol Prog. 2010;9(3):273-80.