

Evaluation of Agricultural Waste Substrates on Growth and Yield Performance of Oyster Mushroom (*Pleurotus ostreatus*)

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Abstract

Oyster mushroom (*Pleurotus ostreatus*) is a commercially important edible fungus valued for its nutritional quality and capacity to grow on diverse lignocellulosic agricultural residues. This study evaluated seven agricultural waste substrates: teff straw, barley straw, wheat straw, grass straw, enset leaf straw, cow dung, and a combined substrate mixture on the growth, yield, performance, and biological efficiency (BE) of *P. ostreatus* under laboratory conditions. A completely randomized design with three replications per treatment was employed. Parameters assessed included mycelial colonization period (MCP), primordia initiation time, pileus diameter, fresh yield per bag, and BE. One-way analysis of variance with Tukey's Honestly Significant Difference post-hoc test ($\alpha = 0.05$) was performed using SPSS v.27. Significant differences among substrates were observed for all parameters ($P \leq 0.05$). MCP ranged from 18.00 ± 0.00 days (combined substrate) to 25.00 ± 0.00 days (enset leaf straw). Primordia initiation was earliest in the combined substrate (17.00 ± 0.01 days) and latest in grass straw (27.33 ± 1.53 days). Fresh yield varied from 50.42 ± 0.40 g/bag (teff straw) to 103.98 ± 0.64 g/bag (combined substrate). BE ranged from $54.07 \pm 0.07\%$ (teff straw) to $86.70 \pm 0.22\%$ (enset leaf straw). The combined substrate excelled in colonization speed and absolute yield, while enset leaf straw demonstrated superior substrate-to-biomass conversion efficiency. These findings establish that locally available agricultural residues, especially substrate blends, constitute viable low-cost alternatives for sustainable oyster mushroom production in highland Ethiopia.

Categories: Food security and sustainability, Food nutrition, Aquaculture for sustainable food production

Keywords: mycelial colonization, oyster mushroom, *pleurotus ostreatus*, agricultural waste, biological efficiency

Introduction

Oyster mushroom (*Pleurotus ostreatus*) is among the most widely cultivated edible fungi worldwide, esteemed for its distinctive organoleptic properties, high protein content (15-30% of dry weight), dietary fiber, essential vitamins (B-complex, D), and minerals such as potassium, phosphorus, and iron [1,2]. Beyond its nutritional attributes, *P. ostreatus* possesses well-documented bioactive compounds conferring antioxidant, anti-inflammatory, and immunomodulatory properties, rendering it a functional food of considerable nutraceutical value [3]. The cultivation of *P. ostreatus* offers significant economic and environmental advantages. Its capacity to efficiently degrade complex lignocellulosic polymers, cellulose, hemicellulose, and lignin through the secretion of laccase, manganese peroxidase, and lignin peroxidase enzymes positions it as a biotechnological tool for valorizing agricultural residues [4,5]. Short production cycles (typically

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45-60 days), low capital requirements, and simple cultivation technology make oyster mushroom production particularly suitable for smallholder farmers and rural enterprises in low- and middle-income countries [4-6]. Substrate composition is the most influential factor governing the growth rate, yield, and biological efficiency of *Pleurotus* spp. The carbon-to-nitrogen (C: N) ratio, lignin and cellulose concentrations, bulk density, water-holding capacity, and particle size collectively determine substrate accessibility and nutrient availability for mycelial growth and fruiting body development [7]. Numerous substrates-including wheat straw, rice straw, cotton waste, sawdust, sugarcane bagasse, corn cobs, and cassava residues-have been evaluated in various geographic contexts, yielding differential performances in colonization rate, primordia initiation, fresh yield, and biological efficiency [7,8]. Substrate blending or supplementation with nitrogen-rich materials consistently improves productivity relative to single-substrate systems by optimizing the nutrient matrix and physical properties of the growing medium [9].

Despite growing research interest, a substantial knowledge gap persists regarding the performance of regionally specific agricultural residues, particularly those endemic to Ethiopian highland farming systems [10]. Locally abundant substrates such as enset (*Ensete ventricosum*) leaf straw and indigenous grass residues have received minimal scientific attention as oyster mushroom cultivation media. Identifying locally adapted, cost-effective substrate alternatives is imperative for scaling up mushroom production in southern Ethiopia, where agricultural residues are abundantly generated but largely underutilized, and where protein-energy malnutrition remains a public health concern [11]. This study was designed to evaluate the growth performance and yield attributes of *P. ostreatus* on seven locally available agricultural waste substrates under controlled laboratory conditions. Specifically, this investigation aimed to: (i) determine the effect of substrate type on mycelial colonization period (MCP), primordia initiation period (PIP), pileus diameter, fresh yield, and biological efficiency (BE); (ii) identify substrates or blends offering superior performance for specific production objectives; and (iii) provide evidence-based recommendations for sustainable, low-input oyster mushroom cultivation in the Ethiopian highland context.

Materials And Methods

Study area and environmental conditions

The experiment was conducted in the Biotechnology Mushroom Cultivation Laboratory of Wachemo University, Hossana, Ethiopia (7°33'N, 37°51'E; altitude approximately 2,300 m above sea level), located approximately 232 km south of Addis Ababa. The facility provided controlled ambient conditions reflecting the cool highland climate of the region (mean temperature 20-25°C; relative humidity 70-80%). The surrounding landscape is characterized by mixed crop-livestock farming systems, generating abundant and diverse agricultural residues.

Experimental design

A completely randomized design (CRD) was employed, consisting of seven substrate treatments each replicated three times, yielding 21 experimental units. The seven treatments were: (1) teff straw, (2) barley straw, (3) wheat straw, (4) grass straw, (5) enset leaf straw, (6) cow dung, and (7) combined substrate mixture (equal proportions by dry weight of all six individual substrates). Each experimental unit consisted of 1.5 kg dry weight equivalent of substrate packed in a polyethylene bag. All bags were incubated under uniform environmental conditions throughout the colonization phase to minimize non-treatment variability [12].

Spawn preparation

Grain spawn of *P. ostreatus* was prepared using sorghum (*Sorghum bicolor* L.) grains following standard protocols [13,14]. Five kilograms of sorghum grains were cleaned, washed, and soaked in clean water for 12 hours. Excess water was drained, and grains were mixed with calcium sulfate (gypsum) at 2% (w/w) to prevent agglomeration and stabilize pH. Moisture content was adjusted to approximately 50-55%. Grains (250 g/bag) were filled into heat-resistant polypropylene bags, sealed, and sterilized at 121°C for 20 minutes. After cooling to 25 ± 2°C under aseptic conditions, sterilized grains were inoculated with pure *P. ostreatus* culture maintained on potato dextrose agar (PDA) and incubated at 25 ± 2°C for 14 days until complete mycelial colonization confirmed spawn readiness [15].

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Substrate collection, preparation, and sterilization

Agricultural residues (teff straw, barley straw, wheat straw, grass straw, and enset leaf straw) were collected from farms around Hossana, Ethiopia. Visible contaminants were removed manually. Straws were chopped into 3-7 cm pieces to increase surface area and improve aeration. Cow dung was air-dried and crushed to a fine texture. For the combined substrate, the six individual substrates were blended in equal proportions (1:1:1:1:1:1 by dry weight) [16]. All substrates were soaked in clean water for 12 hours to achieve uniform hydration. Excess water was drained, and the moisture content adjusted to 65-70% (verified by the hand-squeeze method). Approximately 1.5 kg (dry weight equivalent) of substrate per bag was filled into transparent, heat-resistant polyethylene bags in a layered, lightly compacted form to maintain porosity. Bags were sealed with cotton plugs, covered with aluminum foil, sterilized at 121°C for 30 minutes, and cooled to room temperature ($25 \pm 2^\circ\text{C}$) in a laminar flow hood before inoculation [17].

Inoculation, incubation, fruiting, and harvesting

Sterilized substrate bags were inoculated with grain spawn at 10% (w/w, wet basis) under aseptic conditions. Spawn was applied in three equal layers (bottom, middle, and top thirds) to ensure uniform vertical distribution. Bags were sealed and perforated with sterile holes (0.5-1.0 cm diameter) to facilitate gas exchange. Inoculated bags were incubated in a dark room at $25 \pm 2^\circ\text{C}$ and 70-80% relative humidity and monitored daily for colonization progress and primordia development [17,18]. Upon complete colonization, bags were transferred to a fruiting room with diffuse natural light (12 h photoperiod), temperature 22-26°C, relative humidity 85-95%, and adequate ventilation. Humidity was maintained by manual misting twice daily. Mushroom fruiting bodies were harvested manually at optimal maturity (before cap in rolling and spore release) by gently twisting the entire cluster at its base [19].

Fruiting and harvesting

After complete colonization, bags were transferred to a fruiting room with controlled environmental conditions (diffused light, high humidity, and adequate ventilation). Fruiting bodies were allowed to develop naturally. Mature mushrooms were harvested manually by gently twisting to avoid damage to the substrate. Mushroom was harvested at optimal maturity before spore release [19].

Data collection and measurements

The following parameters were recorded for each experimental unit. MCP: Days from inoculation to complete dense white mycelial coverage of the substrate. PIP: Days from inoculation to first visible pinhead formation (≥ 1 mm diameter). Time to harvest (TTH): Days from inoculation to first harvest. Pileus diameter (PD, cm): Mean diameter of three representative mature fruiting bodies per unit, measured with a digital vernier caliper. Total fresh yield (FY, g/bag): Cumulative fresh mass of all harvested mushrooms per bag, measured immediately after harvest. BE (%) was calculated as in Tarko and Sirna [20]:

$$\text{Biological efficiency} = (\text{weight of fresh mushroom harvested (g)}) / (\text{weight of dried substrate (g)}) \times 100$$

where the dry substrate weight was 1,500 g per experimental unit.

Statistical analysis

All results are expressed as mean $\pm$ SD of three replicates. Data were analyzed using IBM SPSS Statistics v.27.0. One-way Analysis of Variance (ANOVA) was performed for each parameter, followed by Tukey's Honestly Significant Difference (HSD) post-hoc test for mean separation ($\alpha = 0.05$). Means sharing the same superscript letter within a column are not significantly different.

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Results And Discussion

Mycelial colonization, primordia initiation, and harvest period

Substrate type exerted a significant effect ($P < 0.05$) on all temporal growth parameters of *P. ostreatus* (Table 1). Figure 1 illustrates the sequential cultivation stages from substrate inoculation through complete mycelial colonization and early primordial initiation, providing visual documentation of these critical developmental transitions.

Substrate treatment	Mycelial colonization period (days)	Primordia initiation period (days)	Time to harvest (days)
Teff straw	21.67 ± 0.0 ^{8b}	19.33 ± 1.03b	24.00 ± 0.00 ^a
Barley straw	22.00 ± 1.00 ^b	18.67 ± 0.53bc	24.00 ± 0.58 ^a
Grass straw	24.67 ± 1.53 ^c	27.33 ± 1.53a	24.00 ± 0.00 ^a
Wheat straw	18.33 ± 0.53 ^a	18.67 ± 1.23bc	24.00 ± 0.00 ^a
Cow dung	22.34 ± 2.08 ^b	21.00 ± 1.04bc	25.00 ± 1.00 ^a
Enset leaf straw	25.00 ± 0.00 ^c	21.00 ± 1.73bc	24.00 ± 0.00 ^a
Combined substrate	18.00 ± 0.00 ^a	17.00 ± 0.01c	24.00 ± 0.00 ^a

TABLE 1: Table 1. Temporal growth parameters of *Pleurotus ostreatus* on seven agricultural waste substrates.

Values are mean ± SD ($n = 3$). Within a column, means followed by different superscript letters differ significantly at $\alpha = 0.05$ (Tukey's HSD).

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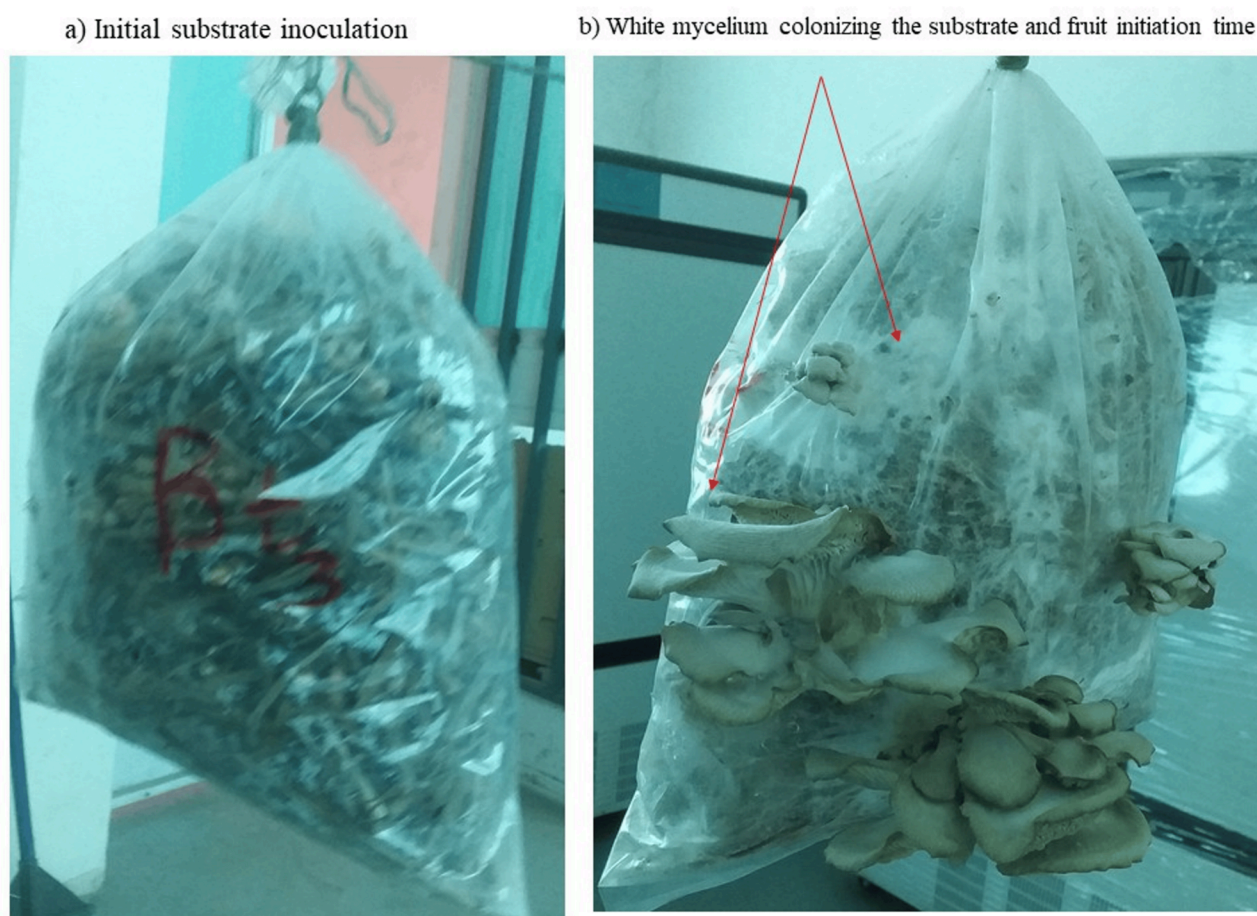


FIGURE 1: Substrate colonization and fruiting body development. (a) Appearance of the cultivation bag immediately following initial substrate inoculation. (b) Complete colonization of the substrate by dense white mycelium and subsequent fruit body initiation. Red arrows indicate areas of active mycelial growth (top) and early primordia formation (left).

The combined substrate achieved the fastest complete mycelial colonization (18.00 ± 0.00 days), followed closely by wheat straw (18.33 ± 0.53 days), while enset leaf straw (25.00 ± 0.00 days) and grass straw (24.67 ± 1.53 days) required the longest colonization periods. The rapid colonization of the combined substrate is likely attributable to improved bulk porosity, enhanced aeration, and a more balanced C:N ratio that collectively facilitated oxygen diffusion and efficient enzymatic hydrolysis across a diverse range of accessible carbohydrate fractions [21,22]. Wheat straw's comparable performance reflects its well-characterized cellulose-to-lignin ratio and moderate nitrogen content, which together support efficient ligninolytic enzyme activity [22].

The prolonged colonization of enset leaf straw and grass straw is consistent with the presence of recalcitrant lignocellulosic polymers, tannic compounds, and high silica content that mechanically and chemically restrict enzymatic substrate attack [23]. Primordia initiation followed the same pattern, with the combined substrate yielding the earliest pinhead formation (17.00 ± 0.01 days) and grass straw the latest (27.33 ± 1.53 days). This is physiologically consistent: faster colonization equates to earlier attainment of the carbon and nutrient thresholds triggering the vegetative-to-reproductive transition in *Pleurotus* spp. [24]. Despite large differences in colonization and primordia initiation, time to harvest was nearly uniform across treatments (24-25 days), suggesting that once primordia are formed, downstream morphogenesis is governed more by microclimate than substrate composition [25].

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Morphological characteristics: pileus diameter

PD differed significantly among treatments ($P \leq 0.05$) (Figure 2; Table 2). The largest fruiting bodies were recorded on cow dung (6.84 ± 0.22 cm), significantly exceeding all other substrates except barley straw (5.35 ± 0.21 cm) and wheat straw (5.18 ± 0.05 cm). Grass straw produced the smallest pileus diameter (3.80 ± 0.17 cm). The superior cap dimensions observed in cow dung are attributable to its elevated nitrogen concentration and rich mineral profile (phosphorus, potassium, calcium, magnesium), which promote protein synthesis and osmotically driven cellular expansion during carpophoresis [22–25]. Importantly, the substrate producing the largest individual fruiting bodies did not yield the highest cumulative fresh mass, indicating trade-offs between individual fruit body size and aggregate productivity [26].

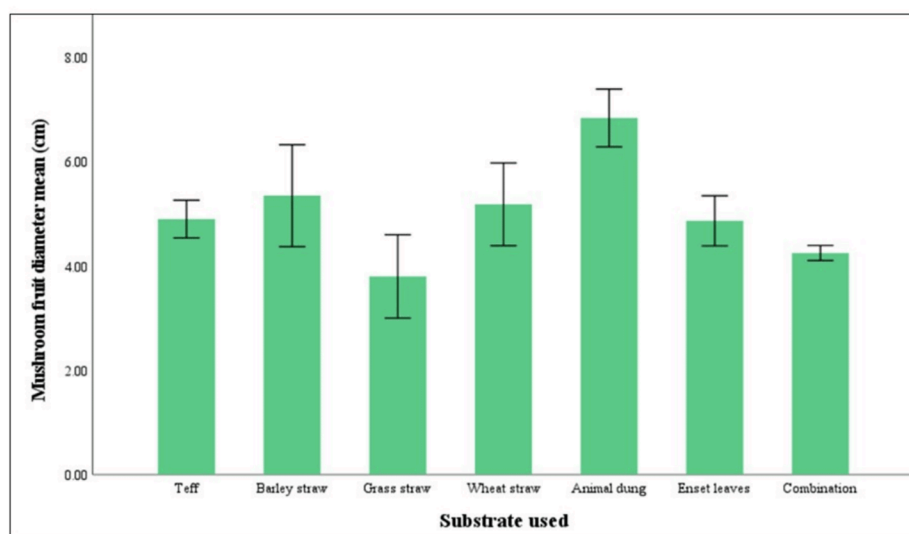


FIGURE 2: The mean value of mushroom fruit diameter (cm) on different substrates used.

Error bars represent standard deviation ($n = 3$).

Fresh yield performance

Substrate type exerted a highly significant effect on fresh mushroom yield per bag ($P \leq 0.05$; Figures 3 and 4; Table 2). The combined substrate formulation yielded the highest fresh biomass (103.98 ± 0.64 g/bag), representing approximately a 2.06-fold advantage over the lowest-yielding substrate, teff straw (50.42 ± 0.40 g/bag). Cow dung ranked second (91.34 ± 0.12 g/bag), followed by enset leaf straw (81.56 ± 0.35 g/bag) and wheat straw (79.34 ± 0.55 g/bag).

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FIGURE 3: Substrate used for mushroom growth and mature mushroom grown on the substrate. The photographs show the range of locally available agricultural residues evaluated and representative mature *P. ostreatus* fruiting bodies.

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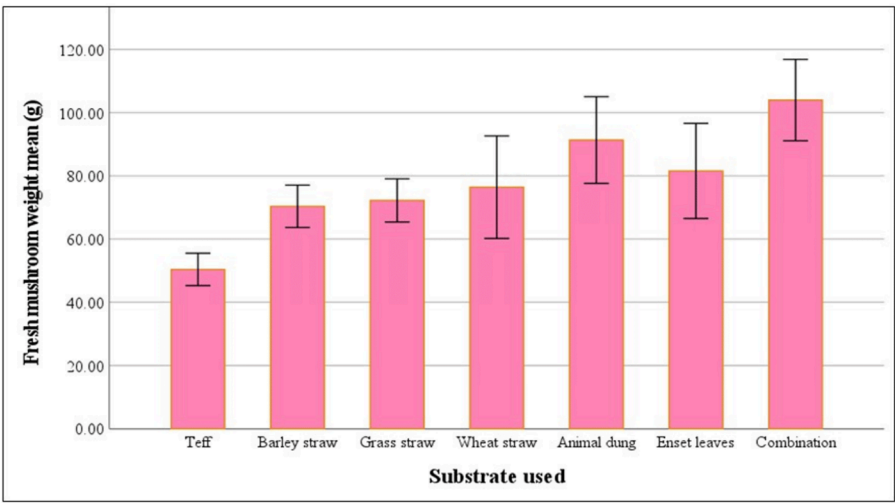


FIGURE 4: Fresh mushroom weight mean (g), measured for all tested substrates.

Error bars represent standard deviation ($n = 3$).

Biological efficiency

Biological efficiency varied significantly across substrate treatments ($P \leq 0.05$; Figure 5; Table 2). Enset leaf straw recorded the highest BE ($86.70 \pm 0.22\%$), marginally exceeding the combined substrate ($85.90 \pm 0.10\%$) and wheat straw ($82.53 \pm 0.60\%$), while teff straw recorded the minimum BE ($54.07 \pm 0.07\%$). The higher BE of enset leaf straw despite lower absolute yield indicates superior dry-matter bioconversion efficiency per unit of dry substrate—a larger fraction of the available substrate carbon and nitrogen was assimilated into fruiting body biomass [25,26]. This dissociation has important practical implications: growers prioritizing maximum food output should select the combined substrate, while those seeking maximum biological value per unit of a single locally available material may preferentially utilize enset leaf straw or wheat straw.

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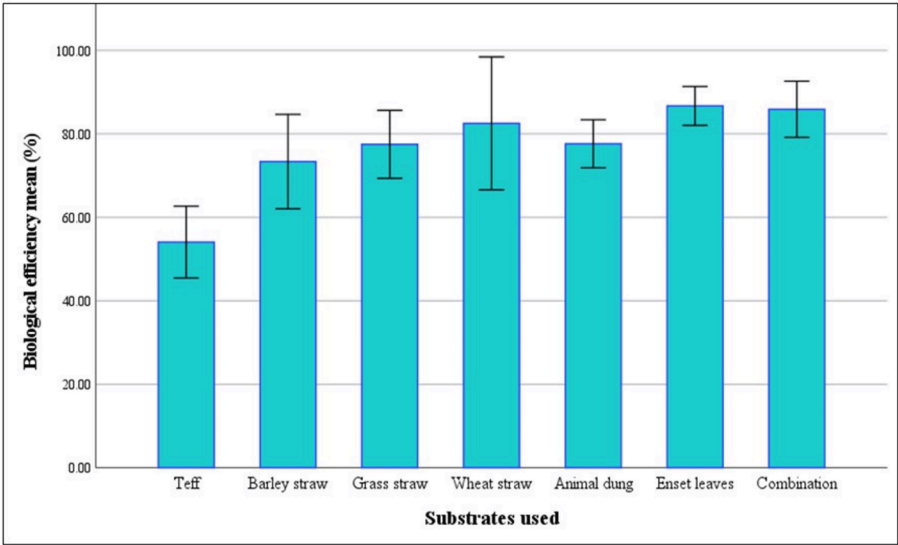


FIGURE 5: Biological efficiency mean (%) for substrates used for mushroom cultivation.

Error bars represent standard deviation ($n = 3$).

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Substrate treatment	Pileus diameter (cm)	Fresh yield (g/bag)	Biological efficiency (%)
Teff straw	4.52 ± 0.18 ^c	50.42 ± 0.40 ^e	54.07 ± 0.07 ^e
Barley straw	5.35 ± 0.21 ^b	68.44 ± 0.52 ^d	73.58 ± 0.14 ^d
Grass straw	3.80 ± 0.17 ^d	65.00 ± 0.45 ^d	57.62 ± 0.32 ^e
Wheat straw	5.18 ± 0.05 ^b	79.34 ± 0.55 ^c	82.53 ± 0.60 ^b
Cow dung	6.84 ± 0.22 ^a	91.34 ± 0.12 ^b	74.30 ± 0.30 ^d
Enset leaf straw	4.88 ± 0.14 ^{bc}	81.56 ± 0.35 ^c	86.70 ± 0.22 ^a
Combined substrate	5.02 ± 0.10 ^{b^c}	103.98 ± 0.64 ^a	85.90 ± 0.10 ^{ab}

TABLE 2: Morphological and yield parameters of *P. ostreatus* on seven agricultural waste substrates

Values are expressed as mean ± standard deviation (n = 3). Superscript letters (a–e) indicate statistical groupings determined by Tukey’s Honestly Significant Difference (HSD) post-hoc test (α = 0.05). Within each column, mean values sharing the same superscript letter do not differ significantly (p > 0.05). Distinct letters represent statistically significant differences (p ≤ 0.05), where 'a' denotes the highest performance value and 'e' denotes the lowest performance value within that specific column.

The suppressed BE values in teff straw (54.07%) and grass straw (57.62%) indicate poor enzymatic digestibility and suboptimal nutrient profiles, confirming their limited utility as mono-substrates. The high residual substrate mass after harvest in these treatments suggests substantial unassimilated lignocellulosic fractions, which could potentially be supplemented or co-composted to improve future utilization [11]. Wheat straw’s robust BE (82.53%) is consistent with its widely established performance as a reference substrate for *P. ostreatus* cultivation globally [24-26].

Conclusions

This study demonstrates that agricultural waste substrate type significantly governs all growth, morphology, and yield parameters of *P. ostreatus* under controlled highland Ethiopian conditions. Four principal conclusions emerge. First, the combined substrate optimized the physical and nutrient environment for mycelial development, achieving the shortest colonization period, the earliest primordial initiation, and the highest fresh yield, confirming that substrate complementarity through blending is an effective strategy for maximizing productivity. Second, enset leaf straw, a locally abundant and largely underutilized residue endemic to southern Ethiopia, exhibited the highest biological efficiency (86.70%), warranting further investigation as a cost-effective single-substrate cultivation medium. Third, cow dung supported the largest individual pileus diameter, reflecting its elevated mineral and nitrogen content, but this morphological advantage did not translate proportionally to maximum cumulative yield. Fourth, teff straw and grass straw consistently underperformed across most parameters, limiting their stand-alone utility as oyster mushroom substrates, though their potential value as blend components deserves further evaluation. Future research should prioritize: (i) physicochemical substrate characterization (C:N ratio, lignin, cellulose, hemicellulose content) to mechanistically explain the observed differences; (ii) optimization of mixing ratios in combined substrates; (iii) multi-flush

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yield assessment to determine cumulative productivity over complete growing cycles; (iv) economic cost-benefit analysis under smallholder farming conditions; and (v) nutritional profiling of mushrooms from highest-performing substrates to assess dietary value implications.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Acquisition, analysis, or interpretation of data: Daniel Mulu Mengistu, Bekele Chakiso Gugero, Solomon Berhanu Sharo

Drafting of the manuscript: Daniel Mulu Mengistu, Bekele Chakiso Gugero, Solomon Berhanu Sharo

Critical review of the manuscript for important intellectual content: Daniel Mulu Mengistu

Supervision: Daniel Mulu Mengistu

Concept and design: Bekele Chakiso Gugero, Solomon Berhanu Sharo

Disclosures

Plant subjects: All authors have confirmed that this study did not involve any herbarium specimen. **Human subjects:** All authors have confirmed that this study did not involve human participants or tissue. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Data Availability Statements

The datasets (and/or code) supporting this study are available from the corresponding author upon reasonable request.

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