Research Square

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Optimisation of different agro-waste substrates and their effects on the yield and nutritional composition of oyster mushrooms in northern Uganda

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Abstract

Background

Food insecurity and malnutrition remain major challenges in developing countries, necessitating nutrient-rich functional foods. Oyster mushrooms (*Pleurotus ostreatus*) are highly adaptable, sustainable, and can be cultivated on agro-waste substrates, offering both nutritional and environmental benefits. This study evaluated the yield, proximate composition, mineral and vitamin content, and biological efficiency of oyster mushrooms grown on four locally available substrates.

Materials and Methods

A completely randomised design (CRD) was employed with four treatments and three replications. The four locally made substrates were sorghum husk + millet husk (A); maize cob + maize straw (B); dried banana fibre + banana leaves (C); paspalum grass + guinea grass (D), each supplemented with 1% lime. Yield data were collected in three phases for three parameters: weight, number of flushes, and diameter. Nutritional composition, including proximate, mineral, and vitamin content, was analysed using standard methods. Data were analysed using SPSS version 26. One-way ANOVA was used, at 95% confidence interval.

Results

Of the four substrate formulae, substrate D failed to yield completely. Mean weight was significantly highest in second harvest for substrate B (151.50 ± 3.11g) ($p < 0.05$). B also had the highest crude protein (29.55%) and ash (9.46%) contents, but the lowest Nitrogen Free Extract ($p < 0.05$). B was significantly higher in Zn (36.76 ± 0.01 mg/kg), substrate A was significantly higher in Ca (496.22 ± 17.08 mg/kg), and substrate C was significantly higher in vitamins A (365.61 ± 37.53µg) and D (1024.39 ± 12.83µg) ($p < 0.05$).

Conclusions

Substrate composition affects mushroom yield and nutritional quality. This study provides a practical strategy for improving food security. Treatment A should be promoted for protein and mineral enrichment, while Treatment C is recommended for higher vitamin content.

Introduction

There is a significant gap between food production and the rapidly increasing population demands in various parts of the world, especially in developing countries [1]. This imbalance raises the risk of malnutrition, resulting in a higher incidence of diseases and increasing the need for functional foods that can help reduce morbidity and mortality. For example, the growing population in these regions places immense pressure on existing food systems, which are often inefficient and unsustainable [2]. Africa, in particular, lags significantly in mushroom production and trade, contributing less than 1% to the global market; this low contribution reflects the region's underutilisation of an economically and nutritionally valuable resource [3]. In some southern African countries, mushroom production technology is almost nonexistent, while in other regions, production remains minimal due to a lack of knowledge, inadequate training, limited access to quality spawn, and insufficient government support, all of which have hindered the growth of the mushroom industry across the continent [2]. However, mushrooms have the potential to become a sustainable and profitable agricultural venture, particularly for smallholder farmers who often face land and resource constraints. Expanding mushroom farming in Africa could create new income opportunities, enhance food security, and contribute to rural development. With proper investment and training, mushroom cultivation could become an accessible means of improving dietary diversity and addressing micronutrient deficiencies in African communities [4]. The oyster mushroom (*Pleurotus ostreatus*) is the third most produced and consumed mushroom worldwide and is considered the second largest commercially produced mushroom after *Agaricus bisporus*. This ranking underscores its importance in global mushroom production and trade. Oyster mushrooms are highly adaptable and can be cultivated using various organic waste materials, making them a sustainable and eco-friendly food source [5]. Their cultivation requires minimal inputs, making them suitable for both small-scale and large-scale farming operations. They can grow on agricultural residues such as straw, corn cobs, and sorghum husk, which also makes them an excellent option for resource-poor farmers seeking to maximise productivity with low investment costs [6]. Furthermore, oyster mushrooms have a relatively short growth cycle, allowing farmers to achieve multiple harvests within a year, thus ensuring a steady food supply and income generation [7]. *Pleurotus ostreatus* represents 19% of global mushroom production, surpassed only by Lentinula edodes (Shiitake), which accounts for 22%. This significant market share highlights the growing demand for oyster mushrooms worldwide, encouraging more farmers in Tropical Africa to engage in mushroom production and helping the region tap into the lucrative global

mushroom market while addressing local food security challenges [8]. Additionally, integrating mushroom farming with other agricultural activities, such as livestock feeding and compost production, can create additional value chains that enhance economic sustainability [3]. Mushrooms, including *Pleurotus ostreatus*, belong to the kingdom Fungi and the order Agaricales [4]. They are achlorophyllous, spore-producing macro-fungi with a heterotrophic mode of nutrition, typically growing above ground with structures including a cap, stem, and hymenium. They are often referred to as the "white vegetable," the "queen of vegetables," or "boneless vegetarian meat" due to their high protein content and meat-like texture [9]. These characteristics make them an excellent dietary alternative for vegetarians and individuals seeking healthier protein sources. Mushroom cultivation is particularly beneficial for small-scale farmers, as it requires relatively little space and can be implemented using shelf-like culture systems such as mud houses. Black shade nets (with 60% density) can create the necessary growing conditions, including moisture and temperature control. These simple yet effective techniques enable farmers to cultivate mushrooms even in resource-limited settings [10]. Ultimately, the findings of this research could contribute to the widespread adoption of mushroom farming as a viable solution to food insecurity and agricultural waste management [11]. Oyster mushroom cultivation relies on nutrient-rich, accessible substrates for growth. Traditional substrates such as millet straw, sawdust, and coffee grounds are commonly used [12], though they vary in cost, availability, and nutrient content, affecting yield and quality. Farmers seek cost-effective substrates that ensure high productivity without sacrificing quality. The ideal substrate would be locally available, nutrient-dense, and affordable. Common options include dry grasses such as guinea grass (*Megathyrsus maximus*) and paspalum grass (*Paspalum notatum*), dry banana leaves and banana pseudostems (*Musa* spp.), maize cob (*Zea mays*), millet husk (*Eleusine coracana*), and sorghum husk (Sorghum bicolor). Thus, finding the most effective substrate requires both research and adaptability to environmental conditions and resource availability [13]. Many studies overlook locally available substrates, often focusing on contexts that may not suit local needs [14]. Research into materials rationing, such as mixing maize cob with straw, banana leaves, and pseudostems to form substrates, is limited in the literature. Urban areas like Lira City face challenges in managing agricultural waste, leading to pollution and sanitation risks. Peri-urban mushroom farming, which requires little space, could use urban agricultural waste as a substrate. Addressing this issue requires a cost-effective, high-yield substrate and a robust cost-optimisation strategy for profitable, sustainable mushroom production. This study aimed to formulate and optimise a locally available, affordable substrate for effective oyster mushroom farming

Materials and Methods

Study Design

The study employed a Completely Randomised Design (CRD) to compare the performance (yield and nutrient profiles) of oyster mushrooms grown on different formulated substrates [15]. Four treatments were included: two known to produce good yields (sorghum husk mixed with millet husk, and maize cob mixed with maize straw), both of which served as controls. The other two treatments – grasses and dry banana leaves and fibre – were tested to determine if they produced yields comparable to the control substrates. Paspalum grass represented all grasses, while guinea grass, dried banana fibre, and dried banana leaves were used in oyster mushroom production, each mixed in a ratio of 50% substrate plus 1% gypsum per treatment. All treatments were managed under identical conditions, regardless of environmental factors such as humidity, temperature, wind, and sunlight [15].

Material and equipment for the experiment

Oyster mushroom spawn

The oyster mushroom spawn used in this study was obtained from Hello Mushrooms Uganda Limited, Plot 1360 Kyebando Ring Road, Kampala -Uganda. It is a reputable commercial dealer of mushroom spawns.

Plain papers and rubber band

They were all used to tie and cover both ends of the mushroom bags [16].

Robes

Polyethene robes were used to tie and suspend the mushroom gardens or treatment in the house.

Disinfectant (JIK)

The disinfectant was used to sterilize the house fully to prevent infections and insects

Panga

This was used to chop the crop residues into 2cm to 3cm pieces

Water

Water was used to soak the materials for example; dry guinea grass, dry paspalum grass, dry banana leaves, dry banana fiber, maize cob, millet husk, and sorghum husk to ensure the materials absorb moisture and make nutrients readily available for the growth of mushrooms. The materials were all soaked for 24 hours [17].

The substrate materials had to be adequately hydrated to support mycelium growth. Oyster mushrooms needed a moist environment, and the substrate typically had a moisture content of around 60–70% [16]with proper hydration to ensure mycelium could access water as it spread through the substrate, because water helped dissolve and transport nutrients within the substrate, allowing the mushroom mycelium to access essential minerals and compounds more efficiently [18], [19].

Mushroom bags

These were white wide polyethylene bags that measured 8 inches; they were cut into pieces to accommodate 1 kg of the substrate mixture [14].

Agricultural Lime (Ash or Calcium Sulfate)

10 grams of lime (ash) per kilogram of substrate were added to the materials being soaked in water. This was to ensure the pH of the materials was neutralized for proper colonization of the dry grass, for example [20].

Sterilization equipment

The substrate was sterilized in boiling water while being stirred with a mingling stick for one hour to eliminate other microbial elements that could affect mushroom growth [18].

Structure of the house

The mushroom house was provided with an environment that supported the optimal growth conditions for mushrooms, particularly in terms of temperature, humidity, ventilation, and light control [21]. The house was covered with materials that provided good insulation to maintain temperatures between 20–24°C (68–75°F) and to retain moisture, such as wood and papyrus mats, with openings for natural or mechanical ventilation to regulate airflow [22]. The floor was fully covered with charcoal dust because it helped maintain moisture for the proper growth of the mushrooms and was sterilized with formalin diluted with water to avoid contaminants at least one time per week [18].

Substrate Treatment

There were four different treatments: Sorghum husk + Millet husk (A), Maize cob + Maize straw (B), Dried banana fiber + dried banana leaves (C) and Paspalum grass + Guinea grass (D) as shown in Table 1.

Table 1
Shows the substrate treatment composition used in the study

Treatment	Mixtures (50% each)	Ash (Lime)
A	Sorghum husk + Millet husk	1%
B	Maize cob + Maize straw	1%
C	Dried banana fiber + dried banana leaves	1%
D	Paspalum grass + Guinea grass	1%

1% = 10g in every 100Kg of ash (lime)

As shown in Table 1, 1% of ash was added to every substrate treatment, in which each treatment was equal to 1kg. The weight of the calcium sulfate was negligible because it was applied in liquid form, and later the substrate was allowed to drip as explained in the process of substrate preparation.

Biological efficiency

Biological efficiency (%) = Weight of fresh mushrooms harvested per packet/treatment divided by weight of dry substrate per bag, all multiplied by 100 [16]

Preparation of substrate

Dry grasses, that is, *Paspalum spp.* and *Megathyrsus maximus*, dry banana leaves, dry banana fiber, millet husk, and sorghum husk were collected from within or around Bar-Apwo from the local farmers, ensuring they were physically free from contamination, mold, or pests. All the residues were thoroughly dried to a little or no moisture content (0% to 15%). Dry grasses *Megathyrsus maximus*, *Paspalum spp.*, dry banana leaves or fiber (*Musa spp.*), maize cob, and maize straw (*Zea mays*), millet husk (*Eleusine coracana*), and sorghum husk (*Sorghum bicolor*) were chopped into (1–2) cm in size to increase surface area for easy colonization[17]. The chopped materials were put in a large container and soaked in water for 12–24 hours. This helped to soften the dry materials and made them more suitable for the mycelium of the oyster mushroom growth [11]. Hydrated lime (hydrated ash, which could also be gypsum) was added to the soaking water of the dry materials. Lime raised the pH of the substrate, reducing the risk of contamination by undesirable microorganisms. A big source pan for cooking the substrate was used for up to about an hour to eliminate unnecessary bacteria, molds, and other microorganisms. This ensured that all the unwanted conditions were cleared [8]. Big, wide white polyethylene mushroom garden bags were washed with soap to remove and kill any unwanted microorganisms and were later sanitized to ensure proper hygiene of the mushroom bags [11]. The cooked materials were allowed to cool to environmental temperature as they were placed in a clean sieve to drip and drain. The moisture content was maintained around 60–65%, meaning when a handful of the substrate was squeezed, not a drop of water came out. The substrate was moist but not wet [8]. The substrate was packed into clean, big, sterilized wide white mushroom bags, ensuring it was not tightly packed to allow for air exchange and to avoid compacting the materials, and the top was covered with a piece of paper tied with a rubber band [23]. At each 5 cm layer of substrate, mushroom spawn was added to ensure all the substrate was evenly colonized. The gardens were then hung in a cool, dark room for about two weeks until all the substrate was colonized [12].

Data Collection

As shown in Table 2, all treatments were replicated four times, and data were collected based on the parameters clearly shown in Table 2.

Table 2
Showing how the data was collected from different parameters

Treatment	Criteria	X_1 replica	X_2 replica	X_3 replica	X_4 replica
A	Weight (Kg) =				
	Number of flushes/ caps =				
	Diameter of caps =				
B	Weight (Kg) =				
	Number of flushes/ caps =				
	Diameter of caps =				
C	Weight (Kg) =				
	Number of flushes/ caps =				
	Diameter of caps =				
D	Weight (g) =				
	Number of Flushes/ caps =				
	Diameter of caps =				

Data Analysis

The data collected were analyzed using the Statistical Package for Social Sciences (SPSS) version 26. Descriptive statistics was presented as mean, standard deviation, and median. Graphs were used to illustrate the levels of performance. One-way analysis of

variance (ANOVA) was conducted to assess significant differences in performance across the substrates. Post-hoc analysis was performed using Tukey HSD to determine where the actual differences occurred. All tests were conducted at a 95% confidence level and a 5% level of significance.

Table 3
Effect of different substrate formulae on yield of oyster mushroom

Treatment	First Harvest			Second Harvest			Third Harvest		
	Weight	Flushes	Diameter	Weight	Flushes	Diameter	Weight	Flushes	Diameter
A	130.25 ± 19.99^{a}	21.75 ± 4.27^{a}	5.14±.91^{a}	140.75 ± 17.46^{a}	25.75 ± 2.75^{a}	5.54±.83^{a}	119.25 ± 17.50^{a}	20.25 ± 7.46^{a}	5.57±.66^{a}
B	107.0 ± 8.87^{a}	25.50 ± 2.08^{a}	4.90±.74^{a}	151.50 ± 3.11^{ab}	23.75 ± 1.71^{a}	5.70±.83^{a}	119.25 ± 22.02^{a}	25.00 ± 2.58^{a}	5.14±.90^{a}
C	121.50 ± 9.33^{a}	24.25 ± 3.86^{a}	5.33±.73^{a}	117.00 ± 8.042^{c}	27.00 ± 4.97^{a}	5.01±.33^{a}	121.50 ± 9.33^{a}	23.00 ± 3.92^{a}	5.19±.72^{a}

Means with different letters in the same columns are significantly different (p < 0.05) by Tukey HSD multiple comparison test

Table 4
Effect of different substrate formulae on proximate composition of oyster mushroom

Treatment	Crude Protein	Crude Fibre	Crude Fat	Ash	Moisture	Nitrogen Free Extract (NFE)	Dry Matter
A	24.84±.70^{a}	13.60±.21^{a}	1.07±.03^{a}	7.39±.01^{a}	5.94±.41^{a}	47.16 ± 0.48^{a}	94.06±.41^{a}
B	29.55±.41^{b}	12.73±.32^{a}	.59±.01^{b}	9.46±.12^{b}	14.91±.75^{b}	32.77 ± 0.98^{b}	85.09±.75^{b}
C	20.32±.49^{c}	13.53 ± 18^{a}	.98±.04^{ac}	7.96±.08^{c}	7.43±.44^{ac}	49.80 ± 0.34^{ac}	93.38±.700^{ac}

Means with different letters in the same columns are significantly different (p < 0.05) by Tukey HSD multiple comparison test

Table 5
Effect of different substrate formulae on mineral and vitamin composition of oyster mushroom

Treatment	Zn (mg/kg)	Fe (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)	Ca (mg/kg)	Vitamin K	Vitamin A	Vitamin D	Vit E
A	27.38 ± 3.55^{a}	74.49 ± 1.36a	1216.16 ± 36.03^{a}	1195.45 ± 22.75^{a}	18740.74 ± 205.54^{a}	496.22 ± 17.08^{a}	85.50±.01^{a}	73.24 ± 2.33^{a}	474.49 ± 58.28^{a}	460.52 ± 22.58^{a}
B	36.76±.01^{b}	86.57 ± 2,52a	1991.86 ± 17.68^{ab}	1014.74 ± 49.46^{a}	18188.94 ± 289.95^{ab}	289.26 ± 13.97^{b}	86.52±.41^{a}	128.82 ± 6.00^{ab}	538.51 ± 26.80^{ab}	411.45 ± 6.35^{a}
C	25.13±.84^{ac}	109.50 ± 49.53a	1175.55 ± 328.18^{ac}	371.10 ± 487.36^{a}	15883.67 ± 107.11^{c}	282.12 ± 8.38^{bc}	86.09±.21^{a}	365.61 ± 37.53^{c}	1024.39 ± 12.83^{c}	457.82 ± 24.99^{a}

Means with different letters in the same columns are significantly different (p < 0.05) by Tukey HSD multiple comparison test

Table 6
Biological Efficiency (BE) of the mushroom grown under different substrates from local materials

Treatment	Biological Efficiency (BE)		
	First Harvest	Second Harvest	Third Harvest
A	13.20±.81^{a}	14.08 ± 1.75^{a}	12.50 ± 1.89^{a}
B	13.43 ± 2.39^{a}	15.15±.31^{b}	14.43 ± 2.63^{b}
C	11.53±.71^{a}	11.70±.80^{a}	10.75 ± 1.56^{a}

Means with different letters in the same columns are significantly different (p < 0.05) by Tukey HSD multiple comparison test

Discussion

The yield performance, flush count, and pileus diameter of *Pleurotus ostreatus* varied according to the substrate formulas across three consecutive harvests (Table 3). In the first harvest, no significant differences ($p > 0.05$) were observed among Treatments A, B, and C in mushroom weight, number of flushes, or cap diameter [24]. This indicates that during the initial cropping phase, all substrate formulations provided sufficient and comparable levels of easily accessible nutrients to support primary mycelial breakthroughs and fruitbody development [25]. However, significant differences appeared in the second harvest, particularly in total fruitbody weight. Treatment B produced the highest yield (151.50 ± 3.11 g), which was significantly greater than Treatment C (117.00 ± 8.04 g), while Treatment A (140.75 ± 17.46 g) was statistically similar to both [26]. This mid-season increase in Treatment B suggests that its substrate formula provided a more sustained release of essential nutrients, such as carbon and nitrogen, which are vital for maintaining high yields after the initial flush [27]. By the third harvest, yields across all groups showed a noticeable decline, with no statistically significant differences remaining among them. This downward trend is well documented in mushroom cultivation and is typically attributed to the progressive depletion of lignocellulosic nutrients and moisture within the substrates as the cultivation cycle progresses. Interestingly, cap diameter and flush numbers remained statistically uniform across all treatments throughout the entire production cycle, indicating that while substrate composition greatly affects the overall biomass (weight) of the yield, the morphological traits and structural density of the flushes are determined primarily by environmental factors and genetics [9]. The nutritional profile of the harvested oyster mushrooms was strongly influenced by the underlying substrate formulations (Table 4) [28]. Treatment B showed a distinct nutritional advantage, yielding a significantly higher crude protein content (29.55 ± 0.41%) compared to Treatment A (24.84 ± 0.70%) and Treatment C (20.32 ± 0.49%). Mushrooms are highly efficient at translocating nitrogen from their growing media into amino acids. Therefore, the superior protein content in Treatment B strongly suggests that its substrate formula had a narrower, more optimal carbon-to-nitrogen (C:N) ratio or contained highly bioavailable organic nitrogen sources [29]. Conversely, Treatment B recorded a significantly lower crude fat content (0.59 ± 0.01%) and nitrogen-free extract carbohydrate value (32.77 ± 0.98%) compared to Treatments A and C [4]. This inverse relationship indicates that the metabolic pathways of the mycelium on Substrate B favoured the synthesis of structural proteins over energy storage lipids and soluble sugars. Treatment B also exhibited the highest moisture retention (14.91 ± 0.75%), which correspondingly lowered its total dry matter content (85.09 ± 0.75%). This higher moisture profile correlates with the larger fruit body weights observed in Table 3, as water accumulation is a primary driver of mushroom cell elongation [30]. Total ash content, an index of overall mineral richness, was significantly elevated in Treatment B (9.46 ± 0.12%), followed by Treatment C (7.96 ± 0.08%) and Treatment A (7.39 ± 0.01%), establishing a clear link between substrate mineral availability and the systemic absorption capabilities of the fungus [3]. The micro-nutritional compositions varied widely among the treatments, reinforcing the premise that the elemental components of a substrate dictate the nutritional value of the final harvest (Table 5). Treatment B excelled in zinc accumulation (36.76 ± 0.01 mg/kg) and magnesium profile (1991.86 ± 17.68 mg/kg), showing a distinct statistical variance from Treatments A and C. Zinc is vital for enzyme regulation in fungal development, and its high concentration in Treatment B complements the high protein synthesis noted in the proximate analysis [31]. Treatment A proved highly efficient for potassium accumulation (18740.74 ± 205.54 mg/kg) and calcium levels (496.22 ± 17.08 mg/kg), outperforming both B and C significantly. Potassium acts as the primary osmoregulator in mushroom cells; hence, the high levels in Treatment A indicate strong cellular structural integrity, even though this did not translate to superior wet biomass compared to Treatment B [32]. For iron, Treatment C registered the highest concentration (109.50 ± 49.53 mg/kg), though large standard deviations across replicates rendered this difference statistically non-significant ($p > 0.05$) [32]. However, Treatment C displayed extraordinary capabilities in vitamin assimilation, showing exponentially higher concentrations of Vitamin A (365.61 ± 37.53 mg/kg) and Vitamin D (1024.39 ± 12.83 mg/kg) compared to the other treatments. Since mushrooms synthesize Vitamin D when exposed to light or specific lipid precursors in the media, the substrate formula of C likely contained raw materials rich in sterol precursors, unlocking unique pathways for fat-soluble vitamin biosynthesis [33]. Biological Efficiency serves as the definitive metric to evaluate how successfully a mushroom strain utilizes a specific substrate mass to convert it into fresh edible biomass [34]. The Biological Efficiency trends observed across the three harvests mirrored the overall yield data, offering a deep look into the long-term viability of each substrate (Table 6). During the first harvest, all treatments yielded statistically equivalent efficiencies, ranging from 11.53% to 13.43%. However, striking differences occurred during the second and third flushes. Treatment B recorded the highest BE during both the second harvest (15.15 ± 0.31%) and third harvest (14.43 ± 2.63%), which was statistically separate from the performance of the other groups. Treatment A maintained moderate, stable efficiencies (14.08% and 12.50%). Treatment C experienced a steep decline, remaining locked at the lowest positions (11.70% and 10.75%) [34]. The sustained high Biological Efficiency of Treatment B in the latter halves of the cropping cycle indicates superior degradation of complex lignocellulosic bonds (lignin, cellulose, and hemicellulose) [1]. While Treatments A and C likely faced rapid moisture loss or nutrient lockups after the initial flush, the structural formulation of Substrate B maintained an optimal physical environment (aeration and water-

holding capacity) and biochemical profile. This allowed the *Pleurotus* mycelium to continuously secrete extracellular enzymes efficiently, sustaining high bioconversion rates until the end of the production trial [35].

Limitations of the Study

Although the study evaluated specific substrate formulas using locally sourced agricultural waste, seasonal variations in the chemical profile or nutrient baseline of these raw materials in other regions may alter mushroom yield outcomes. Furthermore, the experiment tracked performance across three consecutive flushes to measure biological efficiency, but it did not monitor potential late-stage flushes where secondary substrate degradation trends or natural contamination pressures might emerge. Although internal growing conditions were managed, subtle micro-climate fluctuations in ambient temperature and relative humidity within the facility across the different harvest periods may have slightly influenced mushroom moisture absorption and cap diameter. Furthermore, the study utilized a specific mother culture of *Pleurotus ostreatus* provided by a single regional center, meaning the findings may not fully represent how different commercial or wild oyster mushroom strains interact with these exact substrate formulations.

Conclusion

Treatment B is the most effective substrate formula for oyster mushroom cultivation. While all formulas perform similarly in the first harvest, Treatment B provides a superior, sustained nutrient release that maximizes mid-to-late-season yield and Biological Efficiency. Nutritionally, it significantly enhances crude protein, total ash, zinc, and magnesium levels, though it yields lower fat-soluble vitamins compared to Treatment C. Overall, choosing Substrate B optimizes both biometric yield efficiency and macro-nutritional quality, making it the ideal choice for high-yield, protein-rich mushroom production.

Abbreviations

ANOVA Analysis of Variance

BE Biological Efficiency

HSD Honest Significant Difference

SPSS Statistical Package for Social Sciences

Declarations

Ethical approval and consent to participate

Consent to participate were not applicable for this study, as it involved the collection of oyster mushroom spawn from a registered supplier and inoculating with experimental plots. However, ethical approval was necessary and this study got ethical clearance from Lira University Research Ethics Committee (LUREC) with the approval number LUREC – 2025 – 393. As per Ugandan national regulations, there was no specific permission or collection permit was required for the collection of the oyster mushroom species since it was obtained from a registered dealer of mushroom spawn, and oyster mushroom is domesticated, and neither a protected or endangered species in Uganda. All experimental oyster mushroom spawns used in this study were collected in accordance with the guidelines of relevant institutional, national, and international policies on plant collection of Uganda. The oyster mushroom spawn used in this study were identified and authenticated at the National Agricultural Research Organization (NARO) laboratories, Uganda.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Author Contribution

RO served as the principal investigator, leading the overall study design and execution, GK acted as the project mentor, providing strategic guidance and oversight, BA conducted the laboratory analysis and was responsible for editing and supervising manuscript, JA, JJA, and AA, served as co-principal investigators, contributing to the field operations, data collection, and logistical coordination. All authors have read and agreed to the published version of the manuscript

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Data Availability

The data used in this study are available upon a reasonable request from the corresponding author.

References

1. G. Vinci, S. A. Prencipe, L. Pucinischi, F. Perrotta, and M. Ruggeri, “Sustainability assessment of waste and wastewater recovery for edible mushroom production through an integrated nexus. A case study in Lazio,” *Sci. Total Environ.*, vol. 903, no. August, p. 166044, 2023, doi: 10.1016/j.scitotenv.2023.166044.
2. B. Aa, O. Ab, P. M, and Y. Ns, “Mushroom cultivation in tropical Africa: Successes, challenges, and opportunities,” *J. Agric. Food Res.*, vol. 18, no. June, p. 101264, 2024, doi: 10.1016/j.jafr.2024.101264.
3. S. Megersa, T. Shasho, and A. Tolessa, “Enhancing yields of Pleurotus ostreatus and Lentinula edodes mushrooms using water hyacinth (Eichhornia crassipes [Mart.] Solms) supplemented with locally available feedstock as substrate,” *Heliyon*, vol. 10, no. 20, p. e39113, 2024, doi: 10.1016/j.heliyon.2024.e39113.
4. N. A. Mohd Zaini *et al.*, “A narrative action on the battle against hunger using mushroom, peanut, and soybean-based wastes,” *Front. Public Heal.*, vol. 11, no. May, pp. 1–11, 2023, doi: 10.3389/fpubh.2023.1175509.
5. A. Mlambo, M. Maphosa, and A. M, “Mycorrhizal and non-mycorrhizal mushroom cultivation- constraints and opportunities for Sub-Saharan Africa,” *Cogent Food Agric.*, vol. 8, no. 1, 2022, doi: 10.1080/23311932.2022.2147486.
6. M. Anobir, A. Emmanuel, and A. Lawrence, “Motivators and impediments to mushroom commercialisation in Ghana: Implication for policymakers, development partners and extension agents,” *Heliyon*, vol. 9, no. 9, p. e19523, 2023, doi: 10.1016/j.heliyon.2023.e19523.
7. A. Nyakundi, A. O, and A. N, “Impact of Marketing Tactics on the Performance of Oyster Mushroom Cultivation among Small-Scale Farmers: A Study in Mumias Division, Mumias Sub-County, Kenya,” *Inosr Arts Humanit.*, vol. 9, no. 2, pp. 17–25, 2023, doi: 10.59298/inosrah/2023/2.3.4000.
8. Z. Jin, Q. Hou, and T. Niu, “Effect of cultivating Pleurotus ostreatus on substrates supplemented with herb residues on yield characteristics, substrates degradation, and fruiting bodies’ properties,” *J. Sci. Food Agric.*, vol. 100, no. 13, pp. 4901–4910, 2020, doi: 10.1002/jsfa.10551.
9. T. P. Valério, L. A. Szeremeta, J. T. M. R. Pacheco, B. C. B. Barros, E. B. Sydney, and E. D. G. Danesi, “Production of Oyster Mushroom (Pleurotus ostreatus) in Peach Palm By-Products: Effects on Composition and Maximization of Antioxidants Extraction,” *Brazilian Arch. Biol. Technol.*, vol. 67, pp. 1–11, 2024, doi: 10.1590/1678-4324-2024230467.
10. H. Modeste, M. Robert, and M. Japhet, “Effects of grain spawn and substrates on growth and yield of oyster mushroom grown under different cropping shelters,” *African J. Plant Sci.*, vol. 16, no. July, pp. 125–137, 2022, doi: 10.5897/AJPS2022.2244.
11. B. Yohannes *et al.*, “Selection of appropriate substrate for production of oyster mushroom (Pleurotus ostreatus),” *J. Yeast Fungal Res.*, vol. 11, no. 1, pp. 15–25, 2020, doi: 10.5897/jyfr2019.0187.
12. C. Muswati, K. Simango, L. Tapfumaneyi, M. Mutetwa, and W. Ngezimana, “The Effects of Different Substrate Combinations on Growth and Yield of Oyster Mushroom (Pleurotus ostreatus),” *Int. J. Agron.*, vol. 2021, 2021, doi: 10.1155/2021/9962285.
13. Y. Zhou *et al.*, “Evaluation of Corn Stalk as a Substrate to Cultivate King Oyster Mushroom (Pleurotus eryngii),” *Horticulturae*, vol. 9, no. 3, 2023, doi: 10.3390/horticulturae9030319.

14. N. Abou El, I. Hala, and M. A, “Effect of some Organic Applications on Biological Efficiency and Productivity of Mushroom (Pleurotus columbines) Grown under Uncontrolled Conditions,” *J. Plant Prod.*, vol. 12, no. 5, pp. 495–503, 2021, doi: 10.21608/jpp.2021.178923.
15. N. A. Khan, W. Ahmed, M. A. Khan, O. Yasin, S. Asad, and S. Munir, “Effect of Different Kinds of Substrates on the Growth and Yield Performance of Pleurotus sapidus (Oyster Mushroom),” *Asian Food Sci. J.*, no. January, pp. 18–24, 2021, doi: 10.9734/afsj/2021/v20i130250.
16. M. Akter, R. F. Halawani, F. A. Aloufi, M. A. Taleb, S. Akter, and S. Mahmood, “Utilization of Agro-Industrial Wastes for the Production of Quality Oyster Mushrooms,” *Sustain.*, vol. 14, no. 2, pp. 1–10, 2022, doi: 10.3390/su14020994.
17. E. Mesele, A. T. Yaekob, and A. Zeslassie, “Valuation of the growth response of oyster (Pleurotus ostreatus) mushroom on partially composted sesame stalk with different blends of wheat straw,” *Discov. Food*, vol. 4, no. 1, 2024, doi: 10.1007/s44187-024-00147-y.
18. T. Mussie, S. Berhe, S. Teklemichael, and T. Gebrezgiabhier*, “Performance of Waste Paper as a Substrate for Oyster Mushroom (Pleurotus ostreatus L.) Cultivation,” *J. Biomed. Res. Environ. Sci.*, vol. 5, no. 6, pp. 597–606, 2024, doi: 10.37871/jbres1932.
19. D. Dipali, T. Subramanian, and G. S. Kumaran, “A Novel Approach for an Outdoor Oyster Mushroom Cultivation using a Smart IoT-based Adaptive Neuro Fuzzy Controller,” *Int. J. Adv. Comput. Sci. Appl.*, vol. 14, no. 5, pp. 973–981, 2023, doi: 10.14569/IJACSA.2023.01405101.
20. Z. X. Tang, L. E. Shi, Z. B. Jiang, X. L. Bai, and R. F. Ying, “Calcium Enrichment in Edible Mushrooms: A Review,” *J. Fungi*, vol. 9, no. 3, pp. 1–14, 2023, doi: 10.3390/jof9030338.
21. D. Dipali, T. Subramanian, and G. S. Kumaran, “A smart oyster mushroom cultivation using automatic fuzzy logic controller,” *J. Discret. Math. Sci. Cryptogr.*, vol. 26, no. 3, pp. 601–615, 2023, doi: 10.47974/JDMSC-1731.
22. Ponthep, A. K, T. T, and J. J, “Agricultural and Biological Engineering,” *Agric. Eng.*, vol. 1, no. 4, pp. 1–4, 2004.
23. J. R. Zárate-Salazar, M. N. Santos, E. N. M. Caballero, O. G. Martins, and Á. A. P. Herrera, “Use of lignocellulosic corn and rice wastes as substrates for oyster mushroom (Pleurotus ostreatus Jacq.) cultivation,” *SN Appl. Sci.*, vol. 2, no. 11, 2020, doi: 10.1007/s42452-020-03720-z.
24. K. Piska, K. Sułkowska-Ziaja, and B. Muszyńska, “Edible mushroom pleurotus ostreatus (Oyster mushroom) – Its dietary significance and biological activity,” *Acta Sci. Pol. Hortorum Cultus*, vol. 16, no. 1, pp. 151–161, 2017.
25. K. Singh and M. Thakur, “Formulation, organoleptic and nutritional evaluation of value added baked product incorporating Oyster mushrooms (Pleurotus ostearus) powder,” *Int. J. Food Sci. Nutr.*, vol. 1, no. 6, pp. 16–20, 2016, [Online]. Available: www.foodsciencejournal.com
26. O. O, O. Ariyo, and A. O, “Edible Mushrooms: their Impact on Food Security O. O. Ariyo Department of Microbiology. Federal University Oye-Ekiti, Ekiti State, Nigeria.,” vol. 8, 2023, doi: 10.55518/fjpas.QBCF1374.
27. A. Khan *et al.*, “Contribution of mushroom farming to mitigating food scarcity: Current status, challenges and potential future prospects in Pakistan,” *Heliyon*, vol. 10, no. 23, p. e40362, 2024, doi: 10.1016/j.heliyon.2024.e40362.
28. D. Rika Widianita, “No [illegible] [illegible]Title,” *AT-TAWASSUTH J. Ekon. Islam*, vol. VIII, no. I, pp. 1–19, 2023.
29. M. Fagerström, L. Mazzaferro, and M. Martin, “Assessment of oyster mushroom production employing urban-based materials in Stockholm Stad,” 2023.
30. R. Ahmed *et al.*, “Optimizing tea waste as a sustainable substrate for oyster mushroom (Pleurotus ostreatus) cultivation: a comprehensive study on biological efficiency and nutritional aspect,” *Front. Sustain. Food Syst.*, vol. 7, no. January, pp. 1–10, 2023, doi: 10.3389/fsufs.2023.1308053.
31. B. Pryor, “the impact of substrate composition and led light spectra on the In the Graduate College,” 2024.
32. B. Dey *et al.*, “Strategic insights for sustainable growth of mushroom farming industry in Bangladesh: A comprehensive evaluation using SWOT-AHP and TOPSIS frameworks,” *Heliyon*, vol. 10, no. 17, p. e36956, 2024, doi: 10.1016/j.heliyon.2024.e36956.
33. G. Mhlongo and C. M. Mnisi, “Dietary incorporation of brown seaweed spent oyster mushroom substrate alters growth performance, physiological responses and meat quality parameters in Boschveld roosters,” *Sci. Rep.*, vol. 14, no. 1, pp. 1–13, 2024, doi: 10.1038/s41598-024-65338-0.
34. “Studies on cultivation of Pleurotus fossulatus (Cooke) Sacc . on locally available substrates Thesis by Prakriti SAINI Dr . yashwant singh parmar university of horticulture and forestry solan (nauni) H . P . -173230 INDIA in partial fulfilment of the ,” 2021.
35. V. Palangi, A. Kaya, A. Kaya, and I. Giannenas, “Ecofriendly Usability of Mushroom Cultivation Substrate as a Ruminant Feed: Anaerobic Digestion Using Gas Production Techniques,” *Animals*, vol. 12, no. 12, 2022, doi: 10.3390/ani12121583.
36.