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Evaluation of Low-Cost Grain-Based Media for Enhanced Mycelial Growth of Oyster Mushroom (*Pleurotus ostreatus*)

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Abstract— *This study evaluated the efficacy of low-cost, grain-based media for enhancing the mycelial growth of Pleurotus ostreatus (oyster mushroom) as an alternative to expensive commercial potato dextrose agar (PDA). Six locally available grains including wheat, sorghum, maize, millet, hyacinth bean (Lablab purpureus), and pigeon pea (Cajanus cajan), were tested at concentrations of 10, 30, 50, and 70 g/L in a factorial experiment under controlled laboratory conditions. Mycelial radial growth, colonization time, and phenological traits were measured and compared to PDA medium. Results demonstrated that millet and hyacinth bean at 70 g/L supported the most vigorous mycelial expansion, achieving full colonization (90 mm) by day 9, outperforming Potato Dextrose Agar (PDA) medium, which required 15 days. Higher grain concentrations (50–70 g/L) consistently promoted denser mycelium, rhizomorphic hyphae, and faster growth rates (up to 7.89 mm/day). The study highlights the potential of millet, hyacinth bean, and sorghum as cost-effective, locally sourced alternatives for growth medium, offering a sustainable solution for scaling oyster mushroom cultivation in resource-limited settings like Sudan. These findings support the adoption of grain-based media in mushroom biotechnology to enhance food security and agricultural diversification.*

Keywords— ***Grain-based media, low-cost substrates, mycelial growth, Pleurotus ostreatus, Sudan..***

I. INTRODUCTION

Mushroom cultivation is a rapidly expanding biotechnological industry with substantial global growth due to its contribution to food security, nutrition, and environmental sustainability. Among edible fungi, oyster mushroom (*Pleurotus ostreatus*) ranks as the third most commercially cultivated species worldwide, appreciated for its high biological efficiency, rich protein content, palatable flavor, tender texture, and extended shelf life. In many regions, including parts of Africa and Asia, *Pleurotus ostreatus* is consumed as a vegetable, making it a valuable addition to local diets (Sánchez, 2010).

Oyster mushrooms possess the unique ability to degrade lignocellulosic plant residues into edible biomass, enabling their growth on a wide range of agro-wastes and low-cost organic substrates (Rajarathnam et al., 1998). This adaptability, coupled with their tolerance to a broad temperature range (20–30°C), makes them particularly suitable for cultivation in tropical and subtropical regions, such as Sudan (Çağlarırmak, 2007). Importantly *Pleurotus ostreatus* can be cultivated without sophisticated

infrastructure or costly cooling systems, reducing overheads and making mushroom farming accessible to smallholder farmers and low-income research laboratories. Its integration into farm-based crop rotations further enhances its role in sustainable land use and agro-ecological intensification.

In Sudan, the introduction of mushroom farming is a relatively recent endeavor. As part of the Faculty of Agriculture at Al Zaeim Al Azhari University to fit in the national Sudanese strategies to diversify agricultural production and enhance food and income security, efforts are underway to domesticate mushroom production technologies. This research forms part of that process, laying foundational work for a locally adapted, cost-effective, and scalable oyster mushroom cultivation system. Specifically, it contributes to building a home-grown biotechnological platform for edible fungi production, a vision aligned with the role of plant breeders and agricultural scientists in developing region-specific innovations.

The standard protocols for culturing oyster mushrooms rely on ready-to-use commercial media such as potato dextrose agar (PDA). While effective, these media are expensive and pose a significant barrier for underfunded laboratories, extension centers, and grassroots mushroom incubators. Moreover, the reliance on imported inputs undermines the sustainability and self-sufficiency of emerging mushroom production systems in Sudan and similar contexts.

To address these limitations, this study evaluates the efficacy of six locally available grain-based media, including wheat, sorghum, maize, millet, hyacinth bean (*Lablab purpureus*), and pigeon pea (*Cajanus cajan*), as alternative culture substrates for *P. ostreatus*, benchmarking them against the conventional PDA medium. By identifying lower-cost, locally sourced media that can support optimal mycelial growth, this research supports the development of affordable spawn production protocols, which are essential for scaling up mushroom cultivation and embedding it within domestic agricultural systems.

II. MATERIALS & METHODS

2.1 Experimental Design

A laboratory-based factorial experiment was conducted under controlled conditions using a Completely Randomized Design (CRD) with five replications for each treatments. All the experiments were carried out in the growth room of Mushroom Incubator Laboratory, Faculty of Agriculture, Al Zaeim Al Azhari University, Sudan. The study comprised two experimental factors:

2.1.1 Factor A (Grain Type): Six grain types were evaluated as primary substrates for media formulation: wheat (*Triticum aestivum*), maize (*Zea mays*), sorghum (Sorghum bicolor), millet (*Pennisetum glaucum*), pigeon pea (*Cajanus cajan*), and hyacinth bean (*Lablab purp*ureus). All grains were procured from the local grain market in Khartoum, Sudan.

2.1.2 Factor B (Substrate Concentration): Four concentrations of each grain powder were prepared and evaluated: 10 g/L, 30 g/L, 50 g/L, and 70 g/L. These were incorporated into distilled water to formulate semi-synthetic media. The Potato Dextrose Agar (PDA) medium served as a positive control, representing the classical nutrient-rich standard for fungal culture.

2.2 Grain Medium preparation

Wheat agar, maize agar, sorghum agar, Pearl millet agar, Pigeon pea agar, Sorghum agar and Hyacinth agar Medium were prepared under aseptic condition at the Mushroom incubators and laboratory of Al Zaeim Al Azhari University. Each grain was separately boiled in distilled water for 30 minutes at the specified concentrations (10, 30, 50, or 70 g per liter). The resulting decoctions were filtered through clean sterile cheesecloth to obtain grain extracts. To each liter of filtrate, 20 g of agar and 20 g of dextrose were added. The final volume was adjusted to 1 liter using distilled water. All media were sterilized in an autoclave at 121°C and 15 psi for 20 minutes. Streptomycin was added in the sterilized medium at the rate of 1 g/L. The sterilized media were poured into sterile Petri dishes (90 mm diameter) under laminar airflow and Medium were cooled at 40°C. A mycelial disc (19 mm diameter) was cut from an actively growing *Pleurotus ostreatus* culture using a pre-designed sterile circular cutter and aseptically placed at the center of each plate and incubated for fifteen days under sterile conditions .The radial growth was measured following the method reported by Zharare et al. (2010).

2.3 Potato dextrose agar (PDA) medium preparation

Potato dextrose agar (PDA) medium was prepared by adding 20 g each of potato starch, dextrose, agar and 1 L distilled water and autoclaved it at 121°C for 15 minute. Streptomycin was added in the sterilized medium at the rate of 1 g/L. The sterilized media were poured into sterile Petri dishes (90 mm diameter) under laminar airflow and Medium were cooled at 40°C. A mycelial disc (19 mm diameter) was cut from an actively growing *Pleurotus ostreatus* culture using a pre-designed sterile circular

cutter and aseptically placed at the center of each plate and incubated for fifteen days under sterile conditions .The radial growth was measured following the method reported by Zharare et al. (2010).

2.4 Incubation and Data Collection

Petri dishes were incubated in complete darkness at a temperature of 25 ± 2°C using an electric incubator. Mycelial radial growth was recorded every three days, beginning on the day of inoculation and continuing for a total period of 15 days. Radial growth (in mm) was determined by averaging two perpendicular measurements per plate, following the method described by Zharare et al. (2010). Measurements from five replicates were then averaged to obtain the mean radial growth per plate at 3, 6, 9, 12, and 15 days after inoculation. The daily mycelial growth rate (mm/day) was calculated by dividing the total radial expansion (in mm) from the point of inoculation to the fifteenth day by the number of incubation days. The number of days required for complete colonization was estimated as the average number of days taken for the mycelium to fully cover a 90 mm Petri dish. Phenological characterization at total colonization included observations of mycelium color, growth pattern, colony density (mycelial compactness), formation of aerial and rhizomorphic hyphae (Sobal et al., 2007).

2.5 Statistical Analysis

Data collected from both growing seasons were subjected to analysis of variance (ANOVA), appropriate for factorial experiments arranged in a Completely Randomized Block Design (CRBD), following the procedures outlined by Singh and Chaudhary (1985). The main effects and interactions between grain type and concentration were evaluated using Fisher's analysis of variance technique at the 5% and 1% probability levels. Statistical analysis and mean separation were performed using Statistix version 10.0 (Analytical Software, USA). Where significant differences were detected, the Least Significant Difference (LSD) test at $P \leq 0.05$ was applied to compare treatment means, as recommended by Singh and Chaudhary (1985).

III. RESULTS AND DISCUSSION

3.1. Effects of Grain Type, Concentration and Interaction

The analysis of variance (Table 1) revealed highly significant effects ($P < 0.01$) for grain type, grain concentration, and their interaction on mycelial radial growth at all observation intervals (3, 6, 9, and 12 days after inoculation). This confirms that both the type and concentration of grain-based media play a critical role in supporting the vegetative development of *Pleurotus ostreatus*. These findings are consistent with earlier studies demonstrating that nutritional and physicochemical characteristics of culture media strongly influence fungal growth (Zharare et al., 2010; Sánchez, 2010).

Table 1. Mean square values from ANOVA for grain concentration and their interaction

Source	Mycelial radial growth (mm) Days after inoculation (DAI)			
	3	6	9	12
Grain (A)	459.55**	059.64**	48.51**	15.68**
oncentration (B)	727.63**	775.18**	476.16**	8.056**
Grain x Conc.	53.97**	86.9**	34.67**	9.569**
Error	35.55	87.03	33.32	16.181

Note: *Values followed by ** and * are significant at P < 0.01 and P = 0.05, respectively;* ***NS*** *= not significant.*

3.2. Mycelial Growth Performance across Grain Types

As shown in Table 2, among the six grain types tested, millet and hyacinth bean supported the most vigorous mycelial expansion, both achieving 90 mm full colonization by day 12. Millet showed consistent superiority at early stages, registering 52.65 mm by day 3 and 78.55 mm by day (6). Similarly, hyacinth bean recorded the highest diameter at day (6) (80.85 mm), which surpassed PDA medium and most cereal-based media. Mycelial growth of fungus is highly effected by changing the contents of medium, (Miles and Chang 2004). Chittaragi et al, (2018) found that the effect of media on the radial growth of the fungus was influenced greatly by the kind of media used, sorghum meal agar was most favored by the different Oyster strains followed by wheat meal agar and potato dextrose agar in the order of preference. This superior performance may be attributed to the high soluble carbohydrate and protein content of millet and legumes, which are essential for fungal energy metabolism and structural synthesis (Çağlarırmak, 2007; Kim et al., 2011; Muttaqin, et al., 2024).In contrast, pigeon pea exhibited the slowest initial growth (36.15 mm at day 3), though it eventually reached 86.50 mm by day (12), indicating delayed but sustained nutrient release. While wheat, maize, and sorghum showed moderate performance, they all supported complete colonization by day 15.

3.3. Effects of Grain Concentration on Growth Rate

The main effect of grain concentration (Table 2) was also statistically significant at all stages. Media prepared with 70 g/L grain concentration supported the fastest and most extensive mycelial growth across all grain types. At

this level, mean radial growth reached 90 mm by day (12). Lower concentrations (10–30 g/L) produced moderate growth, suggesting suboptimal nutrient availability. This dose-response effect is supported by the work of Obodai et al. (2003), who reported that increased organic content in substrates correlates with improved mycelial growth and colonization efficiency. Ibekwe et al. (2008) reported similar higher growth rates of *Pleurotus ostreatus* mycelia at higher concentrations of grains extracted .Similarly, Royse (2002) Hoa, et al. (2015) and AL-Jbouri, et al. (2025) showed that carbon and nitrogen concentration significantly affect the early vegetative growth of *Pleurotus spp*.

Table 2. Mean performance of mycelial radial growth (mm) for grain type and concentration (g/L) – main effects

Source	Mycelial radial growth (mm) Days after inoculation (DAI)			
	3	6	9	12
Grain (A)				
Wheat	60.90a	70.00cd	78.65c	83.65c
Maize	50.60b	63.00e	79.85bc	88.00ab
Sorghum	49.65b	73.85bc	83.40b	88.35ab
Millet	52.65b	78.55ab	87.70a	90.00a
Pigeon Pea	36.15d	64.55de	80.00bc	86.50b
Hyacinth bean	42.50c	80.85a	88.20a	90.00a
LSD p=0.05	3.745	5.859	3.625	2.526
Mean	48.74	71.8	82.97	87.75
Concentration (B)				
10g/L	38.97d	62.63d	73.93c	82.67b
30g/L	44.37c	68.87c	81.10b	88.50a
50g/L	50.43b	75.43b	87.17a	89.83a
70g/L	61.20a	80.27a	89.67a	90.00a
LSD p=0.05	3.058	4.784	2.960	2.063
Mean	48.74	71.8	82.97	87.75

Note: *Means within a column followed by different superscript letters differ significantly at the 0.05 probability level (P = 0.05). 'NS' denotes a non-significant difference.*

3.4. Interaction Effects between Grain Type and Concentration

As shown in Table 3, interaction effects between grain types and concentrations were significant and informative. Wheat, Millet, sorghum, and hyacinth bean at 70 g/L supported the most rapid and complete radial expansion, recording the highest rapid radial growth in Day (3) and reaching full 90 mm colonization as early as day (9). These treatments consistently outperformed the classical PDA control, which required the full (15) days for colonization and achieved a lower growth rate (4.56 mm/day).It observed that Millet consistently supported rapid and complete mycelial colonization in combination with any of the substrate concentration at 30 g/L, 50 g/L and 70 g/L, reaching full 90 mm colonization as early as day (9)., outperforming the classical PDA control medium.

This indicates that Millet is a highly suitable substrate for promoting fast and robust fungal colonization, even at lower concentrations. The observed trend confirms that both grain type and nutrient density synergistically determine the quality of the growth substrate. The use of legumes (e.g., hyacinth bean and pigeon pea) likely improved the media's nitrogen content, while cereals like millet provided easily fermentable sugars. Millet is a nutrient-rich grain, comprising up to 84.2% carbohydrates and 10.7% protein, along with fats (1.5%) and ash (2.6%), (Kim et al., 2011; Muttaqin, et al., 2024).This makes it an excellent source of essential nutrients that effectively support mycelial growth (Kim et al., 2011;Muttaqin, et al., 2024). Kim et al. (2011) reported that agar-based millet powder medium was superior for the production of thermotolerant entomopathogenic fungal conidia compared to other agar-based media. Higher radial mycelial extension growth has been reported for common soil fungi such as *Aspergillus niger*, *Fusarium moniliforme*, and *Penicillium sp*. on millet-based formulated media compared to commercially produced potato dextrose agar (Ubogu, et al., 2015). *Phellorinia*

herculeana, a mushroom found in sandy soils, is also observed to grow in areas sown with millet crops like sorghum and maize, suggesting millet as a suitable component for its culture media,(Oviya, et al., 2022).

Table 3. Mean performance of mycelial radial growth (mm) for different combinations of grain types and medium concentrations

Grain Type	Conc. (g/L)	Mycelial radial growth (mm) Days after inoculation (DAI)				
		3	6	9	12	15
wheat	10 g/L	46.4^{cdefg}	54.00^{g}	58.00^{f}	64.60^{c}	90.00
	30 g/L	59.60^{b}	69.00^{cdef}	80.60^{cd}	90.00^{a}	90.00
	50 g/L	67.60^{a}	77.00^{abc}	86.00^{abc}	90.00^{a}	90.00
	70 g/L	70.00^{a}	80.00^{abc}	90.00^{a}	90.00^{a}	90.00
Maize	10 g/L	40.80^{gh}	57.40^{fg}	72.60^{e}	86.00^{ab}	90.00
	30 g/L	50.00^{cd}	59.00^{fg}	72.80^{e}	86.00^{ab}	90.00
	50 g/L	51.60^{c}	60.60^{efg}	86.00^{abc}	90.00^{a}	90.00
	70 g/L	60.00^{b}	75.00^{abc}	88.00^{ab}	90.00^{a}	90.00
Sorghum	10 g/L	39.20^{ghi}	61.80^{defg}	76.20^{de}	83.40^{b}	90.00
	30 g/L	42.20^{fgh}	74.80^{abc}	83.40^{abcd}	90.00^{a}	90.00
	50 g/L	46.20^{cdefg}	78.80^{abc}	84.00^{abc}	90.00^{a}	90.00
	70 g/L	71.00^{a}	80.00^{abc}	90.00^{a}	90.00^{a}	90.00
Millet	10 g/L	42.40^{efgh}	72.20^{bcde}	80.80^{bcd}	90.00^{a}	90.00
	30 g/L	43.40^{defg}	76.00^{abc}	90.00^{a}	90.00^{a}	90.00
	50 g/L	49.80^{cde}	81.00^{ab}	90.00^{a}	90.00^{a}	90.00
	70 g/L	75.00^{a}	85.00^{a}	90.00^{a}	90.00^{a}	90.00
Pigeon pea	10 g/L	30.00^{j}	55.00^{g}	71.00^{e}	82.00^{b}	90.00
	30 g/L	32.00^{ij}	55.40^{g}	72.00^{e}	85.00^{ab}	90.00
	50 g/L	41.00^{gh}	72.40^{bcd}	87.00^{abc}	89.00^{a}	90.00
	70 g/L	41.60^{gh}	75.40^{abc}	90.00^{a}	90.00^{a}	90.00
Hyacinth bean	10 g/L	35.00^{hij}	75.40^{abc}	85.00^{abc}	90.00^{a}	90.00
	30 g/L	39.00^{ghi}	79.00^{abc}	87.80^{abc}	90.00^{a}	90.00
	50 g/L	46.40^{cdefg}	82.80^{ab}	90.00^{a}	90.00^{a}	90.00
	70 g/L	49.60^{cdef}	86.20^{a}	90.00^{a}	90.00^{a}	90.00
PDA -Control		**22.80**	**47.80**	**60.00**	**88.00^{a}**	**90.0 ^{0}**
Mean (mm)		**90.00**	**87.75**	**82.97**	**71.80**	**48.74**
LSD (p=0.05)		**7.490**	**11.718**	**7.250**	**5.053**	**NS**

Note: *Means within a column followed by different superscript letters differ significantly at the 0.05 probability level (P = 0.05). 'NS' denotes a non-significant difference.*

3.5. Growth Rate and Days to Total Colonization

As shown in Table 4, the highest mycelial growth rate (7.89 mm/day) was recorded across several treatments, including millet, hyacinth bean, wheat, sorghum, and

pigeon pea at concentrations of 50 and 70 g/L. The time required for full colonization clustered into three distinct groups: a fast group achieving colonization in 9 days, a middle group at 12 days (comprising the majority), and a slow group requiring 15 days. In contrast, the PDA control exhibited the lowest growth rate (4.56 mm/day) and required the entire 15-day incubation period to reach full colonization. These results underscore the superior biological efficiency and cost-effectiveness of the grain-based media evaluated in this study. Similar results were reported by Khandakar et al. (2008), Chittaragi, et al. (2018), Pestsov, et al. (2021), Emayavarman & Singh (2021), who found that *Pleurotus* strains grew faster and colonized more completely on grain-based and organic residue media compared to synthetic agar media.

3.6. Phenological Characteristics of Mycelial Growth

Phenological observations in Table 4 and figure 1 revealed that mycelial colonies exhibited consistent radial regular growth across all treatments. Higher concentrations (≥50 g/L) led to dense and pretty dense mycelium, often accompanied by the development of aerial and rhizomorphic hyphae, features associated with healthy, vigorous fungal cultures and predictive of good fruiting potential, which is critically important for the survival and propagation of fungi (Zervakis et al., 2001; Oviya, et al., 2022). Hyacinth bean and millet treatments, frequently displayed rhizomorphic structures, considered advantageous for substrate penetration and spawn development. The presence of these long rhizoids and extensive mycelial strands allows mushroom to form either parasitic or symbiotic relationships. This capability is believed to be crucial for the mushroom to obtain essential nutrients necessary for its fructification (Royse, 2002; Sánchez, 2010; Oviya, et al., 2022).The presence of dense aerial hyphae in wheat, maize, and sorghum media at higher concentrations suggests that these substrates also supported high metabolic activity, though phenotypic differences in mycelial texture may reflect substrate-specific signaling pathways or nutrient profiles.

Table 4. Mycelia Growth Rate, Days to Total Colonization and Phenology

Treatment	Growth rate mm/day	Days to total colonization	Mycelium phenology at total colonization
Wheat × 10 g/L	4.33	15	radial regular growth, Medium density and white
Wheat × 30 g/L	6.78	12	radial regular growth, Medium density and white
Wheat × 50 g/L	7.44	12	radial regular growth, good density and white
Wheat × 70 g/L	7.89	9	radial regular growth, dense, aerial hypae and white
Maize × 10 g/L	5.96	15	radial regular growth, Medium density and dull white
Maize × 30 g/L	5.98	15	radial regular growth, Medium density and white
Maize × 50 g/L	7.44	12	radial regular growth, Medium density and white
Maize × 70 g/L	7.67	12	radial regular growth, dense, aerial hypae and white
Sorghum × 10 g/L	6.33	15	radial regular growth, Medium density and white
Sorghum × 30 g/L	7.15	12	radial regular growth, Medium density and white
Sorghum × 50 g/L	7.22	12	radial regular growth, Medium density and white
Sorghum × 70 g/L	7.89	12	radial regular growth, dense, aerial hypae and white
Millet × 10 g/L	6.87	12	radial regular growth, dense, Rhizomorfic hypae and white
Millet × 30 g/L	7.89	9	radial regular growth, pretty dense, Rhizomorfic hypae and white
Millet × 50 g/L	7.89	9	radial regular growth, pretty dense, Rhizomorfic hypae and white
Millet × 70 g/L	7.89	9	radial regular growth, pretty dense, Rhizomorfic hypae and white
Pigeon pea × 10 g/L	5.78	12	radial regular growth, dense density, aerial hypae and white
Pigeon pea × 30 g/L	5.89	12	radial regular growth, dense, aerial hypae and white
Pigeon pea × 50 g/L	7.56	12	radial regular growth, dense, Rhizomorfic hypae and white

Pigeon pea × 70 g/L	7.89	9	radial regular growth, pretty dense, Rhizomorfic hypae and white
Hyacinth bean × 10 g/L	7.89	12	radial regular growth, dense and white
Hyacinth bean × 30 g/L	7.64	12	radial regular growth, dense and white, Rhizomorfic hypae and white
Hyacinth bean × 50 g/L	7.89	9	radial regular growth, pretty dense, Rhizomorfic hypae and white
Hyacinth bean × 70 g/L	7.89	9	radial regular growth, pretty dense, Rhizomorfic hypae and white
PDA -control	4.56	15	radial regular growth, pretty dense, Rhizomorfic hypae and white

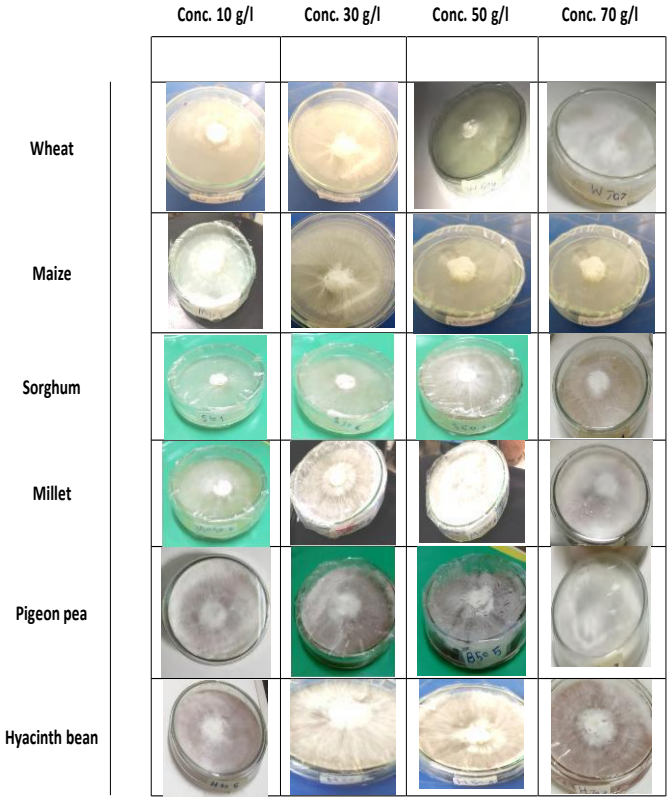

Fig.1. Mycelial Radial Growth and Colonization on Day Twelve from Inoculation

IV. CONCLUSION

This study demonstrated the efficacy of low-cost, locally available grain-based media in promoting the mycelial growth of *Pleurotus ostreatus* under laboratory conditions, offering a viable alternative to the imported PDA medium. Among the six grain types tested, millet, hyacinth bean, and sorghum, particularly at higher concentrations (50–70 g/L), significantly enhanced radial expansion, growth rate, and reduced the time to full colonization. These media also supported dense, rhizomorphic, and aerial hyphal growth, indicating healthy and vigorous fungal biomass suitable for spawn production. Interaction analysis identified specific combinations, such as millet at 30, 50, and 70 g/L, hyacinth bean at 70 g/L, and sorghum at 70 g/L, as optimal for achieving rapid and complete colonization. These findings hold significant implications for the advancement of mushroom biotechnology in Sudan, highlighting the potential of millet, sorghum, and hyacinth bean to support scalable, community-level culture medium and spawn production systems. Their affordability, local availability, short colonization periods, and superior growth performance make them suitable for mushroom technology incubators and integration into sustainable crop rotation systems. Moreover, the adaptability of *P. ostreatus* to these substrates and tropical agro-climatic conditions underscores its role in fostering home-grown, biotechnological solutions for low-cost, climate-resilient mushroom farming in Sudan.

RECOMMENDATION

It is recommended that mushroom technology incubators, laboratories, and rural production units in Sudan adopt millet, hyacinth bean, or sorghum media at 70 g/L concentration as a standard for spawn development of *Pleurotus ostreatus*. Future work should assess the impact of these grain media on fruiting performance, biological efficiency, and postharvest quality under controlled and semi-controlled conditions.

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