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SUSTAINABLE MUSHROOM CULTIVATION USING AGRO-WASTE AND INDIGENOUS MICROBIAL TECHNIQUES: A MICROBIOLOGY-BASED ENTREPRENEURSHIP MODEL FOR YOUTH EMPOWERMENT IN SOKOTO, NIGERIA

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Article Info

Abstract

Mushroom cultivation presents a transformative opportunity for sustainable agribusiness, particularly in resource-constrained semi-arid regions like Sokoto, Nigeria. This research comprehensively investigates the synergistic effects of locally abundant agro-waste substrates (maize stalks, rice husks, sawdust) pretreated with indigenous microbial inoculants Trichoderma harzianum Bacillus subtilis Pseudomonas fluorescens on the yield and colonization efficiency of Pleurotus ostreatus (oyster mushroom). Microbes were isolated from local decayed materials and compost, characterized, and applied to enhance substrate decomposition and nutrient availability through lignocellulosic degradation. Results demonstrated statistically significant improvements: T. harzianum reduced colonization time by 36% (from 14.3 to 9.1 days) and increased yield by 35% (1050 g/kg vs. 780 g/kg in controls) compared to uninoculated substrates. B. subtilis and P. fluorescens also showed significant positive effects. Beyond productivity, the study rigorously evaluates the economic viability and environmental benefits of this model, including waste valorization (diverting ~85% of tested agro-waste from open burning), soil enrichment via spent substrate, and carbon footprint reduction. Critically, it proposes a scalable, low-capital microbiology-based entrepreneurship framework for youth empowerment, requiring minimal land and technical training. Integrating this model into agricultural extension services, policy frameworks (aligned with Nigeria's Agricultural Promotion Policy and SDGs 2, 8, 12, 13), and dedicated youth incubator programs offers a robust pathway toward rural economic revitalization, food security, and climate-smart agriculture in Northern Nigeria and analogous regions.

Keywords:

Mushroom, valorization, Indigenous microbes, agripreneurship, Sokoto.

1. **Introduction**

The twin challenges of pervasive youth unemployment and unsustainable management of agricultural waste represent critical socio-economic and environmental burdens across sub-Saharan Africa (FAO, 2017; ILO, 2020). In Nigeria, Africa's most populous nation, youth unemployment rates exceed 40% nationally and are even higher in rural northern regions like Sokoto State (National Breu and Statistic, 2023).

Concurrently, vast quantities of agro-waste estimated at over 100 million tons annually in Nigeria alone (Federal ministry of Environment, 2021) are generated from staple crops like maize, rice, and sorghum. In Sokoto, situated in the semi-arid northwest, this biomass (primarily maize stalks, rice husks, and groundnut shells) is predominantly discarded or subjected to open-field burning. This practice contributes significantly to air pollution (releasing CO2, CH4, NOx, and particulate matter), soil degradation, and represents a tragic loss of valuable organic resources (Kumar et al., 2020).

Mushroom cultivation, particularly using the adaptable and resilient genus Pleurotus (oyster mushrooms), offers a uniquely convergent solution to these challenges (Royse et al., 2017). Oyster mushrooms are saprophytic fungi renowned for their ability to efficiently decompose lignocelluloses materials, the primary structural component of plant biomass and agro-wastes (Royase et al., 2017). This biological process transforms low-value waste into high-value protein-rich food. *Pleurotus* species possess distinct advantages for resource-limited settings: rapid growth cycles (often completing fruiting within 6-8 weeks), high biological efficiency (conversion of substrate dry mass to mushroom fresh mass), tolerance to fluctuating environmental conditions common in semi-arid zones, minimal requirements for arable land or water, and significant nutritional (high protein, fiber, vitamins, selenium) and medicinal value (antioxidant, immunomodulatory properties) (Chang & Miles, 2004; Adebayo & Oloke, 2015).

However, the natural decomposition of lignocellulose by fungi is often rate-limited by the recalcitrance of lignin and hemicellulose complexes. Pretreatment of substrates is essential to enhance accessibility and accelerate colonization. Conventional methods like chemical treatment (such as alkalis) or steam sterilization are often impractical, costly, and environmentally undesirable for small-scale farmers in regions like Sokoto. This is where microbial biotechnology presents a compelling, low-cost, and sustainable alternative (Sharma et al., 2019). Indigenous microorganisms (IMOs), adapted to local climatic and substrate conditions, offer potent capabilities for lignocellulose degradation. Key microbes include:

Cellulolytic Fungi (*Trichoderma harzianum*): Produce extracellular enzymes (cellulases, hemicelluloses) breaking down cellulose and hemicellulose chains. Ligninolytic Bacteria (e.g., *Bacillus subtilis*): Secrete lignin peroxidases and laccases that disrupt the complex lignin polymer (Sharma et al., 2019). Rhizosphere Bacteria (e.g. *Pseudomonas fluorescens*): Enhance nutrient solubilization (e.g., phosphorus) and produce growth-promoting metabolites. Leveraging these locally sourced microbes for substrate bioaugmentation can dramatically improve substrate quality, reduce colonization times, boost yields, and suppress competitor pathogens (Stamets, 2000; Singh et al., 2011).

This research, therefore, aimed to:

(1) Isolate, identify, and characterize key indigenous lignocellulolytic microbes from Sokoto's environment

(2) Quantitatively evaluate the efficacy of these microbes in pretreating dominant local agro-wastes (maize stalks, rice husks, sawdust) for *P. ostreatus* cultivation

(3) Assess the economic viability and environmental impact of this integrated microbial-mushroom system;

(4) Develop a practical, scalable entrepreneurship model centered on this technology for empowering Sokoto's youth population.

The ultimate goal is to provide a scientifically validated, economically feasible, and ecologically sound model that transforms agro-waste into nutritional and economic assets, creating sustainable livelihoods for young people while promoting circular economy principles and climate resilience in semi-arid Nigeria.

2. Literature Review

2.1. Global Significance and Potential of Mushroom Cultivation

Mushrooms represent a vital component of sustainable food systems globally. The global mushroom market was valued at over USD 50 billion in 2022 and is projected for significant growth, driven by rising demand for nutritious and functional foods (Grand View Research, 2023). Beyond their economic value, mushrooms offer exceptional nutritional profiles. *Pleurotus* species, for instance, contain 20-30% protein (dry weight) including all essential amino acids, are low in fat and calories, rich in dietary fiber (particularly beta-glucans with proven health benefits), B-complex vitamins (riboflavin, niacin), vitamin D precursors (ergosterol), and essential minerals like potassium, phosphorus, iron, zinc, and selenium (Cheung, 2013; Reis et al., 2012). Their medicinal properties are increasingly recognized, encompassing antioxidant, anti-inflammatory, antitumor, immunomodulatory, antiviral, and hypocholesterolemic effects, largely attributed to bioactive polysaccharides (e.g., lentinan-like compounds), terpenoids, and phenolics (Wasser, 2014; Ganeshpurkar et al., 2010). From an agricultural perspective, mushroom cultivation is uniquely sustainable. It requires minimal land space (suitable for vertical farming or small plots), utilizes agricultural by-products as primary inputs, has a very high water-use efficiency compared to traditional crops or livestock, and generates valuable compost (spent mushroom substrate (SMS) as a secondary output, enhancing soil fertility (Grimm & Wösten, 2018). Its rapid production cycle (often 2-3 months from substrate preparation to harvest) allows for quick economic returns, making it highly attractive for small-scale and resource-poor farmers.

2.2. Agro-Waste: Abundance, Challenges, and Valorization Potential

Agricultural activities generate enormous quantities of lignocellulosic residues. Globally, crop residues exceed 5 billion tons annually, with a significant portion underutilized or mismanaged (Lal, 2005). In Nigeria, post-harvest residues from major crops (cereals, legumes, tubers) constitute a major waste stream. Sokoto State, being an agricultural hub in the northwest, produces substantial amounts of maize stalks, rice husks, groundnut shells, sorghum stover, and millet straw. Current disposal practices, primarily open burning, have severe consequences: Environmental Releases, greenhouse gases (GHGs: CO2, CH4, N2O)

and black carbon (a potent short-lived climate pollutant), contributes to air pollution (PM2.5, PM10), causes nutrient loss, and can lead to uncontrolled fires and soil heating (Lal, 2005).

Economic: Wastes are potential resource that could generate income and reduce input costs for other agricultural activities.

Social: Smoke from burning causes respiratory health problems in local communities.

Lignocellulose, comprising cellulose (40-50%), hemicellulose (25-30%), and lignin (15-20%), forms a complex, recalcitrant structure resistant to rapid microbial decomposition (Sánchez, 2009). While oyster mushrooms possess inherent enzymatic machinery (laccases, peroxidases, cellulases) to break this down, the process can be slow and inefficient on untreated substrates, limiting yield and economic potential. Pretreatment is crucial to disrupt lignin's protective shield, depolymerize hemicellulose, and increase cellulose accessibility (Sanchez, 2009).

2.3. Microbial Biotechnology for Substrate Enhancement

The application of specific microorganisms to pre-digest lignocellulosic substrates before mushroom spawning is a promising bioaugmentation strategy.

Key microbial groups and their roles include:

Trichoderma spp. (e.g., *T. harzianum, T. reesei)*: Filamentous fungi renowned for their potent cellulolytic and hemicellulolytic enzyme systems (endoglucanases, exoglycanases, beta-glucosidases, xylanases). They rapidly colonize substrates, breaking down cellulose and hemicellulose into simpler sugars. *Trichoderma* also exhibits strong antagonism against competitor fungi through mycoparasitism and antibiotic production, reducing contamination risks (Benítez et al., 2004; Sharma et al., 2019).

Bacillus spp. (e.g., *B. subtilis, B. licheniformis)*: Gram-positive bacteria capable of producing a wide array of extracellular enzymes, including cellulases, hemicelluloses, and lignin-modifying enzymes (laccases, peroxidases). They are robust, spore-forming, tolerate high temperatures, and often enhance nutrient availability (e.g., phosphate solubilization) and produce plant/fungal growth-promoting substances (Kumar et al., 2012; Singh et al., 2011).

Pseudomonas spp. (e.g., *P. fluorescens*): Gram-negative bacteria prevalent in soil and decaying organic matter. They are effective decomposers, producing cellulases and hemicelluloses. Crucially, many strains solubilize phosphorus and produce siderophores (iron chelators) and phytohormones (e.g., indole-3-acetic acid - IAA) that can stimulate fungal mycelial growth (Sarma et al., 2015). They contribute to microbial consortia synergy.

Aspergillus spp. (e.g., *A. niger*): Common fungi producing strong cellulase and xylanase activities. While used industrially, their potential in mushroom substrate preparation is significant but requires careful management as some strains can become competitors if not properly controlled. Using indigenous strains offers distinct advantages: adaptation to local climate/substrates, potential for higher efficacy under native conditions, ease of isolation and propagation, reduced cost compared to commercial inoculants, and avoidance of introducing non-native species (Oloke & Adebayo, 2015; Adebayo et al., 2013). Microbial

pretreatment operates through enzymatic hydrolysis, breaking complex polymers into fermentable sugars and simpler compounds more readily assimilated by the mushroom mycelium, leading to faster colonization and higher yields.

2. 4 Youth Empowerment through Agripreneurship

Youth unemployment and underemployment are critical issues in Nigeria, particularly in the north. Agriculture, contributing over 20% to Nigeria's GDP, holds immense potential for job creation, yet often fails to attract youth due to perceptions of low returns, high drudgery, and limited access to land, finance, and modern technologies (IFAD, 2019). Agripreneurship entrepreneurial agriculture is increasingly seen as a solution. Mushroom cultivation, especially when enhanced with accessible microbial biotechnology, presents a highly suitable entry point for youth agripreneurship due to:

Low Capital Investment: Requires minimal land (urban/peri-urban spaces, backyards), basic infrastructure (simple shade houses, pasteurization drums), and locally sourced inputs (agro-waste, indigenous microbes).

Rapid Returns: Short production cycle enables quicker income generation compared to many traditional crops.

High Value & Market Demand: Mushrooms command good market prices locally and regionally, with growing demand for nutritious foods.

Technical Accessibility: Core cultivation techniques can be learned relatively quickly. Microbial isolation and application, while scientific, can be taught through practical training modules.

Scalability: usually start at a very small scale (e.g., 50-100 bags) and expand progressively based on market access and capital.

Dual Sustainability Impact: Addresses waste management and provides nutritious food/income.

Gender Inclusivity: Well-suited for both young men and women, offering flexible work schedules. Integrating microbiology skills adds a layer of technical sophistication, enhancing the appeal and potential profitability of the venture, while equipping youth with valuable biotech skills applicable beyond mushroom farming (Oloke & Adebayo, 2015; FAO, 2020). Successful models require integrated support: practical training, access to quality spawn, market linkages, financial services (microcredit, grants), mentorship, and enabling policies.

3. Materials and Methods

3.1. Study Area: Sokoto State, Nigeria

Location: Northwestern Nigeria, bordering Niger Republic. Coordinates: Approx. 13°05'N, 5°15'E.

Climate: Characterized as semi-arid (Köppen BSh). Distinct hot dry season (Feb-May, temps >40°C), rainy season (Jun-Sept, avg. rainfall ~700mm concentrated in peaks), and cool dry season (Oct-Jan). High evaporation rates year-round. Average annual relative humidity ~40%.

Agriculture: Predominantly rain-fed cultivation of millet, sorghum, maize, rice, cowpea, groundnuts, and vegetables. Significant livestock rearing (cattle, goats, sheep). Generates substantial quantities of maize stalks, rice husks, groundnut shells, millet/sorghum Stover, and cowpea husks.

Rationale for Site Selection: High prevalence of target agro-wastes, significant youth unemployment, need for sustainable livelihood options, and representative of semi-arid agricultural challenges across the Sahel.

3.2. Substrate Collection, Preparation, and Characterization

Collection: Maize stalks (post-harvest), rice husks (from local rice mills), and sawdust (mixed hardwood, from carpentry workshops) were sourced directly from farms and processing centers within Sokoto metropolis and environs. Samples were pooled from multiple sources to ensure representativeness.

Pre-drying: Materials were sun-dried for 5-7 days to reduce moisture content below 15% to prevent premature microbial activity during storage.

Physical Processing:

Maize stalks: Chopped into 2-5 cm fragments using manual chaff cut Processing

Rice husks: Used as received.

Sawdust: Sieved to remove large particles and debris.

Chemical Analysis (Proximate Composition - Dry Basis): Representative samples of each substrate were analyzed at the Sokoto State University Laboratory for: Moisture Content (Oven drying at 105°C)

Ash Content (Muffle furnace at 550°C)

Crude Protein (Kjeldahl method, N x 6.25)

Crude Fiber (Acid/alkali digestion)

Lignin, Cellulose, Hemicellulose (Van Soest method

Total Carbohydrates (By difference)

pH (1:10 w/v aqueous extract)

Hydration: Substrates were soaked in clean water for 24 hours to achieve approx. 65-70% moisture content, optimal for microbial and fungal growth.

Pasteurization: Hydrated substrates were packed loosely into perforated polypropylene bags. Pasteurization was achieved using a low-tech drum method: substrates were steamed over boiling water

at 95-100°C for 4 hours, followed by a 24-hour "cook-down" period to allow heat penetration and kill mesophilic competitors. Temperature was monitored using simple stem thermometers. Sterilization (autoclaving) was avoided to mimic realistic small-scale farm conditions.

3.3. Isolation, Identification, and Preparation of Indigenous Microbial Inoculants

Sample Collection: Diverse samples targeting lignocellulolytic microflora were collected aseptically:

Compost: Actively decomposing compost piles (3 sites).

Decayed Plant Matter: Naturally rotting wood, leaf litter (3 sites).

Rhizospheric Soil: Soil adhering to roots of healthy maize plants (3 farms).

Spoiled Fruits/Vegetables: Local market waste piles (2 sites).

Isolation:

Serial Dilution and Plating: Samples were serially diluted (10-fold up to 10^{-6}) in sterile 0.85% saline. 0.1 ml aliquots of appropriate dilutions were spread-plated onto selective media:

Fungi: Potato Dextrose Agar (PDA) amended with Chloramphenicol (50 mg/L) to inhibit bacteria. Incubated at 28°C for 3-7 days.

Bacteria: Nutrient Agar (NA). Incubated at 30°C for 24-48 hours

Actinobacteria: Starch Casein Agar (SCA). Incubated at 30°C for 7-14 days

Purification: Distinct morphotypes (colonies differing in color, shape, texture, margin) were sub-cultured onto fresh plates to obtain pure cultures.

Preliminary Screening for Lignocellulolytic Potential:

Cellulolytic Activity: Plated on Carboxymethylcellulose (CMC) agar. After incubation, plates were flooded with Congo Red solution (0.1%) for 15 min, then rinsed with 1M NaCl. Clear zones around colonies indicated cellulose hydrolysis.

Ligninolytic Activity: Plated on Methylene Blue or Azure B agar. Decolorization zones around colonies indicated lignin-modifying enzyme activity.

Identification:

Morphological: Colony characteristics (color, texture, elevation, margin), microscopic examination (LPCB mount for fungi: hyphal structure, conidiophores, conidia; Gram stain for bacteria: shape, arrangement, Gram reaction).

Biochemical: Standard tests for bacteria (Catalase, Oxidase, Starch hydrolysis, Gelatin liquefaction, Sugar fermentation profiles). Fungi were identified based on conidiogenesis and spore morphology using taxonomic keys (Barnett & Hunter, 1998; Domsch et al., 2007).

(Note: Molecular confirmation was beyond the scope/resources of this study but is recommended for future work).

Selected Strains & Culture Maintenance: *Trichoderma harzianum* (isolated from compost - strain SOK-COMP-T1)

Bacillus subtilis (isolated from Rhizospheric soil - strain SOK-RHIZ-B1)

Pseudomonas fluorescens (isolated from decayed leaves - strain SOK-DECAY-P1)

Aspergillus niger (isolated from spoiled tomato - strain SOK-SPOIL-A1 - included for comparison but results not central to main findings)

Pure cultures were maintained on PDA slants (fungi) or NA slants (bacteria) at 4°C and sub-cultured monthly.

Inoculum Preparation:

Fungi *(T. harzianum, A. niger)*: Grown on PDA plates for 7 days at 28°C. Spores were harvested by flooding plates with sterile 0.05% Tween 80 solution and scraping the surface with a sterile loop. The suspension was filtered through sterile muslin cloth to remove mycelial fragments. Spore concentration was adjusted to 1 x 10^7 spores/ml using a hemocytometer.

Bacteria *(B. subtilis, P. fluorescens):* Grown in Nutrient Broth on a rotary shaker (120 rpm) at 30°C for 24-48 hours (late log phase). Cells were harvested by centrifugation (5000 rpm, 10 min), washed twice in sterile saline, and resuspended to an optical density (OD600) of 0.8 (approx. 1 x 10^8 CFU/ml).

3.4. Experimental Design and Treatments

Factorial Design: 4 Substrates (Maize Stalks, Rice Husks, Sawdust, Mixture - 1:1:1) x 5 Microbial Treatments (Control - No microbe, T. harzianum, B. subtilis, P. fluorescens, A. niger) = 20 treatment combinations.

Replication: Each treatment combination was replicated 5 times (100 experimental units total).

Unit: one experimental unit = one polypropylene bag (40cm x 25cm, ~1.5kg wet substrate capacity) filled with ~1kg dry weight equivalent of pasteurized substrate.

Microbial Inoculation: After pasteurization and cooling to ambient temperature (~30°C), substrates were inoculated with the respective microbial suspensions at 5% v/w (i.e., 50ml suspension per kg dry substrate). For the control, 50ml sterile distilled water was added. Bags were thoroughly mixed by hand (using sterile gloves) and loosely tied at the neck to allow gas exchange.

Microbial Pre-colonization Phase: Inoculated substrate bags were incubated in a clean, shaded room at ambient Sokoto temperature (28-32°C) for 72 hours to allow microbial colonization and initial substrate breakdown.

Mushroom Spawning: After 72 hours, each bag was inoculated with 10g of commercially produced, grain-based *Pleurotus ostreatus* spawn (obtained from a reputable Nigerian spawn laboratory). Spawn grains were mixed into the top 5-7cm of the substrate. Bags were then tightly closed at the neck.

Incubation Conditions: All bags were transferred to a controlled fruiting house designed to mimic optimal conditions:

Temperature: Maintained at 25 ± 2°C using evaporative cooling pads and limited ventilation. **Relative Humidity:** Maintained at 85-90% using humidifiers and regular misting with clean water.

Light: Provided by fluorescent tubes on a 12-hour photoperiod (approx. 1000 lux). CO_2: High CO_2 (>5000 ppm) tolerated during colonization phase; bags were opened and CO_2 levels reduced (<1000 ppm) by increased ventilation upon full colonization to induce pinning.

Casing Layer: Not used, as *P. ostreatus* typically fruits well without casing in bag culture.

3.5. Parameters Measured

Data collection focused on key growth, yield, and biological efficiency metrics:

1.**Colonization Time (Days)**: Time taken for visible white mycelium to fully permeate the substrate (100% colonization), recorded visually every 24 hours.

2. Contamination Rate (%): Percentage of bags per treatment showing visible growth of mmoulds(Neurospora, Rhizopus, Aspergillus competitors) or bacteria before or during mushroom colonization. Bags were discarded upon significant contamination.

3.Pinhead Formation Time (Days): Time from spawning to the first visible appearance of mushroom primordia (pins).

4. Fruiting Time (Days): Time from spawning to the first harvest of mature fruiting bodies.

5. **Number of Flushes**: Total harvest cycles per bag before significant yield drop or contamination.

6. **Yield (g):** Total fresh weight of mushrooms harvested per bag per flush. Only marketable mushrooms (intact caps, firm texture, no pests/disease) were weighed.

7. **Biological Efficiency (BE - %)**: Calculated as: (Total Fresh Mushroom Yield (g) / Dry Weight of Substrate (g)) x 100. A key indicator of substrate conversion efficiency.

8. Spawn Run Rate (mm/day): Measured during early colonization (Days 3-10) by marking the mycelial front daily.

3.6. Economic and Environmental Impact Assessment

Cost-Benefit Analysis (CBA): The detailed recording of all input costs (substrate collection or processing, pasteurization fuel, spawn, microbial inoculum preparation, bags, labor) and output revenue

(mushroom yield x local market price) for each treatment. Profit margin, Return on Investment (ROI), and Break-Even Point (BEP) were calculated.

Waste Diversion Potential: Estimated the percentage of agro-waste (by weight) successfully utilized and diverted from potential burning per unit production.

Spent Mushroom Substrate (SMS) Analysis: Proximate analysis of spent substrate compared to raw substrate to assess nutrient transformation and suitability as organic fertilizer or soil conditioner.

Carbon Footprint Estimation: Simplified calculation comparing GHG emissions avoided by preventing open burning of the agro-waste used vs. emissions from cultivation activities (primarily fuel for pasteurization).

3.7. Data Analysis

Data were recorded in standardized sheets and entered into Microsoft Excel.

Statistical analysis was performed using SPSS Statistics version 25.0 (IBM Corp.).

Normality of data distribution was assessed using Shapiro-Wilk test. Homogeneity of variances was checked using Levene's tesCor

Analysis of Variance (ANOVA): One-way ANOVA was used to determine significant differences ($p < 0.05$) between microbial treatments for each substrate type for key parameters (Colonization Time, Yield, BE). Where ANOVA indicated significance, post-hoc Tukey's Honestly Significant Difference (HSD) test was applied for pairwise comparisons.

Two-way ANOVA: Used to assess the interaction effects between Substrate Type and Microbial Treatment on Yield and BE.

Contamination Rates: Compared using Chi-square (χ^2) tests. Results are presented as Mean ± Standard Deviation (SD). Significance levels: $p < 0.05$, $p < 0.01$, $p < 0.001$.

4. Results and Discussion

4.1. Substrate Characterization

Results confirmed the lignocellulosic nature of the substrates (Table 1). Maize stalks had the highest cellulose content, rice husks were rich in silica and lignin, while sawdust had a balanced lignocellulose profile but lower nitrogen. The mixture aimed to balance nutrients and structure. Initial pH was slightly acidic to neutral.

Table 1: Proximate Composition of Raw Agro-Waste Substrates (Dry Weight Basis)

4.2. Microbial Impact on Colonization and Fruiting

Microbial pretreatment significantly accelerated mycelial colonization across all substrates compared to uninoculated controls ($p < 0.001$). *T. harzianum* consistently demonstrated the most potent effect (Table

2, Fig 1). *B. subtilis* and *P. fluorescens* also significantly reduced colonization times, though less dramatically than *Trichoderma*. *A. niger* showed variable results, sometimes accelerating colonization but increasing contamination risk. Pinhead formation and first fruiting times mirrored colonization speed, occurring significantly earlier in microbially pretreated substrates.

Table 2: Effect of Microbial Pretreatment on Colonization Time (Days) of Pleurotus ostreatus (Mean ± SD)

Treatment	Maize Stalks	Rice Husks	Sawdust	Mixture	Overall Mean
Control	15.8 ± 1.1a	16.2 ± 0.8a	17.5 ± 1.3a	16.0 ± 1.0a	**16.4 ± 1.3a**
T. harzianum	9.4 ± 0.7d	10.0 ± 0.9d	11.2 ± 0.8d	9.0 ± 0.8d	**9.9 ± 1.0d**
B. subtilis	11.2 ± 0.8c	11.8 ± 0.9c	13.0 ± 1.0c	10.8 ± 0.9c	**11.7 ± 1.1c**
P. fluorescens	12.0 ± 1.0b	12.5 ± 0.8b	14.0 ± 1.2b	11.5 ± 0.7b	**12.5 ± 1.2b**
A. niger	13.5 ± 1.2b	14.8 ± 1.1a	16.0 ± 1.5a	13.0 ± 1.0b	**14.3 ± 1.6b**
Substrate Mean	**12.3 ± 2.5**	**12.9 ± 2.4**	**14.3 ± 2.5**	**12.0 ± 2.5**	

Means within a column followed by the same superscript letter are not significantly different ($p > 0.05$, Tukey's HSD).

Figure 1: Colonization Time Comparison Across Treatments (Overall Mean)

4.3. Yield and Biological Efficiency

Microbial pretreatment dramatically increased mushroom yield and BE ($p < 0.001$). *T. harzianum* again yielded the best results, followed by *B. subtilis* and *P. fluorescens* (Table 3, Fig 2). The mixture substrate generally performed best, likely due to complementary nutrients and structure. Maize stalks were the best single substrate, followed by rice husks. Sawdust alone performed poorly without pretreatment, but responded well to microbes. *A. niger* increased yield compared to control but significantly less than the other microbes and showed higher variability. Two-way ANOVA confirmed significant interaction ($p < 0.01$) between substrate and microbe, highlighting the importance of matching microbes to substrate type.

Table 3: Effect of Microbial Pretreatment on Total Yield (g/kg Dry Substrate) and Biological Efficiency (BE%) of Pleurotus ostreatus (Mean ± SD)

Treatment	Maize Stalks (Y / BE)	Rice Husks (Y / BE)	Sawdust (Y / BE)	Mixture (Y / BE)	**Overall Mean (Y / BE)**
Control	825 ± 30a / 82.5a	745 ± 25a / 74.5a	680 ± 35a / 68.0a	780 ± 28a / 78.0a	**758 ± 62a / 75.8a**
T. harzianum	1105 ± 35d / 110.5d	1005 ± 30d / 100.5d	980 ± 40d / 98.0d	1080 ± 32d / 108.0d	**1043 ± 59d / 104.3d**

| *B. subtilis* | 1010 ± 28c / 101.0c | 950 ± 32c / 95.0c | 870 ± 38c / 87.0c| 995 ± 30c / 99.5c| **956 ± 64c / 95.6c** |

| *P. fluorescens* | 965 ± 30b / 96.5b | 900 ± 28b / 90.0b | 820 ± 32b / 82.0b| 950 ± 28b / 95.0b| **909 ± 65b / 90.9b** |

| *A. niger* | 890 ± 40b / 89.0b | 810 ± 35b / 81.0b | 750 ± 40a / 75.0a| 880 ± 36b / 88.0b| **833 ± 67b / 83.3b** |

| **Substrate Mean (Y)**| **959 ± 115** | **882 ± 104** | **820 ± 121** | **937 ± 115** | |

| **Substrate Mean (BE)**| **95.9 ± 11.5** | **88.2 ± 10.4** | **82.0 ± 12.1** | **93.7 ± 11.5** | |

Means within a column for Yield or BE followed by the same superscript letter are not significantly different ($p > 0.05$, Tukey's HSD).

Figure 2: Mushroom Yield (g/kg Dry Substrate) Across Treatments (Overall Mean)

4.4. Contamination Rates

Contamination was significantly lower ($p < 0.05$) in bags pretreated with *T. harzianum* (8%) and *B. subtilis* (10%) compared to the control (22%) and *A. niger* (25) *P. fluorescens* showed an intermediate rate (15%). Trichoderma known antagonistic properties likely suppressed competitor molds. *B. subtilis* may have outcompeted pathogens. *A. niger* sometimes became dominant, hindering Pleurotus growth.

4.5. Economic Viability

The CBA demonstrated clear economic advantages for microbial pretreatment, primarily driven by the significant yield increase:

Cost Increase: Microbial inoculum preparation added approximately 5-7% to total production costs (primarily labor and minor materials for culture maintenance).

Revenue Increase: The 25-35% yield increase (especially with *T. haincreas)* translated to a revenue increase of 30-40%, far outweighing the cost increase.

Profit Margin: Pretreatment with *T. harzianum* increased profit margins by an average of 35% compared to the control across substrates. Profit margins for the mixture substrate with *T. harzianum* were the highest.

ROI: Increased from 120% in the control to ~180% with *T. harzianum* on the mixture substrate within a single 3-month cycle.

BEP: Reduced significantly with microbial pretreatment, meaning youth entrepreneurs reach profitability faster.

4.6. Environmental Benefits

Waste Diversion: The model successfully diverted 85-90% of the agro-waste used from potential open burning or landfill.

GHG Mitigation: Preliminary estimates suggest preventing the burning of 1 tons of agro-waste avoids approximately 1.5 tons CO2-equivalent emissions. Cultivation emissions (mainly pasteurization fuel) were estimated at <0.1 tons CO2-eq per ton substrate, indicating a significant net reduction.

SMS Valorization: Spent substrate analysis showed increased nitrogen content (up by 30-50%), reduced C:N ratio (from ~60-80:1 to ~25-35:1), and enrichment with microbial biomass, making it an excellent organic soil amendment. Field trials showed improved soil structure and crop growth when SMS was applied.

Water Efficiency: Mushroom cultivation used significantly less water per kg protein produced compared to staple crops or livestock common in Sokoto.

4.7. Discussion of Key Findings

T. harzianum Superiority: The dominance of *T. harzianum* aligns with numerous studies (Sharma et al., 2019; Singh et al., 2011). Its effectiveness stems from its potent enzymatic arsenal (cellulases, hemicelluloses, chitinases) rapidly depolymerizing lignocellulose, releasing readily assimilable sugars for Pleurotus Its mycoparasitic ability suppressed competitors like *Trichoderma* sp., *Aspergillus sp.,* and *Mucor* sp., reducing contamination. Its adaptation to the local Sokoto environment likely enhanced performance.

Bacterial Contributions: *B. subtilis* and *P. fluorescens* also significantly improved yields. Beyond lignocellulolysis, their roles in nutrient solubilization (P, K, Fe), production of growth-promoting phytohormones (IAA), siderophores, and possibly induced systemic resistance likely created a more favorable rhizosphere-like environment for the *Pleurotus* mycelium (Sarma et al., 2015). Their synergy with fungi warrants further investigation.

Substrate-Microbe Interaction: The significant interaction effect emphasizes that optimal results require matching the microbial consortium to the substrate's specific lignocellulose composition and nutrient profile. The mixture substrate generally performed best, suggesting blending wastes optimizes physical structure and nutrient balance.

Economic & Empowerment Potential: The 35% average profit margin increase with *T. harzianum* is transformative for small-scale youth enterprises. Starting with 500 bags (requiring ~15m² space and ~$150-$200 USD initial investment for pasteurization drum, bags, spawn, inoculum setup), a youth entrepreneur could generate a net income exceeding the local minimum wage within the first 3-month cycle, with potential for rapid scaling. The integration of microbiology skills adds unique value and marketability.

Environmental Sustainability: The model embodies circular economy principles: waste becomes food and fertilizer, carbon is sequestered in fungal biomass and soil via SMS, pollution is reduced, and resource use is optimized. It contributes directly to SDGs 2 (Zero Hunger), 8 (Decent Work), 12 (Responsible Consumption), and 13 (Climate Action).

5. Conclusion and Recommendations

This research conclusively demonstrates the high potential of integrating indigenous microbial biotechnology, specifically using *Trichoderma harzianum*, with *Pleurotus ostreatus* cultivation on local agro-wastes in Sokoto, Nigeria. Key findings are:

1. Indigenous *T. harzianum*, isolated from Sokoto compost, significantly accelerated mycelial colonization (by ~6.5 days, 36% reduction) and boosted mushroom yield (by 35%) compared to uninoculated controls, while reducing contamination rates.

2. *Bacillus subtilis* and *Pseudomonas fluorescens* also provided significant, though lesser, benefits, highlighting potential for synergistic microbial consortia development.

3. A mixture of maize stalks, rice husks, and sawdust proved the most effective substrate, particularly when pretreated with *T. harzianum*, achieving a Biological Efficiency of 108%.

4. The model is economically viable, increasing profit margins by ~35% compared to traditional methods, with a rapid Return on Investment (ROI ~180%) achievable within a single production cycle, making it highly suitable for youth-led startups with low capital.

5. Significant environmental benefits include substantial agro-waste diversion (~85-90%), avoidance of open burning GHG emissions, production of valuable organic fertilizer (SMS), and efficient land/water use.

6. This approach directly addresses critical challenges in Sokoto and similar regions: youth unemployment, food insecurity, agricultural waste mismanagement, and climate change impacts.

Recommendations for Implementation and Youth Empowerment:

Policy Integration:

Nigerian Federal and Sokoto State Ministries of Agriculture should formally integrate microbial-enhanced mushroom cultivation into agricultural extension programs, youth empowerment initiatives (e.g., N-Power Agro), and climate-smart agriculture strategies.

*Develop specific policy guidelines and financial incentives (e.g., grants, low-interest loans, subsidies for spawn production units) targeting youth agripreneurs in this sector. Promote the recognition and utilization of SMS as a certified organic fertilizer within agricultural input subsidy programs.

Capacity Building & Training

Establish dedicated "Mushroom and Microbial Biotechnology Hubs" within Sokoto's tertiary institutions (e.g., Usmanu Danfodiyo University, Sokoto State University) or Agricultural Development Projects (ADPs) to serve as training centers. Development of standardized, practical training modules covering: Basic microbiology (aseptic technique, microbe isolation, culture maintenance), substrate preparation (pasteurization, microbial inoculation), mushroom cultivation (spawning, incubation, fruiting, harvesting, post-harvest handling), SMS utilization, basic business skills (record keeping, cost-benefit analysis, marketing) Training should emphasize hands-on learning and include follow-up mentorship.

Entrepreneurship Support:

Facilitate access to affordable, high-quality Pleurotus spawn through support for local spawn laboratories or entrepreneur cooperatives. Establish microfinance linkages specifically tailored for youth mushroom ventures.

Develop market linkages: connect producers with local markets (restaurants, hotels, supermarkets), institutional feeding programs (schools, hospitals), and explore processing/value-addition opportunities (drying, powdering).

Create youth cooperatives to leverage economies of scale in input procurement, processing, and marketing.

Research and Development:

Investigate optimal microbial consortia combinations (*T. harzianum* + *B. subtilis* + *P. fluorescens*) for different substrate blends. Optimize inoculation protocols (concentration, timing, application method) Evaluate the long-term productivity and economic sustainability of farms adopting this model. Explore the potential of other high-value mushrooms (e g *T, Shiitake*) adapted to the region. Conduct detailed Life Cycle Assessment (LCA) to quantify the full environmental benefits. Develop simple, low-cost kits for on farm microbial culture maintenance and inoculum production.

Conclusion:

This study provides robust scientific evidence and a practical blueprint for transforming Sokoto's agricultural waste burden into an engine for youth empowerment, nutritional security, and environmental sustainability. By harnessing the power of indigenous microbes like *Trichoderma harzianum*, young people can become pioneers in a profitable, sustainable, and climate-resilient agribusiness sector. The proposed microbiology-based entrepreneurship model is not merely a cultivation technique; it is a pathway to economic inclusion, skills development, and ecological restoration for the youth of Northern Nigeria. Concerted efforts from government, research institutions, the private sector, and development partners are crucial to scale this model and unlock its transformative potential across the semi-arid regions of Africa and beyond.

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