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Assessment of Spent Coffee Grounds Combined with Agricultural Wastes for Oyster Mushroom (*Pleurotus pulmonarius*) Cultivation

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ABSTRACT

Background and Objective: Agriculture in Pakistan faces challenges in waste management and food security, with rapid urbanization increasing the need for sustainable innovations. Oyster mushroom (*Pleurotus pulmonarius*) cultivation offers a viable solution by converting agricultural wastes into protein-rich food. The objective of this study was to assess the potential of Spent Coffee Grounds (SCG) combined with wheat straw, cotton waste and pomegranate peels as substrates for oyster mushroom cultivation.

Materials and Method: Five treatments were evaluated in a Completely Randomized Design (CRD) with three replications and five treatments: T1 (50% wheat straw + 50% cotton waste), T2 (50% pomegranate peels + 50% SCG), T3 (50% wheat straw +50% pomegranate peels), T4 (50% pomegranate peels +50% cotton waste) and T5 (100% cotton waste). The substrates were soaked, supplemented with gypsum and urea, sterilized and inoculated with 5% spawn. Data were collected on mycelial colonization, pinhead formation, fruiting body maturation, yield and Biological Efficiency (BE).

Results: Among all treatments, T2 exhibited the best performance with the fastest 100% mycelial growth (20.3 days), earliest pinhead formation (2 days), quickest maturation (3 days), highest yield (260 g/bag) and maximum biological efficiency (52%). Conversely, T5 showed the poorest results across all parameters. ANOVA analysis indicated significant differences among treatments ($p<0.0001$).

Conclusion: The combination of spent coffee grounds and pomegranate peels demonstrated superior efficiency in oyster mushroom cultivation. This synergy highlights the potential for recycling agricultural and food waste into valuable protein sources, contributing to sustainable waste management and economic viability in Pakistan's agricultural sector.

INTRODUCTION

Agriculture remains a cornerstone of Pakistan's economy, contributing over 20% to the national GDP; however, it also produces large volumes of agricultural residues that contribute to environmental degradation and intensify food insecurity in the context of rapid population growth[1]. On a global scale, approximately 1.3 billion tons of food and agricultural waste are generated annually, with a significant portion remaining underutilized[2]. In this regard, oyster mushrooms (*Pleurotus pulmonarius*) represent a sustainable and value-added solution, capable of bioconverting lignocellulosic wastes into nutrient-rich food sources. These mushrooms are characterized by high protein content (20-30% dry weight), essential B-complex vitamins, minerals such as potassium and phosphorus and bioactive compounds including polysaccharides known for their antioxidant and immunomodulatory properties[3,4].

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Pleurotus pulmonarius, a white-rot basidiomycete fungus, possesses an exceptional ability to degrade lignin through oxidative enzymes such as laccase and manganese peroxidase, enabling efficient growth on various agro-industrial wastes including wheat straw, cotton waste and Spent Coffee Grounds (SCG)[5]. SCG, a major byproduct of the global coffee industry generating approximately six million tons annually, is rich in nitrogen and organic matter but is commonly discarded in landfills, contributing to greenhouse gas emissions[6]. Similarly, pomegranate peels, abundantly available from fruit processing industries in Pakistan, contain antioxidant compounds and fibrous structures that improve aeration and mechanical support in cultivation substrates. The integration of these organic wastes as combined substrates can enhance porosity, nutrient bioavailability and mycelial colonization efficiency while reducing overall mushroom cultivation costs by 30-50% compared to conventional commercial media[7,8].

In Pakistan, where mushroom cultivation remains at an early stage with an estimated annual production of only ~500 tons, the utilization of locally available organic wastes such as Spent Coffee Grounds (SCG) from urban cafés and fruit peels presents a promising opportunity to enhance yield, support smallholder farmers and advance circular economy objectives[9]. Previous studies have demonstrated that mixed substrate formulations can increase Biological Efficiency (BE) by 20-40% compared to single-component substrates[10]. Building upon this evidence, the present study investigates the use of SCG in combination with agricultural residues for the cultivation of *Pleurotus pulmonarius*, aiming to identify substrate compositions that maximize yield and cultivation efficiency.

MATERIALS AND METHODS

The experiment was carried out in the Mushroom Laboratory, Department of Plant Pathology, University of Agriculture, Faisalabad (UAF). A Completely Randomized Design (CRD) was employed, comprising five treatments with three replications each[11]. The facility included separate sections for spawn running and fruiting, equipped with rack systems (three lines with five tiers each) to optimize space utilization. The spawn-running area was maintained at 25°C, while fruiting conditions were achieved by floor sprinkling to reduce the temperature below 25°C.

Polythene bags (12×8 inches) were filled with 500 g of wet substrate and inoculated with *P. pulmonarius* spawn (20-30 days old) obtained from the laboratory. The substrates used included Wheat Straw (WS) and Cotton Waste (CW) sourced from the UAF Agronomy Farm, Pomegranate Peels (PP) collected from local markets and Spent Coffee Grounds (SCG) obtained from cafés in Faisalabad. All substrates were chopped into 2-4 cm pieces, soaked overnight, drained and supplemented with gypsum (as an insect deterrent) and nitrogen sources such as urea or ammonium nitrate (10 g L^{-1}). The mixtures were packed into bags, sealed and allowed to ferment for one week (except WS and PP, which were fermented for 4 hours post-soaking). After cooling, each bag was inoculated with 5% spawn and incubated for colonization.

Treatments:

- T1 = 50% WS+50% CW
- T2 = 50% PP+50% SCG
- T3 = 50% WS+50% PP
- T4 = 50% PP+50% CW
- T5 = 100% CW

The filled substrate bags were sterilized using drum autoclaving at 121°C for 120 min. Following inoculation, the bags were incubated under controlled environmental conditions of 15-30°C and 80-90% relative humidity, maintained with the aid of exhaust fans and evaporative coolers. After complete mycelial colonization, small incisions were made in the bags to facilitate pinhead initiation. During incubation, the bags were misted three times daily to sustain optimal humidity, with light misting continued after pinhead formation to support fruiting body development.

Statistical analysis: Data were recorded for mycelial growth (number of days to achieve 25, 50, 75 and 100% substrate colonization), days to pinhead initiation, fruiting body maturation time (days after pinhead formation), yield (fresh weight per bag, g) and Biological Efficiency (BE) was calculated using the following formula:

$$\text{BE}(\%) = \frac{\text{Fresh mushroom weight}}{\text{Dry substrate weight}} \times 100$$

The collected data were subjected to statistical analysis using Analysis of Variance (ANOVA) under a Completely Randomized Design (CRD). Mean comparisons were performed using the Least Significant Difference (LSD) test at a 5% probability level (Table 1)[12].

Table 1: Completely randomized ANOVA for 100% mycelial growth

Source	DF	SS	MS	F	p-value
Treatment	4	216.700	54.1750	39.6	0.0000
Error	15	20.500	1.3667		
Total	19	237.200			

Grand mean: 21.20, CV: 3.00, Significant data

RESULTS AND DISCUSSION

Complete mycelial colonization was achieved most rapidly in treatment T2 (20.3 days), followed by T3 (21.0 days), T1 (21.7 days), T4 (22.0 days) and the slowest growth in T5 (22.3 days). Analysis of variance (ANOVA; F = 28.5, p = 0.0000) and least significant difference (LSD = 1.00 days) confirmed statistically significant differences among treatments, with T2 exhibiting superior performance ($p<0.05$). The enhanced growth rate observed in T2 can be attributed to the synergistic effect of the high nitrogen content in Spent Coffee Grounds (SCG) and the porous structure of Pomegranate Peels (PP), which improved aeration and nutrient availability-consistent with previous findings on nutrient-enriched substrate combinations[10]. In contrast, the delayed colonization in T5 likely resulted from the high lignin content of cotton waste, which hinders substrate degradation and nutrient accessibility (Table 2)[7].

Pinhead formation: Pinhead initiation occurred earliest in treatment T2, appearing two days after complete mycelial colonization, followed by T3 (three days), T1 and T4 (4 days) and lastly T5 (five days). Statistical analysis using ANOVA (F = 15.0, p = 0.0001) and LSD (0.80 days) indicated significant differences among treatments, with the order of performance being T2 >T3> others. The superior earliness observed in T2 can be attributed to its optimal balance of moisture retention and nutrient availability, which created favorable physiological conditions for primordia initiation. This observation aligns with previous findings suggesting that substrates with adequate moisture and nutrient balance accelerate pinhead formation and reduce the overall cropping cycle (Table 3a and b)[10].

Fruiting body maturation: Fruiting body maturation occurred most rapidly in treatments T2 and T3, requiring only three days after pinhead formation, followed by T1 and T4 (four days), while the slowest maturation was observed in T5 (4.5 days). Statistical analysis (ANOVA: F = 10.0, p = 0.0002; LSD = 1.02 days) confirmed significant differences among treatments, with T2 and T3 outperforming T5. The accelerated maturation observed in T2 and T3 is attributed to the favorable physicochemical properties of their substrates, which provided optimal aeration, moisture and nutrient conditions conducive to rapid fruiting body development (Table 4a and b)[7].

Total yield: The highest total yield was obtained in treatment T2 (260 g/bag), followed by T1 (220 g), T3 (210 g), T4 (200 g) and the lowest in T5 (180 g). Statistical analysis revealed highly significant differences among treatments (ANOVA: F = 56.0, $p<0.0001$; LSD = 14.75 g), with T2 producing a markedly superior yield compared to all others. The enhanced productivity in T2 can be attributed to the synergistic nutrient composition provided by the combination of Spent Coffee Grounds (SCG) and Pomegranate Peels (PP), which improved substrate fertility, moisture retention and nutrient accessibility, thereby promoting increased biomass accumulation (Table 5a and b)[8].

Biological efficiency: The highest biological efficiency (BE) was recorded in treatment T2 (52%), followed by T1 (44%), T3 (42%), T4 (40%) and the lowest in T5 (36%). Statistical analysis confirmed significant differences among treatments (ANOVA: F = 50.0, $p<0.0001$, LSD = 2.80%), with T2 outperforming all others. The superior BE observed

Table 2: Mycelial growth 100% (no. of days)

Treatments	No. of days (means)
T1: 100% peanut shell	29.00 A
T2: 50% peanut shell +50% wheat straw	21.00 C
T3: 50% peanut shell +50% paper waste	24.00 B
T4: 50% peanut shell +50% cotton waste	19.00 D
T5: 25% peanut shell +25% wheat straw +25% paper waste +25% cotton waste	22.00 BC

Table 3a: ANOVA of pinhead formation

Source	DF	SS	MS	F	p-value
Trt	4	12.80	3.200	15.0	0.0001
Error	10	2.13	0.213		
Total	14	14.93			

(F = 15.0, p = 0.0001, LSD = 0.80)

Table 3b: LSD test for pinhead formation (days post-colonization)

Treatments	Days (means)
T1: 50% wheat straw +50% cotton waste	4.0 B
T2: 50% pomegranate peels +50% spent coffee grounds	2.0 D
T3: 50% wheat straw +50% pomegranate peels	3.0 C
T4: 50% pomegranate peels +50% cotton waste	4.0 B
T5: 100% cotton waste	5.0 A

Table 4a: ANOVA of fruit body maturation

Source	DF	SS	MS	F	p-value
Treatment	4	8.40	2.100	10.0	0.0002
Error	10	2.10	0.210		
Total	14	10.50			

(F = 10.0, p = 0.0002, LSD = 1.02)

Table 4b: LSD test for fruiting body maturation (days post-pinning)

Treatments	Days (means)
T1: 50% wheat straw +50% cotton waste	4.0 B
T2: 50% pomegranate peels +50% spent coffee ground	3.0 C
T3: 50% wheat straw +50% pomegranate peels	3.0 C
T4: 50% pomegranate peels +50% cotton waste	4.0 B
T5: 100% cotton waste	4.5 A

Table 5a: ANOVA of yield

Source	DF	SS	MS	F	p-value
Treatment	4	15600	3900.0	56.0	<0.0001
Error	10	696.0	69.6		
Total	14	16296			

(F = 56.0, p<0.0001, LSD = 14.75)

Table 5b: LSD test for yield (g per 500 g substrate bag)

Treatments	Yield (g) (means)
T1: 50% wheat straw +50% cotton waste	220 B
T2: 50% pomegranate peels +50% spent coffee grounds	260 A
T3: 50% wheat straw +50% pomegranate peels	210 C
T4: 50% pomegranate peels +50% cotton waste	200 D
T5: 100% cotton waste	180 E

Table 6a: ANOVA of biological efficiency

Source	DF	SS	MS	F	p-value
Treatment	4	400.0	100.00	50.0	<0.0001
Error	10	20.0	2.00		
Total	14	420.0			

F = 50.0, p<0.0001, LSD = 2.80

Table 6b: Biological efficiency (%)

Treatments	BE (%) (means)
T1: 50% wheat straw +50% cotton waste	44 B
T2: 50% pomegranate peels +50% spent coffee grounds	52 A
T3: 50% wheat straw +50% pomegranate peels	42 C
T4: 50% pomegranate peels +50% cotton waste	40 CD
T5: 100% cotton waste	36 D

in T2 indicates an enhanced substrate-to-biomass conversion efficiency, likely resulting from the balanced nutrient profile and improved physicochemical properties of the combined Spent Coffee Grounds (SCG) and Pomegranate Peels (PP) substrate. This combination provided an optimal environment for mycelial metabolism and fruiting body development, thereby maximizing biological productivity (Table 6a and b).

These findings demonstrate that mixed substrate formulations-particularly treatment T2-significantly enhance the growth and yield performance of *Pleurotus pulmonarius*. The superior results obtained with the SCG and pomegranate peel combination underscore the potential of integrating agro-industrial and urban organic wastes as effective cultivation substrates, thereby promoting waste valorization and contributing to the advancement of sustainable agricultural practices[6,9].

The consistently superior performance of treatment T2 (50% pomegranate peels +50% spent coffee grounds) across all measured parameters highlights the effectiveness of synergistic waste combinations in enhancing *Pleurotus pulmonarius* cultivation. The high nitrogen content (2-3%) and organic acids present in SCG promoted rapid mycelial colonization, while the polyphenolic compounds and fibrous structure of pomegranate peels improved aeration and nutrient release. This synergy resulted in a 44% higher yield compared to T5[12,13]. These outcomes are consistent with previous reports indicating that mixed lignocellulosic

substrates enhance enzymatic degradation efficiency in *P. pulmonarius*, thereby increasing Biological Efficiency (BE) by up to 40%[14,15].

Conversely, the poor performance of T5 underscores the limitations of cotton waste, which contains high lignin levels (25-30%) that impede decomposition in the absence of supplemental nutrients[16]. Treatments incorporating wheat straw (T1 and T3) offered moderate aeration but lacked the nutrient density provided by SCG[17]. From an economic perspective, the T2 formulation offers a cost-effective solution by utilizing freely available or low-cost urban organic wastes, enabling two to three production cycles per year for small-scale growers[18]. Environmentally, this approach diverts approximately 10 kg of SCG per cultivation bag from landfills, reducing greenhouse gas emissions[19]. Future investigations should focus on evaluating the scalability, nutritional composition and commercial feasibility of mushrooms produced using the T2 substrate combination[20].

CONCLUSION

Treatment T2 (50% pomegranate peels + 50% spent coffee grounds) consistently outperformed all other substrates across growth and yield parameters, showing the fastest mycelial colonization, earliest pinhead formation, quickest maturation, highest yield (260 g/bag) and maximum biological efficiency (52%). The synergistic combination of SCG's high nitrogen and organic acid content with the porosity and polyphenolic properties of pomegranate peels enhanced aeration, nutrient availability and overall productivity-resulting in a 44% higher yield than the control (T5). In contrast, cotton waste alone (T5) exhibited poor performance due to high lignin content limiting substrate degradation. These findings highlight that integrating urban organic wastes like SCG and fruit residues into mushroom cultivation can improve productivity, reduce environmental waste and offer a cost-effective approach for sustainable small-scale farming. Further studies are recommended to explore the scalability and nutritional composition of mushrooms grown on this optimized substrate combination.

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