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Research Article

Cultivation of *Pleurotus ostreatus* **on Agricultural Wastes and Their Combination**

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Background. Mushrooms are increasingly becoming an important component of diets worldwide, and it is of paramount importance to choose appropriate substrates to grow them. The objective of this study was to grow Pleurotus ostreatus mushroom using different agricultural substrates. Methods. Corncobs, finger millet straw, and bamboo waste were collected from different sites of the Awi Zone. The substrates were chopped into small pieces, and 500 g of their dry mass alone and their combination was measured, packed in a polythene bag, moistened, and pasteurized. The cooled substrates were inoculated with a spoon of P. ostreatus spawn brought from Debre Berhan University. The bags were placed in the growing room, and growth parameters were recorded continuously with environmental variables. The experimental setup was a complete randomized design, six treatments with three replicates. Results. The fastest spawn running phase of P. ostreatus was 28.71 ± 0.80 days, pinhead formation was 32.36 ± 0.26 days, and fruiting bodies' formation was 5.19 ± 0.74 days after the pinhead was recorded on the corncob substrate. The highest fresh weight and biological efficiency with the significant statistical association were obtained from *P. ostreatus* grown on finger millet straw (253.07 ± 1.05 and 50.20 ± 0.47, respectively). The highest average number of pinheads and fruiting bodies (29.60 and 11.44, respectively) was recorded on finger millet straw. The lowest biological efficiency (20.80 ± 0.41), fresh weight (101.48 ± 0.91), number of pinheads (14.40), and number of fruiting bodies (4.25) were recorded from a mixture of corncob and bamboo waste (50% each) substrates. Conclusion. Finger millet straw is recommended as the best substrate for the cultivation of P. ostreatus. The mixed substrate of corncob and bamboo waste (1:1) for P. ostreatus cultivation is not encouraged due to poor growth performance.

1. Background

One of the world's biggest challenges is food insecurity. This problem is largely common in low- and middle-income countries that mainly have poor food production systems and suffer from serious malnutrition. Mushroom cultivation could be a possible option to alleviate poverty and develop the life style of vulnerable people [1]. Mushroom growing naturally is good for human consumption, and some species are edible, and others are poisonous [2]. The genus *Pleurotus* consists of 40 different species commonly known as "oyster mushroom." Among several species of this genus, *Pleurotus ostreatus* is widely consumed globally due to its taste, flavor, high nutritional content, and medicinal properties [3].

Oyster mushroom is an important nutrient source of protein, carbohydrates, vitamins, calcium, and iron [4]. Edible mushroom has a low crude fat content and a high proportion of polyunsaturated fatty acids [5]. Oyster mushroom production is becoming popular worldwide due to its potential to grow at a wide range of temperatures and utilization of various lignocelluloses [6]. Mushroom cultivation does not always require access to land and significant capital investments, and its production can be practiced by rural, periurban, and urban dwellers [7].

Pleurotus species are widely cultivated worldwide, commonly in Asia, America, and Europe, because of their simpleness, low-cost production technology, and high biological efficiency [8]. The growth of the oyster mushroom

requires high humidity (80-90%) and a temperature of 25–30°C for fruiting body formation [9]. The spent substrate left after harvest could be used as a soil conditioner to plants and animal feed after mushroom cultivation [10]. The substrates that have been used for mushroom production in previous studies include rice straw, rice bran, wheat straw, pulp, corncobs, cocoa shell waste, cotton waste, spent grain, sawdust, maize husks, and cassava peelings [2, 11]. Other substrates are soybean straw, paddy straw, sun flower stalks, sugarcane bagasse, fruit waste, used tea leaves, bamboo leaves, and maize stalk [1, 12, 13]. Enset waste, teff straw, paper waste, finger millet husk, and banana pseudo-stems are additional substrates used for the cultivation of oyster mushrooms [14-17]. Therefore, the current study intended to evaluate Pleurotus ostreatus cultivation on different substrates alone and their combination.

2. Materials and Methods

- 2.1. Study Site. The study was conducted at Injibara University. The university was a governmental institution, the cornerstone for the establishment was laid down in 2015, and it started teaching undergraduate students in 2017. It was located in Injibara town, the capital city of the administrative center of Awi Zone, Ethiopia.
- 2.2. Preparation of Substrates. Corncob (Zea mays) and finger millet straw (Eleusine coracana) substrates were collected from districts of Chagni town. Bamboo waste (Phyllostachys pubescens) was collected from Injibara town. The substrates were transported to Injibara University and chopped into sections of 2 to 5 cm long. Finger millet straw, bamboo waste, and corncob alone and their combination in 1:1 ratio were used as cultivation substrates. The weight of substrates was measured, and they were soaked overnight in a separate clean, freshwater plastic can. Excess water is manually removed by squeezing with the hand. Each substrate was filled in a polythene bag (500 g/bag) with the addition of lime at the rate of 5% (on a dry weight basis), and their mouths were plugged by inserting water-absorbing cotton with the help of plastic rings. The bags were pasteurized in drums at 100°C for 5 hours and kept cool for 6 hours. Figures 1-5 show growth substrate processing during practical work.
- 2.3. Treatment Formulation of Substrates (Table 1). 2.4. Spawning. One tablespoonful of mother culture grain containing mycelia of Pleurotus ostreatus was placed aseptically through the hole of each bag. The bags were placed in a dark cropping room at 25°C for spawn running and 17–20°C for fruiting body formation. Bags were sprinkled with water twice a day to maintain relative humidity.
- 2.5. Harvesting of Mushrooms. Harvesting was performed when gills were well formed. Harvesting was performed by gently pulling or twisting the mushrooms from the substrate.



FIGURE 1: Bamboo waste.



FIGURE 2: Corncob substrate.

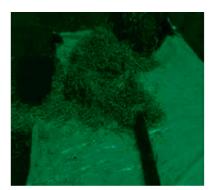


FIGURE 3: Finger millet straw.



FIGURE 4: Bagging substrate.



FIGURE 5: Oil drum pasteurization of the substrate.

Table 1: Treatment formulation for cultivation of *P. ostreatus* on different substrates.

Treatments	Substrates	Composition	Dry weight per bag
T1	CC	CC (100%)	500 grams
T2	FMS	FMS (100%)	500 grams
Т3	BW	BW (100%)	500 grams
T4	FMS + CC	FMS (50%) + CC (50%)	250 gram each
T5	CC + BW	CC (50%) + BW (50%)	250 gram each
T6	FMS + BW	FMS $(50\%) + BW (50\%)$	250 gram each

CC: corncob (Zea mays); FMS: finger millet straw (Eleusine coracana); BW: bamboo waste (Phyllostachys pubescens).

2.6. Fungal Growth Measurement

2.6.1. Days for the Spawn Running Phase. Colonization of substrates with fungal mycelia was monitored at five-day intervals. The number of days with full mycelia coverage of substrates after the day of spawning was recorded.

2.6.2. Days for the Pinhead Formation. After the bags were partially opened, primordia formation was observed every day, and the number of days taken for the first primordia formation was observed and recorded.

2.6.3. Days for the Fruiting Body Formation. The number of days taken to form the fruiting body of *Pleurotus ostreatus* was inspected daily after the formation of effective primordia, and the days were numerically recorded.

2.7. Pileus Diameter. The pileus diameter of the caps was measured by using the measuring ruler and expressed in centimeter.

2.8. Biological Efficiency. The total weight of the fruiting bodies harvested from the substrates measures the efficiency of mushrooms. Biological efficiency was calculated by the following formula: (BE %) = FWM/DWS × 100, where BE is the biological efficiency, FWM is the fresh weight of mushrooms, and DWS is the dry weight of substrates.

2.9. Data Collection and Statistical Analysis. The experiment was a completely randomized design (CRD) with three replications and six treatments. One-way ANOVA techniques were employed to test the overall significance of data in contrast to the least significance difference (LSD) test which compared the differences among variety means. The time taken to complete the growth of the mycelium on substrates, the appearance of pinheads, fresh weight, biological efficiency, and maturation of fruiting bodies of different treatments were compared.

3. Results

Oyster mushroom (*Pleurotus ostreatus*) was grown on various crop residues as substrates. The growth performance of *Pleurotus ostreatus* on substrates alone and their combination is depicted in Table 2.

3.1. Spawn Running Phase. The days for full spawn run ranged from 28.71 days to 43.79 days for corncob and bamboo waste, respectively. The lowest growth period (32.36 days) for primordia initiation was recorded on corncob alone, statistically significant with the longer duration (48.10 days) for primordia development recorded on bamboo waste.

3.2. Pinhead Formation. The maximum number of days taken from pinhead formation to fruiting bodies' formation exhibited a significant difference between the combination

Treatments	Spawn running in days	Appearance of pinheads in days	Days for fruiting body formation	Pileus diameter in centimeter	Fresh weight of fruiting bodies (g)	Biological efficiency
T1 = CC	$28.71^{d} \pm 0.80$	$32.36^{d} \pm 0.26$	$5.19^{b} \pm 0.74$	$4.00^{ab} \pm 0.79$	$144.18^{e} \pm 0.98$	$41.07^{\rm b} \pm 0.37$
T2 = FMS	$34.88^{\mathrm{b}} \pm 0.69$	$38.22^{b} \pm 124$	$5.20^{b} \pm 1.46$	$4.58^{a} \pm 0.15$	$253^{a}.07 \pm 1.05$	$50.20^{a} \pm 0.47$
T3 = BW	$43.79^{a} \pm 1.54$	$48.10^{a} \pm 0.79$	$5.28^{b} \pm 0.59$	$2.99^{b} \pm 1.61$	$180.79^{d} \pm 0.56$	$35.07^{abc} \pm 0.77$
T4 = CC + FMS	$30.99^{c} \pm 1.0$	$35.75^{\circ} \pm 1.50$	$4.64^{\rm b} \pm 1.23$	$3.37^{ab} \pm 0.49$	$233.74^{\rm b} \pm 0.42$	$49.12^{a} \pm 1.11$
T5 = CC + BW	$43.45^{a} \pm 0.95$	$47.84^{d} \pm 0.73$	$12.13^{a} \pm 1.25$	$2.95^{\rm b} \pm 0.41$	$101.48^{\mathrm{f}} \pm 0.91$	$20.80^{abd} \pm 0.41$
T6 = FMS + BW	$36.55^{b} \pm 0.95$	$39.08^{\rm b} \pm 0.72$	$6.24^{\rm b} \pm 0.73$	$3.40^{ab} \pm 0.85$	$211.11^{\circ} \pm 0.98$	$42.27b \pm 0.78$
CV%	2.84	2.55	16.37	24.09	0.45	1.77
LSD	1.83	1.71	1.87	1.52	1.52	1.25

Table 2: Morphological parameters of Pleurotus ostreatus mushroom grown on different agricultural wastes and their combination.

CC: corncob; FMS: finger millet straw; BW: bamboo waste. Mean values within a column sharing the same superscript letters are not significantly different using the LSD test at P = 0.05.

of corncob and bamboo waste (12.13 days). The lowest growth period for fruiting bodies' formation was recorded on corncob and finger millet straw (4.64 days).

3.3. Fruiting Body Formation. The bags of treatments took four to twelve days after primordial formation to maturation of fruiting bodies. There are no statistically significant variations between days taken for fruiting body formation among different treatments of substrates except for a combination of corncobs and bamboo waste (12.13 \pm 1.25).

3.4. Pileus Diameter. In our experiment, the pileus diameter was the highest (4.58 cm) on finger millet straw which was significantly different from the lowest (2.95 cm) diameter observed on a combination of corncobs and bamboo waste.

3.5. Fresh Weight of Fruiting Bodies. The highest (253.07 g) fresh weight of harvested mushrooms was recorded on finger millet straw alone, and the lowest (101.48 g) was recorded on a combination of corncob and bamboo waste. Biological efficiency varied significantly among the substrates used.

3.6. Biological Efficiency. The highest percentage of biological efficiency (50.20%) was reported from finger millet straw which was significantly different from the least (20.80%) observed in the corncob and bamboo waste.

4. Discussion

The oyster mushroom (*Pleurotus ostreatus*) was successfully grown on substrates used for cultivation. Diana et al. [18] reported that agricultural wastes make an ideal form of materials needed for substrate production, and nearly all types of agricultural wastes are useful for mushroom production.

4.1. Spawn Running Phase. In our study, the spawn running phase took 28.71 days on corncob 100% and 43.79 days on bamboo waste 100%. Similarly, Taskirawati et al. [19] reported that the length of the mycelium running phase ranged from 39 to 45 days. Our finding disagreed with that of

Buah et al. [20], who reported that the vegetative growth phase takes 2-3 weeks after inoculation on corncob and sawdust 100%. Also, Girmay et al. [16] found that the mycelium run took about 16 days after inoculation. The variation in the number of days taken for full mycelia colonization of substrates might be due to growth conditions, humidity, temperature, CO₂, and the substrate type (C:N ratio) [14].

4.2. Pinhead Formation. Pinhead formation is the second stage in mycelia growth in mushroom cultivation. The pinhead formation was observed between 2.53 and 4.76 days for all substrates after a period of complete mycelium running phase. Similarly, Mondal et al. [21] found that pinheads appeared 5.50 days after the spawn running. Buah et al. [20] also reported that pinheads formed over six days for all substrates used in the experiment. In support of our studies, Samuel and Eugene [2] reported that the lowest time (5.80 days) for primordia initiation was recorded on sawdust which was statistically similar with corncobs and palm cones in both 1:3 and 3:1 after the mycelium running phase.

4.3. Fruiting Body Formation. The maximum number of days taken from pinhead formation to the development of fruiting bodies in our experiment was 12.13 days recorded on the combination of corncob and bamboo waste (1:1). The lowest period (4.64 days) for fruiting body formation was recorded on corncob and finger millet straw (1:1). On the contrary, the fruiting body formation of all agroindustrial wastes used took between 2 and 4 days after pinhead formation [22]. Primordial development to maturation of fruiting bodies took 3 to 5 days, mushroom cultivated on a combination of sawdust with teff straw, cotton seed with teff straw, sawdust with enset waste, and teff straw with enset waste substrates [15].

The time taken for the fruiting body formation of the mushroom after inoculation on different substrates was recorded to be between 37.55 and 59.97 days. This finding was comparable with that of Tavarwisa et al. [23] who found that 30 to 50 days were taken for the fruiting body formation of mushrooms grown on baobab fruit, wheat straw, sawdust, and maize cobs. Also, Girmay et al. [16] recorded that the time elapsed between spawn inoculation and harvesting of

mushrooms grown on different substrates was found to be 27 days to 40.75 days. However, our result was far from the report of Muswati et al. [14] who demonstrated that the number of days taken for harvest ranged between 26 and 35.5 days. The difference in the duration of fruiting body formation was probably due to the variation in the nutrient content of the substrate used for cultivation.

4.4. Pileus Diameter of the Fruiting Body. The pileus diameter of harvested mushrooms was recorded to be between 2.95 and 4.58 cm on a combination of corncobs and bamboo waste (1:1) and finger millet straw, respectively. It was comparable with the finding of Muswati et al. [14] with the fruiting body pileus diameter range of 4.1 to 5.5 cm of all treatments investigated. The finding was in disagreement with the recorded cap diameter of 8 cm-14 cm on waste paper [24]. Similarly, our finding contradicted the highest cap diameter (12.65 cm) recorded on T12 (25% brewery spent grain + 75% cotton seed), and the lowest cap diameter (8.75) was recorded on T4 (100% sawdust) [21].

4.5. Fresh Weight of Fruiting Bodies. The greatest (253.07 g) weight of harvested mushrooms was recorded on finger millet straw (100%), and the lowest (101.48 g) was recorded on a combination of corncob and bamboo waste (50%). However, the finding was significantly higher than that of Ashraf et al. [25], who reported that cotton waste produced a maximum yield of 41.27 g followed by paddy straw and wheat straw with a total yield of 35.87 g and 32.87 g, respectively. On the contrary, Tavarwisa et al. [23] revealed that the weight of fruiting bodies ranges from 482 to 682 g cultivated on wheat straw, maize cobs, and sawdust. Similarly, a higher yield of fruiting bodies (391.7 to 469 g) of mushrooms was reported from agricultural substrates [26]. Our observation was opposite with Sitaula et al. [27], who found that the highest yield of the mushroom was recorded on a combination of maize cob and paddy straw (401.30 g), and the lowest (276.80 g) was cultivated on paddy straw. Fruiting body yield from finger millet straw in our experiment was high probably due to its higher C: N ratio.

4.6. Biological Efficiency. As shown in Table 2, biological efficiency varied significantly among various substrates used. The highest biological efficiency (50.20%) was obtained from finger millet straw which was significantly different from the least (20.80%) observed in the combination of corncob and bamboo waste. The substrates with higher moistureretaining capacity perform better than those with lower moisture-retaining capacity; it was true in our experiment case of finger millet straw. Similarly, a lower biological efficiency from rice straw (13.5%), corncob (12.8%), and sawdust (0.01%) was reported by Chikwendu et al. [17]. However, the finding of this study was lower than the reported literature of Zakil et al. [22], who found that the highest biological efficiency was 79.72% and lowest biological efficiency was 48.5% in Pleurotus ostreatus grown on different substrates. Also, the results of this study were far

from reports of Asafa and Geda [24] with the biological efficiency of 114%–136% from the substrates of cotton seed wastes, maize leaves, and maize sheaths.

5. Conclusion

The present study revealed that oyster mushrooms (*Pleurotus ostreatus*) could grow on corncob, finger millet straw, bamboo waste, and their combination with varying growth performances. The least biological efficiency and fresh weight were recorded from a mixture of corncob and bamboo waste substrates. Finger millet straw was the best substrate in terms of yield and biological efficiency.

Data Availability

All the data generated or analyzed during this investigation are included within this published article and its supplementary information files.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Picture 1: *Pleurotus ostreatus* mycelium grown on PDA Petri plates. Picture 2: *Pleurotus* ostreatus mycelium colonizing grain spawns within the flask. Picture 3: corncob grinding using the local pestle and mortar. Picture 4: arrangement of substrates' bags (shelve A). Picture 5: arrangement of substrates' bags (shelve B). Picture 6: Pleurotus growth on substrates. (*Supplementary Materials*)

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