Research Article

Grey Oyster Mushroom Biofarm for Small-Scale Entrepreneurship

Desta Berhe Sbhatu , Haftom Baraki Abraha, and Hiluf Tekle Fisseha

Department of Biological and Chemical Engineering, Mekelle Institute of Technology, Mekelle University,
P.O. Box 1632, Mekelle, Ethiopia

Correspondence should be addressed to Desta Berhe Sbhatu; desta.sbhatu@mu.edu.et

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This paper reports the productivity of a small-scale pilot biofarm of grey oyster mushroom (*Pleurotus sajor-caju* (Fr.) Sing.). The pilot was tested in Mekelle city (Ethiopia) in a brick-walled dark room. Growing structures were constructed by erecting three wood poles and fixing them with three wooden side bars at multiple locations to make a prism-shaped rack with multiple triangular open shelves, each capable of carrying one bag of spawned substrate. Mushroom substrates were prepared from maize stalk and wheat bran supplement. Pasteurized chopped maize stalk and wheat bran were mixed at the ratio of 10:0, 9:1, 8:2, and 7:3—yielding four treatments. Five kilograms of substrate was taken from each treatment and was mixed with one kilogram of gypsum to produce a growing mass. Each mass was spawned with 200 g of inoculum under aseptic conditions and put in polyethylene bags. The treatments were replicated thrice and the bags were put on the growth racks in completely randomized design. The growing room was maintained at optimum conditions. Maize stalk substrates supplemented with 10% and 20% of wheat bran have resulted in statistically comparable productivities but statistically significantly higher than those grown on nonsupplemented and highly supplemented maize stalk substrates ($p \leq 0.05$). The ingenuity of the design and the convenience of the construction of the racks, the availability of the substrates, and the simplicity of the management and maintenance of the biofarm rendered the piloted design suitable for home-based and small- and medium-scale mushroom biofarm entrepreneurship.

1. Introduction

Mushrooms are reproductive structure of multicellular fungi. They are sources of low-calorie food, eaten raw or cooked, sources of carbohydrate, protein, vitamins, and minerals [1, 2]. They are popularly called the vegetarian's meat. Raw dietary mushrooms are good sources of B vitamins (such as thiamine, riboflavin, niacin, and pantothenic acid), vitamin C, zinc, calcium, phosphorus, potassium, sodium, carbohydrates, proteins, and fats and are becoming more popular [3]. Mushrooms and/or their extracts are also used in medications, biological remediation, bio-degradable packaging, dyeing wool, etc. [4].

Oyster mushrooms (*Pleurotus* spp.) are the most preferred ones among the edible mushrooms due to their ability to grow quickly and productively in various lignocellulosic media [5–10], their versatility, and absolute ease of cultivation (e.g., [11]) and their nutritional value especially as source of

protein [10, 12, 13]. They are cultivated throughout the world for producing flavouring and aromatic as well as medicinal stuff [14–17]; lignin and phenol degrading activities [18, 19]; antimicrobial activities and antioxidants [20–25]; immune enhancing activities (e.g., [26]) and producing secondary metabolites like terpenoids, alkaloids, and phenols (e.g., [27]). The medicinal properties and activities of *Pleurotus* species is compiled by Patil and coworkers [2].

Mushroom cultivation offers ample opportunities by turning agroindustrial wastes into new forms of resources and protein-rich food by biodegradation, bioremediation, and biotransformation [28–31]. This is because mushrooms are excellent converters of cheap cellulosic materials into valuable proteins [32]. In fact, many studies have shown that agroindustrial effluents are outstanding supplements that shorten crop period and increase mushroom productivities of oyster species [31, 33, 34].

Indoor mushroom biofarm by using cheaply and amply available substrates coming from agricultural, industrial, forestry, and domestic wastes and by exploiting all available horizontal and vertical space helps us produce the highest protein per unit area. Protein yield of indoor mushroom biofarm is estimated to be greater than 100 times compared to the yield of conventional agriculture or animal husbandry [35]. In Ethiopia, indoor mushroom cultivation would contribute in: (a) promoting household food and nutritional security, (b) opening opportunities for home-based and small-scale entrepreneurs, and (c) assisting efforts of biotransformation of various wastes. This paper reports the result of grey oyster mushroom (*P. sajor-caju*) cultivation project piloted to acquire empirical data for developing a cultivation manual, including design and construction of growing racks and protocol of simple mushroom biofarm, for home-based and small/medium-scale producers in and around Mekelle city, Tigray, Ethiopia.

2. Methods and Materials

2.1. Selection of Suitable Mushroom Species. The choice of the mushroom species used in this knowledge and technology transfer project was made based on four overriding parameters, ease of cultivation, local temperature, sources of growth substrates, and availability of spawn vendors. Literature research and consultation with experts brought the researchers to consensus to choose grey oyster (*P. sajor-caju* (Fr.) Sing. (syn. *Lentinus sajor-caju* (Fr.) Fries) piloting its cultivation in Mekelle city, Ethiopia (alt.: 1979 m; lat.: 13° 30' 0" N; long.: 39° 28' 11" E; average high/low yearly temperatures: 24.3°C/17.6°C). *P. sajor-caju* grows easily with relatively less complicated procedures in moderate to high temperature areas in the tropics and subtropics [6].

2.2. Preparation of Aseptic Growing Substrate and Spawning. Maize stalk (C-source) and wheat bran (N-source) were collected from around Adigrat and Mekelle cities, Ethiopia. Preparations of mushroom growing substrate began with sun-drying and chopping and shredding of the maize stalk into 1.5 – 2.0 cm cuts. The resulting maize stalk biomass was soaked in tap water overnight and pasteurized using boiling water in 100 litres metal barrels at 70–100°C for 30 minutes by stirring as needed. Then, the pasteurized maize stalk biomass was cooled to 30°C. The wheat bran supplement was pasteurized separately following similar procedures. Then, the maize stalk biomass and wheat bran were aseptically and thoroughly mixed at a ratio of 10:0, 9:1, 8:2, and 7:3 yielding four stalk-bran combinations for four treatments. Then, 5 kg of biomass was taken from each combination and was mixed with 1 kg of gypsum to produce a roughly spherical mass (like cotton boll) of substrate ready for spawning. Spawning was done aseptically by ensuring that spawns reach all surfaces of the substrates. Aseptic measures include using alcoholic flames, closing the working spaces, finishing the spawning quickly, spawning in cold night, and combination of measures. Spawned substrates were then put into polyethylene bags and sealed leaving space on the top of the bags for air circulation. Holes, about 10 cm apart, were

FIGURE 1: Growth rack.

opened in all sides of the bags to ensure that enough oxygen reaches the substrates. Then, spawned substrates were readied for incubation on racks. The racks were erected in a dark room with a dimension of 340 cm × 360 cm × 350 cm (LWH).

2.3. Infrastructure and Experimental Designs. The home-based mushroom biofarm infrastructure was designed and built in such a way that the available horizontal and vertical space is maximally utilized without affecting workers' movement and safety and the room's aseptic conditions. The growth racks were erected inside a rectangular stone and brick room (LWH: 340 cm × 360 cm × 350 cm) with one wooden door and two glass windows. The glass windows were equipped with wire screen to keep out insects and other objects while allowing air movement. The temperature and humidity of the room were monitored. Air movement between the room and the outside was controlled by opening and closing the winds as needed. The darkness of the room was also controlled by covering the glass window with cartons and newspapers.

The growth rack was constructed by erecting three wood poles (length: 300 cm; bottom diameter: 4 – 5 cm) and fixing them together with wooden side bars (length: 20 cm; diameter: 3 – 4 cm) at 45, 115, 185, and 255 cm from the bottom to make a prism-shaped structure with four triangular open shelves (Figure 1). Each triangular open shelf carries one bag of spawned substrate. The triangular opening of the

TABLE 1: Effects of different substrates on the growth rate of grey oyster mushroom (*P. sajor-caju*).

Treatment	Percent of Substrates		Colonization	Mean Number of Days	
	C-Source	N-Source		Pinhead Formation	1 st Harvest
1	100	0	21.00 ^a	30.00 ^a	40.00 ^a
2	90	10	19.00 ^b	24.67 ^b	32.67 ^c
3	80	20	18.00 ^c	23.33 ^b	33.67 ^c
4	70	30	17.00 ^d	25.00 ^b	35.33 ^b
	Mean		18.75	25.75	35.42
	CV		0	4.29	1.94
	LSD		0	2.20	1.37

Means with different letters in the same column are significantly different at $p \leq 0.05$. LSD = least significant different, CV = coefficient of variation (%).

shelves is narrow enough to hold back the bags of spawned substrates. The treatments were placed in the growing racks in a completely randomized design with three replications.

2.4. Experimental Conditions and Their Maintenance. The spawn-run was carried out at dark conditions for 17 to 21 days. The mushroom house was maintained at relative humidity of 65–75% and temperature of 22–30°C where gaseous exchange was kept low to guarantee high CO₂ and low O₂ concentrations. After the completion of the spawn run, the bags were sliced open and removed. During the period of pinhead and fruiting body formation (run for 25 – 30 days), the environmental conditions were modified by raising the relative humidity to 75–85% and by lowering the temperature to 18–25°C while high gaseous exchange and light were allowed. Maintenance of the conditions of the mushroom house was carried out with conventional procedures as appropriate, by spraying water to the air, flooding the floor, wetting the walls, covering the floor with wetted rags, hanging clean wet sacks on the walls, allowing a greater volume of outside air, using live steam, covering the door and window with papers and cartons when needed, and turning the fluorescent light off or on.

2.5. Data Collection and Analyses. Sources of research data include (a) qualitative (descriptions of the nature and colour of sprouted mycelia and size of fruiting bodies) and (b) quantitative (number of days to colonization, pinhead formation, and first harvest, yield per flush and total yield, number of fruiting bodies, and biological efficiency (B.E. calculated as the percentage of fresh weight of mushroom divided by dry weight of substrate)). Data analyses and comparisons were carried out using descriptive and inferential statistical methods. All comparisons are made at *a priori* probability level of $p \leq 0.05$.

3. Results

3.1. Spawn Run. The effects of the nature (composition) of the growing substrates on the performance of the mushroom were studied by observing the density and colour of surface mycelia and by comparing the days to: substrate colonization, pinhead formation, and first harvest. Maize stalk substrate with no wheat bran supplement (control or treatment 1) showed poor growth of mycelia. Maize stalk substrate with

30% supplement of wheat bran (treatment 4) resulted in full growth of mycelia but it was not completely colonized. On the other hand, maize stalk substrate supplemented with 10% (treatment 2) and 20% (treatment 3) of wheat bran was completely colonized.

Maize stalk supplemented with 30% wheat bran required shortest spawn run time compared to the rest of the treatments. On the other hand, the control required longest spawn run time compared to the other treatments ($p \leq 0.05$). Spawn run time to pinhead formation was higher in the control than in the rest of the treatments. The treatments with 10% and 20% wheat bran supplements required comparable spawn run time to first mushroom harvest but fewer mean number days compared to the control and treatment 4 (Table 1).

3.2. Mushroom Yield and Biological Efficiency. This study has showed that mushroom yield, biological efficiency (B.E.), and numbers of fruiting bodies are strongly related to the good or full colonization of the substrates. Apparently, treatments with full colonization (i.e., treatments 2 and 3) resulted in statistically significantly better yield per flush (thus better yield per bag) and higher B.E. than the control (treatment 1) and treatment 4 ($p \leq 0.05$) (Table 2). The treatments that resulted in better performances and productivities have produced statistically significantly greater number of fruiting bodies than those resulted in lower performance and productivities (Table 3). In fact, the more productive treatments had many but small fruiting bodies as opposed to few but large fruiting bodies.

4. Discussion

4.1. Effects of the Substrate Composition on Mushroom Growth and Development. Some researchers have reported similar findings where maize cop substrate supplemented with 20% wheat bran has lowered the mean number of days for pinhead formation (e.g., [36]). Likewise, other workers have observed that maize stalk substrate without any supplements resulted in delayed colonization, pinhead formation, and first harvest compared to maize stalk substrate with supplements (e.g., [37]). Very slow growth rate of mushroom to maturity with similar growth substrate was also reported by other researchers [38]. Furthermore, Pala and coworkers have reported 22–24 days to spawn running, 28–30 days to pinhead formation, and 32–34 days to fruiting body formation

TABLE 2: Yield and B.E. of grey oyster (*P. sajor-caju*) mushroom.

Treatment	Percent of Substrates		Yield per Flush and Total Yield (in grams)					B.E. (%)
	C-source	N-source	First	Second	Third	Fourth	Total	
1	100	0	98.5 ^c	80.2 ^b	34.0 ^b	17.0 ^c	229.8 ^c	22.8 ^c
2	90	10	374.7 ^a	242.4 ^a	84.7 ^a	50.1 ^a	751.9 ^a	75.2 ^a
3	80	20	337.4 ^a	217.3 ^a	77.5 ^a	36.2 ^{ab}	668.5 ^a	66.8 ^a
4	70	30	227.6 ^b	124.0 ^b	62.5 ^{ab}	29.8 ^{cb}	444.8 ^b	44.4 ^b
	Mean		259.6	166.99	64.67	33.29	523.52	52.31
	CV		17.22	22.43	26.14	26.02	19.73	19.96
	LSD		89.30	74.41	33.77	17.30	206.44	20.87

Means with different letters in the same column are significantly different at $p \leq 0.05$. LSD = least significant different, CV = coefficient of variation (%).

TABLE 3: Number of fruiting bodies of grey oyster (*P. sajor-caju*) mushroom.

Treatment	Percent of Substrates		Mean Number of Fruiting Bodies per Flush				
	C-source	N-source	First	Second	Third	Fourth	Total
1	100	0	9.3 ^c	6.3 ^b	2.3 ^c	1.7 ^c	19.7 ^c
2	90	10	33.3 ^a	22.0 ^a	8.3 ^{ab}	5.0 ^a	68.7 ^a
3	80	20	30.0 ^{ab}	20.3 ^a	8.7 ^a	5.3 ^a	64.3 ^a
4	70	30	22.3 ^b	12.7 ^b	5.7 ^b	3.3 ^b	44.0 ^b
	Mean		23.75	15.33	6.25	3.8	49.16
	CV		19.14	21.60	21.82	20.85	18.82
	LSD		9.08	6.61	2.72	1.59	18.48

Means with different letters in the same column are significantly different at $p \leq 0.05$. LSD = least significant different, CV = coefficient of variation (%).

when cultivating *P. sajor-caju* on wheat straw substrate (e.g., [10]). On the other hand, other researchers have observed quicker induction of primordia of inoculums (15–17 days) and mushroom maturity for first flush harvesting (20 days) (e.g., [39]).

4.2. Substrate Composition on the Performance and B.E. of the Mushroom. It is evident that maize stalk supplemented with wheat bran results in better performance in terms of yield, B.E., and number of fruiting bodies than without supplement. But, it is also apparent that the supplementation needs to be 10 to 20%. This observation could be attributed to the fact that nitrogenous supplements stimulate the proliferation of mycelia and thus increase the yield of mushrooms, while excess organic or mineral nitrogen would inhibit the synthesis of lignin-degrading enzymes and thus causes a decrease in productivity (e.g., [28, 39, 40]). Apparently, treatments 2 and 3 have resulted in significantly higher B.E. than treatment 1 (control) and treatment 4 ($p \leq 0.05$). Treatments without (treatment 1/control) or with higher nitrogenous supplements (treatment 4) have resulted in lower B.E. in growing *P. sajor-caju* (Table 2).

The variability of B.E. depending on the nature (composition) of growing substrates and its enhancement with the addition of optimum nitrogenous supplements has been observed by many workers (e.g., [37, 39, 41]). As it has been observed by many researchers working with *P. sajor-caju* (e.g., [8, 37, 42]), the present study has showed that the yields decrease over the course of the four flushes (harvesting times). It is believed that the nature and amount of nitrogen available in the growing substrates after each flush affect the

growth of the species, thus the productivity of the biofarm. Interestingly, contrary to ours, others working with *Pleurotus flabellatus* have reported an increase of yield with increasing time of growth till the last harvest [38]. The researchers have attributed the observation to the capacity of the species in degrading available cellulosic fiber as it can have a different enzyme profile.

5. Concluding Remarks

This paper is prepared based on a knowledge and technology transfer project aiming at designing and piloting an easy and effective small to medium-scale mushroom biofarm. It has, thus, reported the protocol of cultivating grey oyster mushroom (*P. sajor-caju*) using amply available maize stalk enriched with wheat bran in Mekelle city, Ethiopia, and established the nature of the growing substrate that resulted in good yield. It has also reported the design and construction of a growth rack that maximizes the utilization of horizontal and vertical space of any growing rooms. We have shown that the design of the growing racks is ingenious in that it maximizes the use of available space and the construction of the racks is quite easy requiring very limited inputs. In fact, the number of growth racks can be increased from four to nine by separating each shelf by 25 cm in about 350 cm high growth rooms.

The federal and the state governments of Ethiopia and development partners are aggressively encouraging and incentivizing citizens, especially the educated youth, to initiate knowledge-based micro and small businesses. Also, the governments are working towards ensuring and sustaining food and nutrition security at household and national levels

through increasing production and productivity and diversifying the produces. And yet, there is a growing demand for mushroom in many urban and semi-urban settlements of the Country. Thus, the authors believe that the protocol of grey oyster cultivation and the design of the ingenious and easily erectable growth racks are timely in opening opportunities for home-based as well as small- and medium-scale entrepreneurs.

Data Availability

The data is available as personal file and can be presented up on request.

Conflicts of Interest

The authors declare that there are no actual or potential conflicts of interest regarding this publication.

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