

Responses of Oyster Mushroom (*Pleurotus Ostreatus*) As Influenced by Substrate Difference in Gamo Gofa Zone, Southern Ethiopia

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Abstract: Most developing countries like Ethiopia should look forward to solve acute protein deficiency in the diets of its increasing population and providing employment opportunity by cultivating mushrooms in the urban area, without heavily depending on agricultural lands and capital. To this end, this experiment was conducted to identify the best basic substrate for yield and yield contributing attributes of oyster mushroom in Gamo Gofa zone in two locations at low (1278m.a.s) and high (2741m.a.s) altitude, Arba Minch and Chencha towns respectively. Three types of substrate namely groundnut shell, maize stalk and wheat straw were arranged in a completely randomized design (CRD) with three replications. Percent biological efficiency (%BE), fresh yield and yield components of mushroom was analyzed by using SAS (9.1) statistical procedure. Total highest fresh yield (1466.8g/kg) and % BE (146.68%) and total lowest fresh yield (319.83g/kg) and %BE (31.98%) were achieved on wheat straw and groundnut shell, respectively at Chencha. Maize stalk had maximum yield, BE and yield components at 3rd flush compared with wheat straw. The same scenario was also repeated at Arba Minch with less total fresh yield (203.97g/kg) and biological efficiency (20.40%) could be due temperature and other ecological variations. Thus, cooler temperature at Chencha is more favorable for the maximum production of Oyster mushroom compared to hot temperature at Arba Minch.

Keywords: Oyster Mushroom, Percent biological efficiency, substrate, fresh yield and Yield component,

I. Background And Justification

1.1. Background of the study

Mushrooms are fleshy, spore-bearing reproductive structures of fungi. For a long time, wild edible mushrooms have played an important role as a human food. Even though, mushrooms are being recognized as important food items from ancient time, empirical methods for their cultivation are relatively recent [1]. Their usage is being increased day by day for their significant role in human health, nutrition and disease prevention. In Ethiopia, there are mainly three species of mushroom namely, white button (*Agaricus bisporus*), oyster (*Pleurotus ostreatus*) and shiitake (*Lentinula edodes*) mushrooms that are grown commercially. Oyster mushroom contains 20-35% protein in dry weight which makes it higher than that of vegetables and fruits [2]. They are also known to contain substances that enhance the immune system, and lower blood pressure and cholesterol levels [2]. The mushroom production is a global and expanding industry, its world production is 6,535,542 ton in 2009 [3].

Mushroom cultivation is a new activity in Ethiopia but wild mushrooms are harvested in forests in Ethiopia during the rainy season, even though they are not a staple part of the diet and were not cultivated previously. But currently small scale production has been started in cities like Addis Ababa, Beshoftu, Hawassa, Bair Dar, etc. This technology of artificial mushroom cultivation is a recent innovation, which stemmed from the realization that the incorporation of non-conventional crops in existing agricultural systems can help in improving the social as well as the economic status of disabled people, youth, women and small farmers. Since, the land required for cultivation is very small, as mushrooms do not need light for growth and they are commonly produced on shelves indoor. Thus, mushrooms can be produced and harvested throughout the year with relatively little investment, due to this mushroom cultivation has reported as other effective way of alleviating poverty in developing countries [4].

Crop residues are largely abundant as agricultural waste after harvest in Gamo Gofa zone. Consequently, needs conversion in to food or to other usable products. Mushroom cultivation on crop residues is considered as potential source to generate a significant cash income, an alternative food production, and provision of employment opportunity. Besides, it is used to dispose agricultural waste in environmentally friendly in this era of climate change.

Gamo Gofa zone is one of the zones in Southern Ethiopia that has been experiencing out migration of its rural labor force for a long period. This could be due to average household land holding size is very small,

women and youth unemployment or underemployment are among others. This is so because of the fact that population growth is increasing largely and resulted in increased pressures on agricultural land and natural resources especially on highlands. Inadequate supply and high cost of animal protein necessitate search for and cultivation of locally available and cheap protein sources. The ability of fungus to degrade agricultural materials and the readily availability of many agricultural by-products may contribute in addressing unemployment and protein deficiency. All crop residues cannot perform equally, so that needs substrate selection to produce a medium that is nutritionally sufficient and selective for the mushroom mycelium growth, besides a sufficient supply with moisture, oxygen, nutrients and a balanced C/N ratio. In line with this, the yield and quality of mushrooms depends on the type of substrate used, the method of preparation and the suitability of environmental conditions (temperature and humidity) for growth and fruiting-body formation. There is no enough information available on the potentials of using locally available agricultural residues/wastes for mushroom cultivation in the area. Therefore, the aim of this research is to identify best basic substrate for oyster mushroom yield and yield components at low and highland areas of the zone.

II. Research Methodology

2.1. Description of experimental sites

The experiment was conducted in 2015 in Gamo Gofa zone at two locations with different climatic conditions viz: Chench and Arba Minch. At Arba Minch the research was conducted at Arba Minch University Horticulture laboratory and at Chench towns in local houses in uncontrolled environment. Arba Minch tow is located at 6° 2' N latitude and 37° 33' E longitude, far about 500 km south of Addis Ababa, the capital city of Ethiopia and at an altitude of about 1278 meters above sea level (m.a.s.l). Its average temperature and annual rainfall is 29°C and 900 mm, respectively. Chench woreda is found in the Gamo Gofa administrative zone of the South Nations, Nationalities and Peoples' Region (SNNPR) of Ethiopia. It is geographically located at 6°15'N latitude and 37°34'E longitude and at an elevation of 2741 m.a.s.l.

2.2 Treatments and experimental design

The experiment was conducted on substrate types namely Groundnut shell, Maize stalk and Wheat straw which were arranged in a completely randomized design (CRD) with three replications.

2.3 Substrate preparation, inoculation, and incubation

All the three substrates (Groundnut shell, Maize stalk and Wheat straw) were soaked in water overnight and drained, resulted in a moisture content of approximately 70%. All the substrates were chopped before use. Substrates were allowed to ferment for 3 days. Each substrate(1kg) was filled in plastic bag and autoclaved at 15 psi, 121 °C for 90 min. Sterilized substrate bags were inoculated with grain spawn layer wisely at a rate of 5% (w/w), heat sealed, and transferred to the incubation room for spawn running. The pinholes were also made in the bags manually for exhaust of gases. The bags were incubated at about 20-25°C at Arba Minch and 16-20°C at Chench location covered with black polythene to initiate complete darkness. The humidity of bags was accomplished by spraying of water on them three times a day at Chench and six times a day at Arba Minch. Polythene covers were removed after three weeks at Arba Minch and after eight weeks at Chench to allow maximum light to speed up growth and improve shiny color of mushroom basidiocaps when incubation period was over.

2.4. Data collection

Pileus Diameter: This was measured in centimeters with transparent ruler from one edge of the pileus across to the center of the cap and divided by the number of samples. Then final mean value was worked out per bag.

Length of Stipe: Stipe length was measured in centimeter using transparent ruler from the base of the stipe to the pileus and divided by the number of samples. Then final mean value was worked out per bag.

Fresh yield (g/kg bag): All Fresh harvested fruit bodies per bag was weighed by using an electrical weighting balance.

Biological Efficiency: Biological efficiency is indicating the conversion of substrate mass to mushroom fruiting bodies, was calculated according to (Wasantha Kumara and Edirimanna, 2009)[5].

$$\text{Biological efficiency \%} = \frac{\text{Fresh weight of fruit bodies}}{\text{Total weight of substrate used}} \times 100$$

2.5. Data analysis

The mean value of each parameter was computed and subjected to analysis of variance (ANOVA) using SAS (9.1) statistical package. The LSD test was employed to compare means at 5% probability levels where significant differences were detected [6].

III. Results And Discussion

Maximum and minimum temperatures persisting at two experimental sites, Chencha and Arba Minch are presented in Figure 1. Mean maximum and mean minimum temperatures at Arba Minch remained consistently higher than Chencha in all the 8 months of testing period. Therefore, the spawn running time, i.e., mycelium growth took much longer time (53 days) at Chencha as compared to that of Arba Minch (21 days). Furthermore, mycelium growth in two specified locations were indicated a strong positive relationship between temperature and the growth of mycelium. The higher the temperature, the faster the growth of mycelium resulted to shorter spawn running time, but with lower yield and BE. The production period of mushroom under Chencha condition was found to be considerably longer than Arba Minch. Such differences could be attributed to cooler temperatures of Chencha as more favorable for the longer period taken for Oyster mushroom fruit body formation compared to hot temperatures at Arba Minch. The results of current experiment are substantiated by the results of earlier reports who recommended cooler temperature than hot temperature for oyster mushroom cultivation [7, 23].

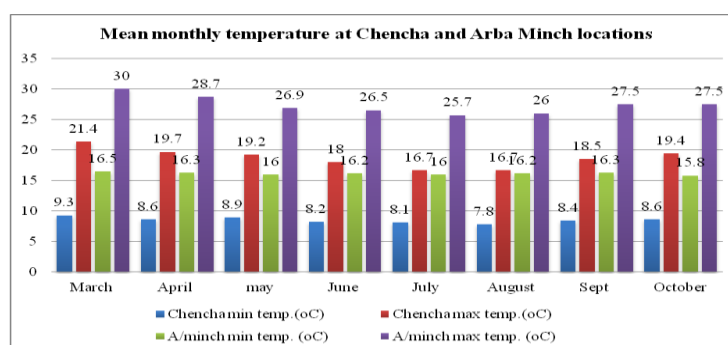


Figure 1. Mean monthly minimum and maximum temperature at Chencha and Arba Minch

3.1 Yield, biological efficiency (BE) and yield component at Chencha location in three flush

1st Flush

Flush of mushroom is also known as break of mushroom. In the 1st flush, oyster mushroom yield, BE and yield components were significantly affected by substrate types at ($P < 5\%$). Higher yield (616.68g) and biological efficiency (61.67%) on fresh weight basis were recorded on wheat straw followed by maize stalk (325.31g & 32.53%) and the lowest yield and BE were observed on groundnut shell (230.5g & 23.05%), respectively, as shown in Table 1. The duration and yield of breaks depends upon the type of mushroom strain, substrate and environmental conditions of mushroom growing room [8]. These results are in conformity with reports of earlier workers, who reported maximum yield was obtained on wheat straw (Uddin *et al.*, 2012) [9]. On the contrary to the current study, better yield in first flush was obtained on cotton seed hull and paddy straw than wheat straw (Ashraf *et al.*, 2013) [10]. The variation in stipe length and mushroom pileus diameter were observed in the three substrates used in this study. Longest stipe length were recorded on maize stalk (11.73cm) followed by wheat straw (6.8cm) and shortest stipe was recorded on groundnut shell (6.10cm) which was statistically not significantly different with wheat straw (Table 2). The widest pileus diameter (8.33cm) was observed on wheat straw followed by maize stalk (6.1cm) but they were not significantly different. The narrowest pileus diameter was recorded on groundnut shell (4.9cm). The result was in agreement with findings of Kumar and Achal (2008), who reported fruit bodies produced on wheat straw substrate had maximum pileus diameter ($8.55 \text{ cm} \pm 0.186 \text{ cm}$) as compared to other substrates used [11].

2nd Flush

In the 2nd flush, substrates were significantly affected yield and BE at ($P < 5\%$). Yield and BE on wheat straw (464.6g & 46.46%) and maize stalk (440.49g & 44.05%) were not significantly different respectively, but groundnut shell had significantly lower yield and BE (46g & 4.6%) (Table 1). Substrates difference were also

significantly affected stipe length and piles diameter. Longest stipe was recorded on maize stalk (12.33cm) followed by wheat straw (7.67cm) and the shortest stipe length (4.10cm) was recorded on groundnut shell. Widest pileus diameter was obtained on wheat straw (11.67cm) followed by maze stalk (8.00cm) and the narrowest pileus diameter was recorded on groundnut shell (Table 2).

3rd Flush

Analysis of variance for substrate means showed that mushroom yield, BE and yield components at 3rd flush differ significantly. Highest yield and BE was obtained on maize stalk (540.53g & 54.05%) followed by wheat straw (385g & 38.5%) and the lowest yield and BE were obtained on groundnut shell (43.33g & 4.33%) as given in Table 1. In 3rd flush maize stalk gave higher stipe length and pileus diameter followed by wheat straw and the least on groundnut shell. At the beginning mycelium colonization on maize stalk was slower than wheat straw but higher yield obtained at later flushes. Increasing trend from 1st flush to the 3rd flush for yield, BE and yield components were observed on maize stalk but decreasing trend was observed on wheat straw. This result is in agreement with Abena *et al.* (2015), who reported maize stalk had their highest peaks during the fourth week [12].

Total fresh yield and BE of Oyster mushroom at Chenchha location

Maximum total fresh yield and BE (1466.8g/kg & 146.62%) were obtained on wheat straw followed by maize stalk (1306.3g/kg & 130.63%) and the lowest yield and BE (319.83g/kg and 31.98%) were obtained on groundnut shell, respectively. This result can be explained by the easy breakdown and fast decomposition of wheat straw. Assan and Mpofo (2014) reported that highest yield of oyster mushroom was obtained on wheat straw and the next best option was maize stalk [13]. Gitte *et al.* (2014) reported that biological efficiency of different substrate ranged from 51.57 - 146.3 % of which the BE 146.3% was observed on wheat straw [14]. According to the report of Yildiz (2002), a total of 79.4% of BE was attained when the wheat straw alone used [15]. The main function of wheat straw is to provide a reservoir of cellulose, hemicellulose and lignin, which is utilized during the growth of mycelium running and during fruit body formation [16]. A variable quantity of nitrogen is also provided by wheat straw. The groundnut shell was the poorest of all substrates in present study. This might be due to nature of minimum water holding capacity and it remained tough which lacks spongy nature to maintain moisture. Substrate moisture content is a very important factor affecting the cultivation of *Pleurotus ostreatus* mushroom because it influence yield and BE [17]. These results are concurrence with Borkar, *et al.* (2014), who reported groundnut shell substrate was the poorest performer among the 7 substrates studied [18].

Table 1. Yield and biological efficiency of oyster mushroom as influenced by substrate on three flushes at Chenchha

Substrates	Yield (g/kg substrate)				Biological efficiency (%)			
	1 st Flush	2 nd Flush	3 rd Flush	Total yield	1 st Flush	2 nd Flush	3 rd Flush	Overall BE (%)
Wheat straw	616.68	464.6	385.00	1466.8	61.67	46.46	38.50	146.62
Maize stalk	325.31	440.49	540.53	1306.3	32.53	44.05	54.05	130.63
Ground nut shell	230.50	46.00	43.33	319.83	23.05	4.60	4.33	31.98
CV (%)	26.14	12.28	21.32		26.14	12.28	21.32	
LSD(0.05)	204.09	77.80	137.69		20.41	7.78	13.77	

Table 2. Yield components of oyster mushroom as influenced by substrate on three flushes at Chenchha

Substrates	1 st Flush		2 nd flush		3 rd flush	
	Stipe length(cm)	Pileus diameter(cm)	Stipe length(cm)	Pileus diameter(cm)	Stipe length(cm)	Pileus diameter(cm)
Wheat straw	6.80	8.33	7.67	11.67	6.57	4.20
Maize stalk	11.73	6.10	12.33	8.00	9.03	8.77
Ground nut shell	6.10	4.90	4.10	2.40	3.83	1.67
CV (%)	19.82	17.88	16.29	11.24	18.22	11.70
LSD(0.05)	3.25	2.30	2.61	2.65	2.36	1.13

3.1 Yield, biological efficiency (BE) and yield component at Arba Minch location

1st Flush

In first flush different substrates used affected yield and BE. Relatively higher yield and BE were obtained on wheat straw (94.63g and 9.46% BE), followed by maize stalk (68.03 and 6.80% BE) which were not significantly different with wheat straw. The lowest yield and BE were observed on groundnut shell

(24.53g/kg and 2.45% BE) as indicated in Table 2. Yield and BE obtained at Arba Minch is much more less than obtained at Chenchu due to variation in climatic condition in both locations. The longest stipe (10cm) and widest pileus (7cm) were recorded on wheat straw which were significantly higher than maize stalk (Table 4). Similarly, shortest stipe (2.50 cm) and narrowest pileus diameter (0.47cm) were obtained on groundnut shell.

2nd Flush

In the second flush, yield and BE were not significantly different for wheat straw and maize stalk at (p<5%). Similarly, stipe length and pileus diameter were also not significantly influenced by wheat straw and maize stalk (Table 3). However, the lowest yield, BE, stipe length and pileus diameter were recorded on groundnut shell (Table 3 & 4).

3rd Flush

In the 3rd flush the relative performance of maize stalk in yield and BE were better, followed by wheat straw and the lowest recorded on groundnut shell. Current results were in line with reports of Naraian *et al.* (2009), who reported due to maize stalks have higher lipid components than cobs and husks [19]. The lipids present in the maize stalks might have contributed to the high biological efficiency. In other report, maize stalk resulted biological efficiency about 90–97% after 50–60 days with a spawn rate of 10% (Fan *et al.*, 2000) [20]. Significantly longest stipe obtained on maize stalk (7.33cm), followed by wheat straw (4.17cm) and the shortest was obtained on groundnut shell (1.33cm) (Table 3). However, significantly widest pileus was resulted on wheat straw (6cm), followed by maize stalk (3.17cm) and the narrowest pileus of all was obtained on groundnut shell (0.2cm). When mushroom quality is concerned, widest pileus is more important than longest stipe. In both locations maize stalk resulted to largest yield and BE at third flushes. It is the general experience that the second flush results to the largest fresh yield. It is not uncommon for the first flush to be the largest and with a few substrates the third flush may occasionally be the largest [21].

Total fresh yield and BE at Arba Minch

The yield and BE obtained at Arba Minch were much less than the yield and BE obtained at Chenchu location as indicated in Table 3 & 4. The differences in yield and BE observed between the two locations are not surprising since these locations greatly differ in their temperature, altitudes and latitudes. Thus, mean comparison of all parameters in these locations (fresh yield, BE, stipe length and Pileus diameter at all flushes) had greater values at Chenchu as compared to those found in Arba Minch. This could be due to temperature and other ecological variation in this two locations. Current results are well supported by an earlier observation of AMGA (2004), who reported the major ecological factors that affect stalk height, stalk diameter and cap size in mushroom cultivation were temperature, humidity, fresh air, and material compactness [22]. Yield of *P. ostreatus* was higher (232.0 g/packet) in January at relatively lower temperature (14-25°C) and minimum yield (46.0 g/packet) was obtained in September relatively at higher temperature (25-30°C) (Uddin *et al.*, 2011) [23].

Table 3. Yield and biological efficiency of mushroom as influenced by substrate at Arba Minch

Substrates	Yield (g/kg substrate)				Biological efficiency (%)			
	1 st Flush	2 nd Flush	3 rd Flush	Total yield	1 st Flush	2 nd Flush	3 rd Flush	Overall BE (%)
Wheat straw	94.63	59.67	49.67	203.97	9.46	5.97	4.97	20.4
Maize stalk	68.03	57.67	80.67	206.70	6.80	5.77	8.07	20.67
Ground nut shell	24.53	17.67	14.00	56.2	2.45	1.77	1.40	5.62
CV (%)	35.00	19.16	11.36		35.00	19.16	11.36	
LSD(0.05)	43.63	17.22	10.92		4.36	1.72	1.09	

Table 4. Yield components of mushroom as influenced by substrate on three flushes at Arba Minch

Substrates	1 st Flush		2 nd flush		3 rd flush	
	Stipe length	Pileus diameter	Stipe length	Pileus diameter	Stipe length	Pileus diameter
Wheat straw	10.00	7.00	6.00	4.83	4.17	6.00
Maize stalk	5.83	4.00	6.00	4.50	7.33	3.17
Ground nut shell	2.50	0.47	1.67	0.43	1.33	0.20
CV (%)	22.82	33.79	18.03	10.29	13.09	5.34
LSD(0.05)	2.79	2.58	1.64	0.67	1.12	0.33

IV. Conclusion

Mycelium growth in both locations indicated a strong positive relationship between temperature and the growth of mycelium, i.e., the higher the temperature, the faster the growth of the mycelium resulting in to shorter spawn running time, but with lower yield and BE. The production period of mushroom under Chenchu condition was considerably longer than Arba Minch. The yield and BE on wheat straw substrate at first flush were maximum compared to second and subsequent flushes which resulted to overall maximum yield and BE. Therefore, wheat straw substrate is suitable for oyster mushroom cultivation in the area. Additionally, maize stalk is also a potential substrate for oyster mushroom production. However, yield and BE on maize stalk substrate was maximum at 3rd flushes at both locations. Yield and BE at groundnut shell were very poor in all flushes in both locations. Therefore, it would not be recommended in the area for oyster mushroom cultivation. We suggest for the future mixing of wheat straw and maize stalk at different ratio to combine advantages existed in two different substrates (fast mycelium colonization on wheat straw and increased yield in subsequent flushes on maize stalk) for oyster mushroom cultivation. Considering the availability of different crop residues, further researches are required under controlled environment to increase production of this mushroom.

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