

Cultivation of Oyster Mushroom (*Pleurotusostreatus* Kumm, 1871) using Agro-Industrial ResiduesTsegaye Z^{1*}
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Abstract

This study was conducted to compare effects of different agro-industrial residues on growth and bioconversion efficiency of oyster mushroom. Oyster mushroom were grown on different substrates namely cotton waste, coffee pulps, wood chips and tef straw. Agro-industrial residues were collected from different areas of Addis Ababa and sterilize at 121°C and 15 P for 60 minutes. Pure culture of the oyster mushroom was maintained on potato dextrose agar plates and inoculated in the sterilized grain. Fastest mycelia growth took less than two weeks on the sugarcane bagasse. Healthy and fully-grown spawn was transferred into sterilize substrates. Maximum mycelia colonization, primordial initiation, fruiting bodies formation and yield of oyster mushroom (790g/kg) was observed on combination of cotton waste + coffee pulp in 1st, 2nd and 3rd flushes. Combination of cotton waste and coffee pulp was found to be a better substrate than others because it recored 79% of bio-conversion efficiency and yield of mushroom in three consecutive flushes. The fresh mushroom yield was directly related to chemical and biological composition of the substrate that important for the growth of mushroom than other substrates.

Key words: Mushroom; Coffee pulp; Cultivation; Sterilization.

Introduction

A huge amount of ligno-cellulosic agricultural crop residues by-products rich in organic compounds are annually generated. On surface of our planet, around 200 billion tons per year of organic matter were produced through the photosynthetic process [1]. However, the majority of this organic matter is not directly edible by humans and animals; in many cases, becomes a contaminate source of environmental medium.

In Ethiopia there is a big waste from agricultural and agro industrial activities. There are approximately 39 sawmills and a total of 5-10 factories involved in production of wood. Saw mill residue is estimated to a total about 25,000 tons per year Agricultural and agro industrial residues used in domestic sector for cooking and baking, using very low efficiency devices constitute 15% of total energy consumed in Ethiopia. But rest 85% of residue mostly tends to be disposed as waste [2].

Among bioconversion processes, mushroom cultivation is an appropriate technology for management of agricultural and agro-industrial residues [3]. Mushroom which is a fleshy saprophytic fungus are found growing in nature on damp rotten log of wood trunks of trees, decaying organic matter and in damp soil rich in organic substances. Cultivation of mushroom can be viewed as an effective way to extract bio resources left behind in agricultural residues and environmental protection strategy [3].

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Mushrooms are seasonal, commercial cultivation is therefore necessary to ensure constant availability. However, large scale cultivation and processing of mushroom requires a good knowledge of the growth requirements, and influence of the substrate on their growth rate and nutritional composition. Some researchers have already observed that the yield and the quality of oyster mushroom depend on the chemical and nutritional content of substrates [4]. The use of the residues in bioprocesses may be one - bioconversion solution of inedible biomass residue into nutrition protein rich food in form of edible mushroom. Cultivation of any type of mushroom implies principles of microbiology, environmental engineering and solid-state fermentation in the conversion of domestic agricultural, industrial, forestry wastes into food for humans.

In most countries, there is a well-established consumer acceptance for cultivated mushrooms such as *Agaricus bisporus*, *Pleurotus* spp., *Lentinus edodes*, *Volvariella volvacea* and *Auricularia* spp.[5]. The oyster mushroom is grown under natural conditions on living trees as parasite or dead woody branches of trees as saprophyte and primary decomposer. The chemical composition of the fresh fruiting bodies of oyster mushroom, *Pleurotus ostreatus* indicates a large quantity of moisture (90.8%), whereas fresh as well as dry oyster mushrooms are rich in proteins (30.4%), fat (2.2%), carbohydrates (57.6%), fiber (8.7%) and ash (9.8%) with 345 Kcal energy value on 100 g dry weight basis; while vitamins such as thiamin (4.8 mg), riboflavin (4.7 mg) and niacin (108.7 mg), minerals like calcium (98 mg), phosphorus (476 mg), ferrous (8.5 mg) and sodium (61 mg) on 100 g dry weight basis, are also found.

Rambelli and Menini [6] reported that oyster mushroom is anti-tumoural presumed because their chemical composition. Oyster mushrooms are known to bear therapeutic ingredients such as dietary fibers (chitins and chitosans) and phenolic compounds [7-10]. Various bioactive compounds isolated from *P. ostreatus* culture extracts of Ethiopian higher fungi showed other biological properties such as antiprotozoal, antihelminthic, phytotoxic and brine shrimp lethality activities [11].

Inchausti [12] investigated Leishmanicidal and Trypanocidal activity of the extracts and secondary metabolites of some Basidiomycetes and their spent can be used as cattle feed, fertilizer or landfills. The mushroom

consumption habit and cultivation practice of people in Ethiopia has not well documented so far though it believed that many part of country is suitable for mushroom cultivation.

Mushroom cultivation is a useful method of environmental waste management and waste disposal. Many agricultural and industrial by-products are important in mushroom production. Tef straw, coffee pulp, wood chips and cotton waste have high cellulose, hemi-cellulose and lignin contents, and their low protein content and digestibility. Therefore, present study focused to evaluate the growth, economic feasibility of small scale production and yield (bioconversion efficiency) of oyster mushroom on agro-industrial by-products at Ethiopian Biodiversity Institute.

Methods and Materials

Sample collection

Agro-industrial residues namely cotton waste, coffee pulp, tef straw, and wood chips were collect from different Addis Ababa region. The fungus was taken from stock culture in Mycology Department of Microbial Biodiversity Directorate, Ethiopia Biodiversity Institute. Pure culture of mushroom was maintained on potato dextrose agar and malt extract agar plates.

Study period

This study was carried out in Ethiopia biodiversity institute, Microbial Biodiversity directorate laboratory from April to October 2014 G.C.

Laboratory work

Preparation of culture media: Pure cultures of oyster mushroom maintained on potato dextrose agar (PDA) and malt extract agar (MEA) slant at plus four were obtained from Mycology Laboratory Figure 1. Thirty-nine (39) g of PDA (potato extract- 4.0 g, Dextrose- 20.0 g and Agar- 15 g) and MEA 50 g (malt extract-30 g, mycological peptone-5 g, agar-15 g) was added to 1 L distilled water into two liters flask. Then it was placed on Bunsen burner to dissolve agar. It was autoclaved at 121°C for 15 min. Fifteen milliliters (15 ml) of the medium was then dispensed into 9 cm diameter Petri dishes. These were inoculated with the oyster mushroom cultures Figure 1 by using spatula and incubated at 25°C. Mycelia growth in terms of diameter on culture plate was measured using calibrator Figure 1.



Figure 1: Mycelia invasion on agar plates.

Spawn production: Spawn production is a highly technical operation and is generally done in laboratory. Spawn was prepared usually on grains (wheat, sorghum, millet). The grains were washed and soaked in water overnight. The water was changed often to prevent fermentation. Once the grains have been prepared, they were boiled till they become soft but remain firm, then the water was drained and spread on a cheese cloth. Calcium carbonate (2%) was mix with the grains. These grains were filled in half litter size; empty bottles to three-fourths their capacity. These grains were sterilized in an autoclave for 30 min at 121°C temperature and 15 psp (Pascal pressure). Inoculation was carefully done in total aseptic conditions using pure culture or previously prepared grain spawn. After the inoculation, the bottles were incubated at 25°C temperature until mycelia fully cover the grains.

Substrate preparation and inoculation: Cotton waste, tef straw, coffee pulp, and wood chips which were soaked separately in water for moisture absorption. For the best results, substrates were mixed with wheat bran (10%) and gypsum (3%) supplement at similar concentration Figure 2. Mixed substrates were placed in heat resistant polypropylene bags and sterilized in an autoclave at 121°C for 60 min and allowed to cool at room temperature. After sterilization, each of experimental polypropylene bags was inoculated at center of the substrate with 10 g of pure culture of *P. ostreatus* under aseptic condition and bags tiled with wrap. They were kept in dark room at 25 - 30°C and 90% relative humidity for 20 days.

Product evaluation

The bags were regularly disinfected using alcohol and hypochlorite to avoid contamination of substrates by unwanted microorganisms. When mycelia had fully covered the substrates bags, the bags were moved to another room for fructification. Bags were opened and regularly watered for fructification. They were harvested after 27 days of inoculation. The weights of the harvested mushroom were taken and recorded. The pileus and stipe of the mushrooms were measured in cm using metric ruler. Weight fruiting bodies of the mushroom were harvested in three different flushes.

Bio conversion efficiency

Weights of all fruiting bodies harvested from polypropylene bags were recorded as total yield of mushroom. The biological efficiency (BE) was calculated

using the formula given by Chang [13]. The BE was determined as the ratio of fresh mushrooms harvested (g) per dry substrate (g) and expressed as a percentage as shown by the formula. Biological efficiency BE = Weight of fresh mushroom harvested (g × 100 Weight of dry substrate (g) 1. Yield performance and bio conversion efficiency of oyster mushroom on the 4 kinds of substrates were calculated for three Flushes.

$$BE = \frac{\text{Fresh weight of the mushroom}}{\text{Dry weight of the substrate}} \times 100$$

Data analysis

Data analysis were carried out using tables and frequency ratio.

Results

Mycelia growth performance on different grains

After inoculation of spawn in prepared grains, the grains were fully covered by mycelia with in 14 to 20 days. i.e (mycelia coverage for sugarcane Bagasse it takes 14 days, for sorghum it takes 16 days, for wheat it takes 18 days and for millet it takes 20 days Figure 3. The spawn which is now ready, should be used as soon as possible, otherwise it was compacted with time and make spawning difficult.

Mycelia growth performance on different mixed substrates

Mycelia invasion started after 48 h of spawning the substrate. Complete mycelia invasion of the whole substrate was seen within 21 ± 6 days Figure 4. The highest running rate was observed in mixture of cotton waste and coffee pulp, oyster mushroom mycelia growth coverage after 7th days of incubation was 3 cm, in the 15th days of the incubation oyster mushroom mycelia growth coverage was 9.8 cm, in 21th days of incubation of oyster mushroom mycelia growth was fully cover the substrates. Followed by the mixture of the cotton waste and tef straw substrates, oyster mushroom mycelia growth coverage after 7th days of inoculation was 2.7 cm, in the 15th days of incubation oyster mushroom mycelia growth coverage was 9.6 cm of the growing substrates, and in the 23th day of incubation of oyster mushroom mycelia was fully invaded Figure 3 and 4 the substrates, in mixture of coffee pulp and tef straw mushroom mycelia growth coverage within 7th days of incubation was 2.5 cm, in the 15th days of the incubation mushroom mycelia growth coverage was 9.0 cm, in 25th days of incubation mushroom mycelia



Figure 2: Mycelia invasion of different grains.

growth fully cover the substrate; in mixture of cotton waste and wood chips mushroom mycelia growth coverage within 7th days of incubation was 2.1 cm, in the 15th days of the incubation mushroom mycelia growth coverage was 8.7 cm, in 26th days of incubation of mushroom mycelia growth was fully cover the substrates; The lowest mycelia running rate was observed in the mixture of wood chips and tef straw (0.52 cm/day), mushroom mycelia growth coverage within 7th days of incubation was 1.9 cm, in the 15th days of the incubation mushroom mycelia growth coverage was 7.8 cm, in 27th days of incubation mushroom mycelia growth was fully cover the substrates. The lowest mycelia growth colonization was recorded in the combination of the wood chips and tef straw Table 1.

Periods of oyster mushroom fruiting bodies maturation

Most of the experimental bags took 3 to 5 days from primordial formation to maturation of mushroom fruiting body. After 3 days, mushrooms became ready for picking. Figure 4 shows the progressive development of oyster mushroom. Duration for the maturation of fruiting bodies after primordial formation showed variations among different substrates and replicates. oyster mushroom cultivated on mixture of cotton waste and coffee pulp recorded the highest fruit body followed by on mixture of cotton waste + tef straw; coffee pulp + tef straw; cotton waste + wood chips; wood chips + tef straw Table 2. Mushroom grown on mixture of cotton waste and coffee pulp had the highest stipe diameter (2.75 cm). The lowest stipe and piles diameter of the cultivated oyster mushroom was recorded in the mixture of wood chips + tef straw Table 2.

Bioconversion efficiency of oyster mushroom

Mushroom bioconversion efficiency on different substrates mixtures having 45:45 and 90:10 ww main materials and additive wheat bran for three consecutive flushes: mixtures of cotton waste and coffee pulp their bioconversion efficiency was 79%, followed by 76% of cotton waste and Tef straw mixture, 63% of coffee pulp and tef straw mixture, 61% of cotton waste and wood chips mixture, 58% of coffee pulp and wood chips mixture, 52% of wood chips and tef straw mixture Table 3.

$$BE = \frac{\text{Fresh weight of the mushroom}}{\text{Dry weight of the substrate}} \times 100$$

$$BE = \frac{790g}{1000g} \times 100, BE = 79\%$$

Discussion

Mushroom cultivation requires carbon, nitrogen and inorganic compounds as their nutritional sources, and main nutrients are carbon sources such as cellulose, hemi cellulose and lignin. Oyster mushrooms require less nitrogen and more carbon source. Thus, most organic matters containing cellulose, hemi cellulose and lignin can be use as mushroom substrate i.e rice and wheat straw, cottonseed hulls, sugarcane, bagase, sawdust, waste paper, leaves, and so on. Mushrooms are reported to be easily grown on different lignocelluloses wastes such as banana leaves, cereal straw,



Figure 3: Different substrates used for mushroom cultivation.



Figure 4: Pictures from mycelia invasion, primordial formation and matured fruiting bodies formation.

Table 1: Mycelia invasion periods.

No	Substrates	Mycelial invasion in (cm)		Mean invasion value(cm/ days)	Complete invasion (days)
		7 th days	15 th days		
1	Cotton waste + coffee pulp	3	9.8	0.65	21
2	Cotton waste + tef straw	2.7	9.6	0.64	23
3	Coffee pulp + tef straw	2.5	9	0.6	25
4	Cotton waste + wood chips	2.1	8.7	0.58	26
5	Wood chips + tef straw	1.9	7.8	0.52	27

Tables 2: Growth of oyster mushroom fruiting bodies.

No	Substrates	Piles size in cm/days				Stiple size in cm/days					
		2 nd days	3 rd days	4 th days	5 th days	Piles Mean size	2 nd days	3 rd days	4 th days	5 th days	Stiple Mean size
1	Cotton waste + coffee pulp	2	2.5	3	4	2.9	1.9	2.2	2.5	4.4	2.8
2	Cotton waste + tef straw	1.8	2.1	2.4	3.2	2.3	1.8	2.1	2.3	4.2	2.6
3	Coffee pulp + tef straw	1.5	2	2.2	3	2.2	1.5	1.9	2.2	4.5	2.5
4	Cotton waste + wood chips	1.3	1.8	2	2.9	2	1.33	1.7	2	4.3	2.1
5	Wood chips + tef straw	1.22	1.6	1.9	2.8	1.9	1.21	1.4	1.9	4	1.9

Table 3: Harvesting total yield and bio conversation efficiency of oyster mushroom on mixed substrate having 45:45ww and 90:10ww main materials and additive (wheat bran).

Content of substrate (100ww and 45:45ww)			Flushes(grams)			Total	BE in%
90:10ww (main substrate: additives)			1 st	2 nd	3 rd		
Mixed	1	Cotton waste + coffee pulp + wheat bran	300	264	227	790	79
	2	Cotton waste +Tef straw + wheat bran	270	250	240	760	76
	3	Coffee pulp + Tef straw + wheat bran	230	210	190	630	63
	4	Cotton waste + wood chips + wheat bran	220	202	188	610	61
	5	Coffee pulp +wood chips + wheat bran	200	190	180	580	58
	6	Wood chips +Tef straw + wheat bran	190	172	158	520	52

paper wastes, sawdust and poultry droppings [14-16].

Amongst these different grains used during current investigation, oyster mushroom mycelia invasion took minimum number of days (14) for spawn running on Sugarcane bagasse followed by on Sorghum (16 days), Wheat (18 days) and Millet (20days). Thus, results supported by other investigators such as Rana [17] on oyster mushroom showed significantly rapid growth on different grains as compared to rest of other mushroom species. Oyster mushroom mycelia growth was very fast on the mixture of cotton waste and coffee pulp compare to length of oyster mushroom mycelia on mixture of wood chips and tef straw. This is also supported by other investigators such as Khan. After spawn running, to primordial formations took 7-8 days. It took different durations for primordial emergence in coir fiber soybean stover and cotton stalk substrate. Oyster mushroom can be grown successfully on five different mixtures of substrates as shown in Table 2 and it colonized all the substrates.

The ability of oyster mushroom to grow successfully on the combination of cotton waste and coffee pulp may be associated with the essential chemical composition of selected substrates that important for growth of mushroom.

Variations observed in the number of fruiting bodies produced may be associated with the physical nature of the substrates as well as the nature of the mushroom species. The number of fruit bodies recorded is related to their mycelia colonization. The mixture of cotton waste and coffee pulp yielded the highest total weight and number of fruit bodies and also had a wider pileus diameter. This result is greatly related to the findings of previous investigators perhaps early primordial emergence could be dependent upon nutrient contents in substrate.

The yield recorded from mixtures of different substrate media were (790 g/kg) of mushroom fruit bodies from combination of (cotton waste + coffee pulp) followed by (760 g/kg) of mushroom fruit bodies from (cotton waste + tef straw), (630 g/kg) from combination of (coffee pulp + tef straw), (610 g/kg) of mushroom fruit bodies from combination of (cotton waste + wood chips), (580 g) of mushroom fruit bodies from combination of (coffee pulp + wood chips), (520 g/kg) of mushroom fruit bodies from combination of (wood chips + tef straw) in three flushes. Bioconversion efficiency were recorded in different combination of substrates (main 45:45 ww) and (additives 90:10ww) was 79, 76, 63, 61, 58, and 52% respectively.

The highest bioconversion efficiency was recorded in a combination of cotton waste + coffee pulp and the lowest bioconversion efficiency was recorded in a combination of substrates such as wood chips + tef straw Table 1.

Conclusion and Recommendation

Cultivation of edible oyster mushroom is a prime factor for the conversion of these low value inedible wastes into a higher value commodity which can serve as food material for humans as well as source of the commercially important metabolites. Also, their spent can be used as cattle feed, fertilizer or landfills [18].

Therefore, cultivation of oyster mushroom on Agro-industrial residues provides multi-disciplinary advantages for human being, animals as well as for the ecosystem. The highest yield (bioconversion efficiency) of oyster mushroom was obtained from the combination of cotton waste and coffee pulp which are easily available substrates and a large biomass exists in the country. The fresh mushroom biological efficiency was directly related to nutritional composition of the substrate used for growing mushrooms.

The observed differences in the substrates media may be due to the percentage composition of cellulose materials and essential chemicals and biomolecules that are important for the growth of oyster mushroom. Based on the result of this study, the following recommendations are given:

(i) For sustainable oyster mushroom production, combination of high cellulose containing substrate formulation must be used.

(ii) Further study must be carried out using a combination of different agricultural and industrial wastes for production of mushroom to prevent malnutrition, create job opportunity and also prevent environmental contamination. The government and other stake-holders can redirect thus low value inedible substance namely, agricultural and industrial waste into mushroom growth substrate.

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