
Introduction and Cultivation of *P. ostreatus* and *L. edodes* Using Sugar Cane Bagasse, Leaves of *Prosopis juliflora* and Waste Paper at Oda Bultum University, Chiro, Ethiopia

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Abstract: A research of introduction and cultivation of two edible mushroom; shiitake mushroom (*Lentinus edodes*) and oyster mushroom (*Pleurotus ostreatus*) was conducted on three different substrates namely waste paper, leaves of *Prosopis juliflora* and sugarcane bagasse during 2017/18 at Chiro, Oda Bultum University to determine the effective substrate/substrate combination for cultivation of shiitake mushroom and oyster mushroom and to identify mushroom species that provides high biological efficiency. Thirteen different combinations of three substrates were used for cultivation of both mushrooms. The substrate combination were substrate one (75%SCB + 25%WP), substrate two (50%SCB + 50%WP), substrate three (25% SCB+75%WP), substrate four (75%SCB + 25%LPJ), substrate five (50%SCB + 50%LPJ), substrate six (25%SCB + 75%LPJ), substrate seven (75% WP + 25%LPJ), substrate eight (50%WP + 50%LPJ), substrate nine (25%WP + 75%LPJ), substrate ten (100% SCB), substrate eleven (100%LPJ), substrate twelve (100%WP) and substrate thirteen (33% SCB+33%WP+ 33%LPJ) replicated three times for both mushrooms. Among two varieties of edible mushroom cultivated, shiitake mushroom was not germinated, not harvested and no analysis of variance was conducted while oyster mushroom was success fully colonized the substrate, germinated, grown and harvested except for substrate six (S6), substrate nine(S9) and substrate eleven (S11) due to presence of high proportions of leaves of *Prosopis juliflora*. Presence of high proportions of leaves of *Prosopis juliflora* was affected colonization, germination and growth of oyster mushroom in comparison with the remaining other ten different substrates. On these ten substrates oyster mushroom was success fully grown, harvested and analyzed. Based on their analysis substrate thirteen, substrate four, substrate seven and substrate three were highly significant for fresh weight, dry weight and for biological efficiency. Hence they were the best substrate combination for good harvest of oyster mushroom under the study area.

Keywords: Oyster Mushroom, Shiitake Mushroom, Biological Efficiency, Waste Paper, *Prosopis Juliflora* and Sugar Cane Bagasse

1. Introduction

One of the world's biggest challenges is food insecurity. This problem is largely common in low and middle income countries which mainly have poor food production system and hence, suffer from series malnutrition [12]. Such countries must find ways of improving food production so as to feed alarmingly increasing human population. Mushroom cultivation could be a possible option to alleviate poverty and develop the life style of the vulnerable peoples [12].

Mushrooms are fruiting bodies of fungi especially of ascomycetes or basidiomycetes and a macro fungus with a distinctive fruiting body, large enough to be seen with naked eye and to be picked up by hand [3].

Mushroom with their flavor, texture, nutritional value and high productivity per unit area have been identified as an excellent food source to alleviate malnutrition in developing countries [8]. Among the reasons for the quick acceptance of mushroom is its nutritive content. To alleviate hunger and malnutrition in a world of rising food prices, cultivation of

mushrooms is a very reliable and profitable option [11].

Among edible mushroom fungi, *L. edodes* and *P. ostreatus* have received considerable attention for their nutritional value, medicinal properties and biodegradation abilities. Both are efficient colonizers and bioconverters of lignocellulosic agro industrial residues into pleasant human food with medicinal properties, with the productivity of the conversion being expressed by biological efficiency [23]. Oyster mushrooms are one of the most popular edible mushrooms and belong to the genus *Pleurotus* and family *pleurotaceae*. Like oyster mushroom (*Pleurotus ostreatus*), many of the *Pleurotus* mushrooms are primary decomposers of hard wood trees and are found worldwide. The oyster mushroom (*Pleurotus*) species are grown under natural conditions on living trees as parasites or dead woody branches of trees as saprophytes and primary decomposers, the oyster mushrooms can be cultivated successfully under semi-controlled conditions in small scale by using agricultural as well as industrial wastes and other refuse as substrate [23].

Shiitake mushroom (*Lentinus edodes*) is special mushroom with distinctive flavor that are grown on oak logs. Behind the common button and oyster mushroom, shiitake mushrooms is the third most widely produced mushroom in the world and in some countries such as in America, the production of shiitake has increased faster than any other specialty mushrooms. The shiitake is large, umbrella shaped mushroom that is dark brown and is prized both for its culinary and its medicinal benefits including antiviral, antifungal, and antitumor effects. Shiitake contain all eight essential amino acids in better proportions than in soybean, meat, milk, or eggs, as well as a good blend of vitamins and minerals, including vitamins A, B, B12, C, D and niacin [6]. The cultivation of mushroom requires the use of cellulosic materials or residues such as cereals, waste paper, *Prosopis juliflora*, sugar cane bagasse, straw, tea waste, cotton stalks, maize and sorghum stover, coffee pulps and coffee husk, and chips are some examples of residues or substrates that can be recovered and up graded to higher value and useful products as growth substrates [7].

Prosopis juliflora is an exotic evergreen tree with compound leaves, leaflets in 13-25 pairs and its root system include a deep tap roots. *P. juliflora* is xerophytes and is adapted to many soil types under a wide range of moisture conditions. It is some times said to dry out the soil and compete with grasses, particularly in dry areas, hence, in some areas it is considered as weed. The *P. juliflora* reduces grass availability and impacts the plant biodiversity by creating a physical barriers on seedlings of other plant species, preventing sunlight to reach to the under canopy vegetation, lowering the water table and by releasing various chemicals that may have negative effects on the native plants species. *P. juliflora* negative impacts are it invades range lands, destroys other plant biodiversity and hinders easy movement of pastoralist's indigenous trees [22].

Paper is almost 100% cellulosic composition. Waste paper refers to paper and cardboard from the industries, offices or other organizations and which is collected, stored and

discarded. It has the environmental and health impacts. At a global level about 40-65% of paper wasted to the environment [21].

Sugarcane bagasse is the matted cellulose fiber residue from sugar cane that has been processed in a sugar mill. Inside the mill, cane preparation for extraction usually involves washing the cane to remove trash and dirt, chopping and then crushing. Juice is extracted in the milling portion of the plant by passing the chopped and crushed cane through a series of grooved rolls. The cane remaining after milling is bagasse [17].

The study area is highly and frequently affected by drought and famine hazards. The district has 54% dry land and characterized by shortage of rain fall to produce sufficient food crops for the society. Mushroom production requires much more less water and produced under the shade. It requires also only about 35 days to be matured and reach for consumption. Therefore, it is essential to introduce such early maturing and less water demanding important crops to the study area to reduce shortage of food and malnutrition especially for the poor farmers in the study area.

2. Materials and Methods

2.1. Description of the Study Area

The study was conducted at Oda Bultum University, department of plant science located at 326km east of Addis Ababa at Chiro, capital city of west hararghe zone, Oromia regional state. Chiro is geographically located at latitude and longitude of 8°08'78"N and 42°07'12"E, respectively and at an altitude of approximately 1800meters above sea level. The area receives an annual rainfall of 700mm to 900mm and the mean annual minimum and maximum temperatures of the area are 12°C to 27°C respectively. In the district, agriculture is the major activity which covers 91% total economy. The economy of the whole society in the district is highly and frequently affected by drought and famine hazards.

2.2. Experimental Design

Thirteen different growth substrates (sugar cane bagasse, waste paper, leaves of *Prosopis juliflora* and their ten different combinations) with two edible mushrooms namely: *L. edodes* and *P. ostreatus* were arranged in a completely randomized design (CRD) replicated three times.

2.3. Materials Used

2.3.1. Spawn

Pure culture of *Lentinus edodes* and pure culture of *Pleurotus ostreatus* spawn were obtained from mushroom spawn producing private limited company (P.L.C), Addis Ababa.

2.3.2. Substrates

Leaves of *Prosopis juliflora* were collected from the surrounding field of Gumbi-bordode district, west hararghe

zone, Oromia regional state and transported to Oda Bultum University. Waste paper was collected from waste container of Oda Bultum University, department of plant science. Sugar cane bagasse was obtained from Metahara sugar factory and transported to Oda Bultum University.

2.4. Methods

2.4.1. Substrate Preparation

Thirteen different substrates: pure waste paper, pure leaves of *Prosopis juliflora*, pure sugar cane bagasse and their ten different combinations were prepared based on their substrate ratio as mushroom growing substrate for both *Lentinus edodes* and *ostreatus* separately and replicated three times. Each substrate had 300gm weight on dry weight base. Transparent plastic bags (20*30 cm) were used for both *L. edodes* and *P. ostreatus* growing.

The prepared substrates were measured on dry weight, based on their substrate ratio and mixed together. The measured substrates were soaked in the water and become wet. Then excess water was removed from the wet substrates by decanting and manually squeezing by hand. When the water stopped dripping, the substrates were ready for spawning and the moisture content of the substrates were approximately 60% and then moist substrates were filled into seventy eight plastic bags for sterilization.

The seventy eight plastic bags filled with moist substrates were labeled and sterilized in autoclave turn by turn to avoid contamination. The sterilized substrates were kept in a clean room for 12 hour until they cooled down to facilitate inoculation of spawn [1]. Then, the sterilized substrates with plastic bags were arranged according to their substrates type.

Table 1. Growth substrates (treatments) used for cultivation of both shiitake mushrooms (*L. edodes*) and oyster mushrooms (*P. ostreatus*).

Growth substrate	Substrate Combination
S1 (Substrate one)	75% SCB+ 25%WP
S2 (Substrate two)	50%SCB+50% WP
S3 (Substrate three)	25% SCB+75% WP
S4 (Substrate four)	75% SCB+25%LPJ
S5 (Substrate five)	50%SCB+50% LPJ
S6 (Substrate six)	25% SCB+75% LPJ
S7 (Substrate seven)	75% WP+25%LPJ
S8 (Substrate eight)	50% WP+50% LPJ
S9 (Substrate nine)	25%WP +75% LPJ
S10 (Substrate ten)	100%SCB
S11 (Substrate eleven)	100%LPJ
S12 (Substrate twelve)	100%WP
S13 (Substrate thirteen)	33%SCB + 33%WP + 33%LPJ

Where; S = Substrate, SCB = Sugar Cane Bagasse, WP = Waste Paper and LPJ = Leaves of *Prosopis juliflora*.

2.4.2. Spawning, Incubation and Cropping of Mushroom

The sterilized and cooled substrates were spread on a clean plastic material swabbed with 96% alcohol. Then thirty gram spawn (10% of substrate) of both *L. edodes* and *P. ostreatus* were inoculated to each substrate while re-filling the substrate in the polythene bags. The spawn was inoculated to the substrate in the labeled polythene bags using sterile hand gloves under laminar air flow hood found in the biology

laboratory. The open ends of the labeled polythene bags were tied and a number of small holes were made using sterile needle to allow air exchange of the polythene bags. Finally, both *P. ostreatus* containing bags and *L. edodes* containing bags were incubated at plant science laboratory in the carton box to maintain full darkness for colonization of mycelia as there is no dark room in the laboratory for this purpose.

All inoculated bags with spawn were incubated in clean and disinfected carton box four weeks for *P. ostreatus* containing bags and ten weeks for *L. edodes* containing bags to maintain darkness. Darkness environment was maintained during incubation period to enhance the quick colonization of the substrates. After full colonization of *Pleurotus ostreatus* containing polythene bags with mycelia (substrate one, substrate two, substrate three, substrate four, substrate five, substrate seven, substrate eight, substrate ten, substrate twelve and substrate thirteen) were taken out of the carton box and transferred to shelves for cropping in plant science laboratory. The humidity was maintained through watering the polythene bags twice a day and spraying water on the floor of the cropping room.

2.5. Data Collection and Analysis

After 35 days cultivation of *Pleurotus ostreatus*, fully matured oyster mushroom grown on ten different substrates were collected and analyzed for fresh weight, dry weight and biological efficiency. The collected data were subjected to analysis of variance (ANOVA) recommended by [10] with statistical analysis system (SAS) version 9. Means were compared for their statistical difference using Turkey's multiple range test at $p < 0.05$.

2.5.1. Determination of Fresh Weight and Dry Weight

Fresh weight and dry weight of oyster mushroom (*Pleurotus ostreatus*) collected after 35 days of cultivation from each ten different substrates were determined by measuring the fresh harvest of mushroom and dried mushroom after moisture was removed from fresh mushroom respectively.

2.5.2. Determination of Biological Efficiency

The biological efficiency (BE) of *Pleurotus ostreatus* harvested from each substrate was calculated by the formula recommended by [4];

$$\% \text{ BE} = \frac{W1}{W2} \times 100$$

Where:

W1 = weight of fresh harvested mushroom

W2 = weight of dry substrate used

3. Result and Discussion

3.1. Result of Shiitake Mushroom (*Lentinus edodes*)

Among the two varieties of mushroom cultivated, *Lentinus edodes* was not germinated totally on all substrate used for

growing of mushroom in the plant science laboratory. The possible reason for non germination of *Lentinus edodes* was probably *Lentinus edodes* requires less temperature than *Pleurotus ostreatus* as both of them were inoculated under normal room temperature of the laboratory. The timing of shiitake mushroom production in nature depends on both temperature and the timing of precipitation [13]. It was also reported that the temperature for mycelia growth of shiitake mushroom ranges between 5-35°C [5] compared with mean minimum temperature of study area 12°C. The temperature during incubation of shiitake mushroom is also reported that it ranges between 18-25°C and the optimum temperature for spore germination of shiitake mushroom ranges 22-26°C [5]. Generally, there was no colonization of mycelia, no germination, no growth of mushroom, no harvesting of mushroom and finally no analysis of variance was conducted for *Lentinus edodes*.

3.2. Result of Oyster Mushroom (*Pleurotus ostreatus*)

Concerning *Pleurotus ostreatus*, *Pleurotus ostreatus* was successfully colonized the substrates, germinated, grown and harvested except substrate six, substrate nine and substrate eleven. These three substrates were containing almost about 75% LPJ and 100% LPJ in their substrate combination. The presence of too much leaves of *Prosopis juliflora* in these three substrates affected the colonization and germination of *Pleurotus ostreatus* in comparison with the remaining other ten different substrates used for the growing of *Pleurotus ostreatus*. It was reported that the nutrient composition of the substrate is one of the factors limiting colonization as well as quantitative and qualitative yield of cultivated mushroom [20]. On these ten substrates namely; substrate one, substrate two, substrate three, substrate four, substrate five, substrate seven, substrate eight, substrate ten, substrate twelve and substrate thirteen the oyster mushroom was success fully grown, harvested and analyzed by statistical analysis system (SAS) version 9.

3.2.1. Analysis of Variance for Fresh Weight

The analysis of variance for fresh weight of *Pleurotus ostreatus* at maturity revealed that there was significant difference among substrates due to variation in substrate combination used for the growth of oyster mushroom. That is substrate thirteen, substrate four, substrate seven and substrate three were highly significant from substrate two, substrate one, substrate twelve, substrate ten, substrate eight and substrate five (Table 2 and Table 3). Substrate thirteen, substrate four, substrate seven and substrate three scored the highest fresh weight of 126.60, 124.20, 123.30 and 122.70gm respectively in decreasing order. Substrate two was statistically different from substrate one, substrate twelve, substrate ten, substrate eight and substrate five. Substrate one and substrate twelve were not statistically different from one another.

Substrate two, substrate one and substrate twelve, substrate ten, substrate eight and substrate five were significantly different from one another in descending order and they

scored intermediate fresh weight of 116.10, 111.30, 103.20, 80.10 and 76.20gm respectively.

The lowest fresh weight of 80.10 and 76.20gm were observed from substrate eight and substrate five due to presence of 50% LPJ that could affect the growth of *Pleurotus ostreatus*, and then the fresh weight was affected. The research result conducted on waste paper supplemented with rice husk, chicken manure and peat for *Pleurotus ostreatus* cultivation confirmed that highest fresh weight was recorded as 350.2gm in the substrate containing 20% rice husk [2].

3.2.2. Analysis of Variance for Dry Weight

The analysis of variance for dry weight (yield) of oyster mushroom showed that there was statistically significant difference among substrates. Substrate four, substrate thirteen and substrate seven were highly significant from substrate three, substrate two, substrate twelve, substrate one and substrate ten (Table 2 and Table 3). Substrate three, substrate two, substrate twelve, substrate one and substrate ten were significantly different from substrate five and substrate eight.

The highest dry weight were observed from substrate four (72.20 gm), substrate thirteen (26.97gm) and substrate seven (25gm) due to presence of high proportion of sugarcane bagasse and waste paper relatively with low proportion of leaves of *Prosopis juliflora* as high proportion of leaves of *Prosopis juliflora* affect the growth of oyster mushroom.

The intermediate dry weight were observed from substrate three (20.73gm), substrate two (19.97gm), substrate twelve (19.43gm), substrate one (18.57gm) and substrate ten (16.50gm). The lowest dry weight were obtained from substrate five (9.87gm) and substrate eight (8.90gm) as a result of presence of high proportions of leaves of *Prosopis juliflora* in both substrates. This indicates that substrates with high proportions of leaves of *Prosopis juliflora* affected the growth of *Pleurotus ostreatus*. Thus, the reason why on substrate six, substrate nine and substrate eleven in which the proportions of leaves *Prosopis juliflora* were 75%, 75% and 100% the spawn of *Pleurotus ostreatus* was not colonized, germinated totally. Leaves of *Prosopis juliflora* were not effective to be decomposed and not suitable for cultivation of oyster mushroom and affected its growth. Various findings have documented different substrates for the growth of oyster mushroom. For instance, cotton waste and saw dust, respectively noticed as the good substrates to obtain the highest yield of mushroom so far [14], [18]. In another finding, the maximum yield was obtained from cotton waste substrate for *Pleurotus Sajar-caju* [24].

3.2.3. Analysis of Variance for Biological Efficiency

The analysis of variance for biological efficiency revealed that statistically there was significant difference between the substrates. Substrate thirteen, substrate four, substrate seven and substrate three were highly significant from substrate two, substrate one, substrate twelve, substrate ten, substrate eight and substrate five (Table 2 and Table 3). Substrate two, substrate one and substrate twelve were significantly different from substrate ten, substrate eight and substrate five. Substrate ten was statistically different from substrate

eight and substrate five. This result confirmed that the biological efficiency of *Pleurotus ostreatus* was also affected by presence of high proportion of leaves of *Prosopis juliflora* in these two substrate; namely substrate eight and substrate five. Substrate eight and substrate five were not statistically different from one another.

The highest biological efficiency of *Pleurotus ostreatus* were observed from substrate thirteen, substrate four, substrate seven and substrate three and recorded as 42.20, 41.40, 41.10 and 40.91% respectively. The intermediate biological efficiency were observed from substrate two, substrate one, substrate twelve and substrate ten with record of 37.72, 37.10, 37.00 and 34.40% respectively. The lowest biological efficiency was observed from substrate eight (26.7%) and substrate five (25.4%) due to presence of high proportion of leaves of *Prosopis juliflora* in these substrate as

high proportion of leaves of *Prosopis juliflora* affect the growth of *Pleurotus ostreatus*. The result of biological efficiency of oyster mushroom under this investigation was in line with the results of [19] that have reported the biological efficiency of mushroom grown on substrates of agro-industrial residues ranged from 50-75%. It was also reported that cultivation of oyster mushroom on different agro wastes like cotton stalks, waste paper, maize cobs; cotton waste, wheat and paddy straw were utilized for achieving higher biological efficiency [15]. In other finding researchers have reported that saw dust recorded the highest biological efficiency being 65.22% while sugar cane bagasse had the lowest biological efficiency being 45.71% [16]. The biological efficiency of mushroom was also reported that it reached the maximum of 90-97% [9].

Table 2. Effect of substrates on fresh weight, dry weight and biological efficiency of oyster mushroom (mean values) evaluated at Chiro, 2017/2018.

Treatment	Fresh Weight	Dry Weight	Biological Efficiency
S1 (75% SCB + 25% WP)	111.30d	18.57bc	37.10b
S2 (50% SCB + 50% WP)	116.10c	19.97b	37.72b
S3 (25% SCB + 75% WP)	122.70b	20.73b	40.91a
S4 (75% SCB + 25% LPJ)	124.20ab	27.20a	41.40a
S5 (50% SCB + 50% LPJ)	76.20g	9.87d	25.40d
S7 (75% WP + 25% LPJ)	123.30ab	25.00a	41.10a
S8 (50% WP + 50% LPJ)	80.10f	8.90d	26.70d
S10 (100% SCB)	103.20e	16.50c	34.40c
S12 (100% WP)	111.00d	19.43b	37.00b
S13 (33 SCB + 33WP +33% LPJ)	126.60a	26.97a	42.20a
LSD	3.6554	5.1874	3.7109

Means with the same letter are not significantly different.

4. Conclusion

Among the two cultivated species of edible mushroom; *Lentinus edodes* and *Pleurotus ostreatus* on different substrates, *Lentinus edodes* was not colonized the substrates, not germinated and not harvested while *Pleurotus ostreatus* was successfully colonized substrates, germinated, grown, harvested and analyzed except substrate six (25%SCB+75% LPJ), substrate nine (75% LPJ + 25% WP) and substrate eleven (100% LPJ). These three substrates were containing about 75% LPJ and 100% LPJ in their substrate combination. The presence of too much leaves of *Prosopis juliflora* in these three substrate affected colonization and germination of *Pleurotus ostreatus* in comparison with the remaining other ten different substrate used for growing *Pleurotus ostreatus*.

The result of the experiment concluded that different combinations of the three substrates have effect on the growth and production of edible *Pleurotus ostreatus*. That is substrate thirteen, substrate four, substrate seven and substrate three were found to give the highest fresh weight of oyster mushroom, the highest dry weight of oyster mushroom and the highest biological efficiency of oyster mushroom over the other substrates, hence they found to give highest yield.

5. Recommendation

Even though the experiment needs to be repeated, from the

result of the experiment above substrate thirteen, substrate four, substrate seven and substrate three were recommended for cultivation and high yield production of edible *Pleurotus ostreatus* at Chiro environmental condition. For shiitake mushroom further research should be conducted to confirm about its colonization, germination, growth and production at Chiro environment and it leads to an opportunity for further research in the future.

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Appendix

Table 3. Analysis of variance (ANOVA) for fresh weight, dry weight and biological efficiency of oyster mushroom conducted at Chiro, 2017/18.

Source of Variation	DF	Mean Squares		
		Fresh weight	Dry weight	Biological efficiency
Treatment	9	7647.45**	305.62**	844.80**
Réplication	2	0.26ns	1.17 ns	0.42ns
Error	24	1.52	2.96	1.45
CV1.46	11.594.31			

** = Significant at 0.01 level of probability, ns = non significant at 0.05 level of probability.

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