
Effect of Substrate (Waste Paper, Leaves of *P. Juliflora* and Sugar Cane Bagasse) on Nutritional Composition of *P. ostreatus* at Oda Bultum University, Chiro, Ethiopia

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Abstract: An experiment of cultivation of oyster mushroom (*Pleurotus ostreatus*) was conducted on three different substrates namely waste paper, leaves of *Prosopis juliflora* and sugarcane bagasse at Chiro, Oda Bultum University to determine the effect of substrate on nutritional composition of oyster mushroom. Thirteen different combinations of three substrates were used for cultivation of oyster mushroom. 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. The oyster mushroom was success fully grown and harvested except for substrate six, substrate nine and substrate eleven due to presence of high proportions of leaves of *Prosopis juliflora* in these substrates. The leaves of *Prosopis juliflora* were not easily decomposed. Hence, affect the germination and growth of oyster mushroom in comparison with the other ten different substrates. On these ten substrates oyster mushroom was success fully grown, harvested and analyzed for their nutritional composition. Based on their analysis substrate thirteen, substrate four, substrate ten, substrate seven, substrate twelve, substrate two, and substrate three were statistically significant for their crude protein and crude fiber content while substrate ten, substrate four, substrate two, substrate one and substrate three were highly significant for their carbohydrate content. Hence they were the best substrates for their nutritional composition of oyster mushroom under Chiro condition.

Keywords: Oyster Mushroom, Waste Paper, Leaves of *Prosopis juliflora*, Sugarcane Bagasse

1. Introduction

In the world, mushroom production started in the 1800's. The demand of mushroom has been increasing due to population growth, market expansion, changing of consumer behavior and developments in the manufacturing industries, storage, transportation and retailing. Gradually, the world mushroom production has reached 33.4 million tons in 2007 while it was 26 million tons in 2008. China, United States of America and Netherlands rank as the first three in mushroom production in the world [5].

Mushroom cultivation and production culture is more

developed in china, Japan, Korea, Thailand, America. However, least known in Africa countries like Nigeria, Egypt, Kenya, Zimbabwe and South Africa and Ethiopia relatively are making good trial. Despite the high diversity of wild edible mushrooms in Africa including Ethiopia, very little of it is known. Cultivation and production of mushrooms has not been practiced on commercial scales in most developing countries which has consequently affected commercial mushroom marketing which is yet to be embraced by most farmers [12]. In developing countries, governmental and nongovernmental organization have not given due attention to mushrooms as an important crops that

can fetch farmers a substantial income to alleviate poverty [19]. Similarly, it is well accepted that mushrooms are a national necessity to combat poverty and malnutrition [11]. However, there is not enough mushroom cultivation practice in our countries to fill the demands of peoples interested in the mushrooms consumption. Mushroom cultivation is a very recent activity in Ethiopia with almost no mushroom consumption and cultivation techniques known except for few trials in small scale on *agarics*, *bisporus*, *Lentinula edodes* and *Pleurotus ostreatus* with few peoples under known substrate formula [12].

The edible mushrooms are excellent foods that can be incorporated into well balanced diets due to their low content of fat and energy and high contents of dietary fiber and functional compounds. Their benefits to health includes: antitumor, immunomodulatory and hypocholesterolemic effects [8]. 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 [28].

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 [12].

Waste paper refers to paper and cardboard from the industries, offices and collected, stored, discarded which has the environmental and health impacts. At a global level about 40-65% of paper wasted to the environment [23]. *Prosopis juliflora* is an exotic evergreen tree with deep tap root system. *P. juliflora* is xerophytes and 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 [27]. 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 mature and reaching for

consumption. Therefore, it is essential to cultivate such early maturing and less water demanding important crops to the study area to reduce shortage of food and malnutrition as well as to maintain nutritional balance 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° 87' 898' N and 42° 712' 46' E, respectively and at an altitude of approximately 1800meters above sea level. The areas receives an annual rainfall of 700mm to 900mm and mean annual minimum and maximum temperatures of the area are 12°C to 27°C respectively. In the district, agriculture is the main 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 (waste paper, leaves of *Prosopis juliflora*, sugar cane bagasse and their ten different combinations) with *Pleurotus ostreatus* were arranged in a completely randomized design (CRD) replicated three times.

2.3. Materials Used

2.3.1. Substrates

Waste paper was collected from waste container of Oda Bultum University, department of plant science. Leaves of *Prosopis Juliflora* were collected from the surrounding field of Gumbi-bordode district, west hararghe zone and transported to Oda Bultum University chopped and dried well under shade. Sugar cane bagasse was obtained from Metahara sugar factory.

2.3.2. Spawn

Pure culture of *Pleurotus ostreatus* spawn was obtained from mushroom spawn producing private limited company (P.L.C), Addis Ababa.

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 of waste paper, leaves of *Prosopis juliflora* and sugar cane bagasse were prepared based on their substrate ratio as mushroom growing material for growing *Pleurotus ostreatus* separately, then replicated three times. Each substrate had 300gm weight on dry weight base. Thirty nine transparent plastic bags (20*30 cm) were used.

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 then moist substrates were filled into thirty nine plastic bags for sterilization.

Thirty nine 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 [5]. Then the sterilized substrates with plastic bags were arranged according to their substrates type.

Table 1. Growth substrates (treatments) for cultivation of oyster mushrooms (*Pleurotus 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 the substrate) of edible *Pleurotus ostreatus* was inoculated to each substrate while re-filling the substrate in polythene bags. The spawn was inoculated to the substrates in the labeled polythene bags using sterile hand gloves under laminar air flow hood. The open end of the polythene bags were tied by rubber bands and a numbers of small holes were made using sterile needle to allow air exchange of the polythene bags. Finally, *Pleurotus ostreatus* containing bags were incubated in the carton box to maintain full darkness for colonization of mycelia as there is no dark room for this purpose.

All inoculated polythene bags with spawn were incubated in the disinfected carton box for four weeks to maintain darkness. Darkness environment was maintained during incubation period to enhance the quick colonization of the substrates. After full colonization of polythene bags with mycelia the substrates were taken out of the carton box and transferred to shelves for cropping in the laboratory. The humidity was maintained through watering the polythene bag twice a day and spraying water on the floor of the cropping room.

2.5. Data Collection and Analysis

After 35 days of cultivation, matured oyster mushroom

grown on ten different substrates were collected and analyzed for their nutritional composition (crude protein, crude fat, carbohydrate, moisture content and crude fiber). The collected data were subjected to analysis of variance (ANOVA) recommended by [13] with statistical analysis (SAS) version 9. Means were compared for statistical difference using Turkey's multiple range test at $p < 0.05$.

2.6. Determination of Nutritional Composition

2.6.1. Determination of Crude protein Content

The crude protein content of oyster mushroom was determined by kjeldahl method as described by [14] and [10] in which the nitrogen content of a sample was first determined and then converted to percentage crude protein by multiplying nitrogen content with 6.25 as suggested [3].

2.6.2. Determination of Crude Fat Content

The crude fat content was determined by soxhlet solvent extraction methods of [14].

$$\text{crude fat (\% of DM)} = \frac{\text{weight of fat}}{\text{weight of sample}} \times 100$$

2.6.3. Determination of Moisture Content

The moisture content (MC%) of the harvested mushroom was determined by formula recommended [3].

$$\text{MC\%} = \frac{\text{weight fresh sample} - \text{weight dry sample}}{\text{weight fresh sample}} \times 100$$

2.6.4. Determination of Carbohydrate

Carbohydrate content was determined by equation recommended [24];

2.6.5. Determination of Crude Fiber Content

The crude fiber content in percentage was calculated using formulae recommended [2].

$$(\%) \text{ crude fiber} = \frac{(W3 - W2)}{W1} \times 100$$

Where;

W1 = weight of sample used, on dry matter bases

W2 = weight of crucible with ash after ashing and

W3 = weight of the crucible with dry residue before ashing.

3. Result and Discussion

Oyster mushroom was successfully colonized ten different substrates, grown and harvested among thirteen substrates used. The remaining three substrates (substrate six, substrate nine and substrate eleven) were containing almost about 75% LPJ, 75% LPJ and 100% LPJ in their substrate combination. The presence of too much leaves of *Prosopis juliflora* in these three substrates were affected the colonization and growth of *Pleurotus ostreatus* in comparison with the remaining other ten different substrates used for the growing of oyster mushroom. 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 [22]. On the remaining substrates namely; S1, S2, S3, S4, S5, S7, S8, S10, S12 and S13 the oyster mushroom was successfully grown, harvested and analyzed for their nutritional composition by statistical analysis system version 9.

3.1. Analysis of Variance for Crude Protein

The analysis of variance for crude protein indicated that statistically there was significant difference among the substrates. Crude protein obtained from the mushroom grown on S13, S10, S7, S4, S12, S2, S1 and S3 were highly significant from S5 and S8 (Table 2 and Table 3). High crude protein content was observed from the mushroom grown on S13, S10, S7, S4, S12, S2, S1 and S3 with record of 36.78, 34.45, 34.08, 32.01, 29.44, 28.40, 27.90 and 26.05% respectively.

The lowest crude protein content was observed from the mushroom grown on S5 and S8 with record of 22.02% and 20.07% respectively as the mushroom was not grown satisfactorily with the presence of high proportion of leaves of *Prosopis juliflora*. The values of crude protein observed in this research ranged from 20.07 to 36.78% in general. The result of this crude protein content is in line with the findings of [9] that reported the values of crude protein content of mushroom ranged between 19 to 39%. The difference in crude protein content of mushroom is attributed to the differences in nitrogen content of the growth substrate and the efficiency of the mushroom for nitrogen utilization and nitrogen fixation [21]. The protein contents of mushrooms are dependent on biological, chemical difference and on the C: N ratio of the substrate [25]. It was also reported that on dry weight basis, the highest protein content (11%) were observed in fruiting body grown on sugar cane bagasse and on wheat straw. The lowest protein content (7.81%) observed from the mushrooms that grown on rice straw [26].

Research findings have reported that among species of mushroom the maximum crude protein was found in *Pleurotus ostreatus* (27.23) and the minimum was found in *Pleurotus djmor* (24.83%) [4]. High nutritional values of oyster mushroom have been reported with protein content of 25-50%. In other findings the total crude protein was reported in the range of 27.95-38.89gm/100gm dry matter [6]. The chemical composition of fresh *Pleurotus ostreatus*, rich 34.4% in protein [20]. Other research conducted on *Pleurotus ostreatus* concluded that *Pleurotus ostreatus* cultivated on citrus limonium and carica papaya wastes contain 26-31.5% digestible protein [16].

3.2. Analysis of Variance for Crude Fat

The analysis of variance for crude fat content revealed that statistically there was no significant difference among substrates (Table 2 and Table 3). This result confirmed that the fat content of the oyster mushroom in all substrates was uniformly low without any variation. The low fat content showed that the quality of the mushroom to be eaten as

nutritionally selected diet for different purpose including for health condition. The crude fat content of the mushroom under this investigation ranges from 0.95% to 1.92% in general. This crude fat content of the oyster mushroom result under this investigation was agreed with the result of [1] that reported the crude fat content of the mushroom ranged from 0.6 to 3.10%.

The edible mushrooms are excellent food that can be incorporated in to well balanced diets due to their low fat content and low energy content and high content of protein and high content of dietary fiber and functional compounds [8]. It was reported that among species of mushroom the highest fat content was found in *Pleurotus djmor* (3.07%) while lowest fat content was found in *Pleurotus ostreatus* (2.37%) [4]. High nutritional values of oyster mushroom have been reported with fat content of 2-5%. In other findings researchers concluded that *Pleurotus ostreatus* cultivated on citrus limonium and carica papaya wastes contain 2.0-5.9% fat on dry basis [16]. It was also reported that the chemical composition of fresh oyster mushroom *Pleurotus ostreatus*, fat content was 2.2% [20]. Very low fat content reported in the range of 1.34-6.45gm/100gm makes mushroom a best diet for peoples suffering from heart disease [6].

3.3. Analysis of Variance for Crude Fiber

The analysis of variance for crude fiber revealed that statistically there was significant difference among substrates. Crude fiber obtained from the mushroom grown on S4 was highly significant from the mushroom grown on S13, S10, S7, S1, S12, S3, S2, S8 and S5. Crude fiber obtained from the mushroom grown on S13, S10, S7, S1, S12, S3 and S2 were statistically different from S8 and S5 (Table 2 and Table 3). High crude fiber content was recorded for the mushroom grown on S4, S13, S10, S7, S1, S12, S3 and S2 with value of 15.94, 14.50, 14.41, 14.40, 13.58, 13.00, 12.30 and 12.02% respectively.

The lowest crude fiber content was recorded for the mushroom grown on S8 and S5 with value of 9.50% and 9.03% respectively. The crude fiber content of the oyster mushroom under this investigation ranges from 9.03 to 15.94% in general. This crude fiber content of the oyster mushroom result observed under this investigation agreed with the result of [18] that reported the crude fiber content of the oyster mushroom range between (7.5 to 16). Researchers have reported that among species of mushroom the maximum fiber content was found in *Pleurotus sajor-caju* (26.28%) while the minimum fiber content was found in *Pleurotus djmor* (22.03%) [4]. It was also reported that the chemical composition of fresh *Pleurotus ostreatus*, fiber content was 8.7% [20]. Oyster mushroom riches in fiber and low in fat contents and this characteristic are highly beneficial for heart patients.

3.4. Analysis of Variance for Moisture Content

The analysis of variance for moisture content showed that statistically there was significant difference between the

substrates. The moisture observed from mushroom grown on S8 was highly significant from mushroom grown on S5 while the moisture observed from mushroom grown on S5 significantly different from moisture observed from mushroom grown on S10, S1, S3, S2, and S12. The moisture observed from mushroom grown on S10, S1, S3, S2 and S12 was also significantly different from mushroom grown on S7, S13 and S4 (Table 2 and Table 3).

The moisture content of each substrate was ranged between 78.10% and 88.90%. The variation occurred with moisture content in this observation may be due to variation in water holding capacity of the substrate and variation in cropping area position towards sunshine through the window. The result of the moisture content of the oyster mushroom under this investigation was ranged from 78.1 to 88.9% in general. The result under this investigation was within the range of the result of [15] that reported the moisture content of the oyster mushroom range between 70% and 90%. It was also reported that the chemical composition of fresh oyster mushroom *Pleurotus ostreatus* indicate a large quantity of moisture content was 90.8% [20].

3.5. Analysis of Variance for Carbohydrate Content

The analysis of variance for carbohydrate content revealed that statistically there was significant difference among substrates. The carbohydrate obtained from mushroom grown on S4, S2, S1, S3 and S10 were highly significant from carbohydrate obtained from mushroom grown on S12, S13, S8, S7 and S5 (Table 2 and Table 3). They had high carbohydrate content with record of 37.29, 36.18, 35.48,

34.20 and 33.74% respectively. Carbohydrate obtained from mushroom grown on S12, S13, S8 and S7 were significantly different from S5. They had intermediate carbohydrate content with record of 29.00, 27.52, 26.48 and 24.77% respectively.

Substrate five had least carbohydrate content with record of 14.03% among all substrate. The carbohydrate content of the oyster mushroom under this research was ranged from 14.03 to 37.29% in general. The carbohydrate content of this investigation was agreed with the results of [7] that reported the total carbohydrate content of oyster mushroom ranges between (16 to 85%). The difference in carbohydrate content of oyster mushroom grown on different substrates could be due to the difference in carbon content of the substrate used for cultivation. It was reported that mushroom is low calorie food and its nutritional value is 27 calorie per 100gm of mushroom [29]. Researchers conducted research on carbohydrate content of mushroom have reported that among species of mushroom highest carbohydrate content was found in *Pleurotus djmor* (37.69%) while lowest carbohydrate content was found in *Pleurotus ostreatus* (36.74%) [4].

The total carbohydrate content was reported also in the range of 42.62-66.78gm/100gm [6]. Different findings have concluded that *Pleurotus ostreatus* cultivated on citrus limonium and carica papaya wastes contain 20.9-33.0% total soluble carbohydrate [16]. In other findings it was also reported that the chemical composition of fresh oyster mushroom *Pleurotus ostreatus* carbohydrate content was 57.6% [20].

Table 2. Effect of substrate on nutritional composition of oyster mushroom (mean values) evaluated at Chiro.

Treatment	Crude Protein	Crude Fat	Crude Fiber	Moisture Content	Carbohydrate Content
S1 (75% SCB + 25% WP)	27.90d	1.92a	13.58bcd	83.30c	35.48ab
S2 (50% SCB + 50% WP)	28.4cd	1.85a	12.02e	82.80c	36.18ab
S3 (25% SCB + 75% WP)	26.05d	1.22cde	12.30de	83.10c	34.20b
S4 (75% SCB + 25% LPJ)	32.01bc	1.48bc	15.94a	78.10e	37.29a
S5 (50% SCB + 50% LPJ)	22.02e	1.34bcd	9.03f	87.00b	14.03e
S7 (75% WP + 25% LPJ)	34.08ab	1.07de	14.40bc	80.10d	24.77d
S8 (50% WP + 50% LPJ)	20.07e	0.95e	9.50f	88.90a	26.48cd
S10 (100% SCB)	34.45ab	1.04de	14.41bc	84.00c	33.74b
S12 (100% WP)	29.44cd	1.49bc	12.00cde	82.50c	29.00c
S13 (33% SCB +33 WP +33% LPJ)	36.78a	1.60ab	14.50b	78.70de	27.52cd
LSD	3.661	0.625	2.529	3.238	2.782

Means with the same letter are not significantly different.

4. Conclusion and Recommendation

The result of the experiment confirmed that different combinations of the three substrates had effect on nutritional composition of oyster mushroom. That is substrate thirteen (33%SCB + 33WP + 33%LPJ), substrate four (75%SCB + 25%LPJ), substrate ten (100% SCB), substrate seven (75% WP + 25%LPJ), substrate twelve (100%WP), substrate two (50%SCB + 50%WP) and substrate three (25% SCB + 75% WP) were given high crude protein and crude fiber content while substrate ten (100%SCB), substrate four (75%SCB +

25% LPJ), substrate two (50% SCB + 50% WP), substrate one (75% SCB + 25%WP) and substrate three (25% SCB + 75%WP) were given high carbohydrate content. That is substrate either with high sugar cane bagasse, high waste paper, with lower proportions of *Prosopis juliflora* or substrates with their equal proportions were produced acceptable nutritional composition for oyster mushroom

Therefore, even though the experiment needs repetition, from the result of the experiment above substrate thirteen, substrate four, substrate ten, substrate seven, substrate twelve, substrate two, substrate one and substrate three were recommended for their acceptable nutritional composition for

edible oyster mushroom at Chiro environmental condition.

Appendix

Table 3. Analysis of variance (ANOVA) for crude protein, crude fat, crude fiber, moisture content and carbohydrate content of oyster mushroom conducted at Chiro, 2017/18.

		Mean Squares				
Source of		Crude	Crude	Crude	Moisture	Carbohydrate
Variation	DF	Protein	Fat	Fiber	Content	Content
Treatment	12	555.19**	1.38ns	106.65**	3985.79**	628.40**
Réplication	2	0.42ns	0.01ns	0.12ns	1.78ns	0.29ns
Error	24	1.48	0.04	0.73	1.19	0.88
CV		5.43	19.60	8.61	1.71	4.08

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