

Evaluation of Millet Straw (*Elusine coracana*) with the Supplement of Cotton Seed Waste for Cultivation of Oyster Mushroom (*Pleurotus ostreatus*)

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Abstract: Mushroom production has been considered as one of the microbial biotechnologies which will improve the livelihood of the community as it is a recycle processes which produces nutritionally rich and medicinally useful mushroom biomass from low cost and no cost organic by products. The main objective of this study was to evaluate millet straw (*Elusine coracana*) with the supplement of cotton seed waste for the production of oyster mushroom. The oyster mushroom culture was prepared on potato dextrose agar. The spawn was developed on yellow color sorghum grain. The sterilized substrate was inoculated with 10% on dry / wet basis of substrate/spawn. The experiment was laid in a completely Randomized Design (CRD) with three replications involving a 10x3 factorial arrangement for millet straw and cotton seed waste mix ratio. The inoculated bags were placed in the dark room for vegetative growth and in the mushroom production house from January 2020 to April 2020 in the main campus of Ambo University. The fastest complete mycelia colonization was recorded from T10 10 days, while the slowest mycelia colonization was recorded on T1 (20 days). The fastest primordial formation was observed on T7 (14 days) while, T1 (24 days) showed slowest primordial formation. The fastest first harvest was recorded from T2 (16 days) while, the slowest first harvest was from T1 (29 days). The highest numbers of fruiting body was recorded from T4 (80) while the least number of fruiting body was from T1 (50). The highest total biomass was recorded from T3 (1618 gm) while the least total biomass was from T1 (871 gm). In this study, the highest biological efficiency was recorded from T3 (302.4%) while, the least biological efficiency was from T1 (138.25%). All the treatments investigated in this, gave the highest yield, total biomass and highest biological efficiency more than those reported in the literature with other substrates so, this will open up the new oyster mushroom substrate mixture based on finger millet could be used for pilot, farm or at industrial scale production.

Keywords: Biological Efficiency, Cotton Seed Wastes, Millet Straw, Oyster Mushroom, Yield

1. Introduction

Mushrooms are eukaryotic heterotrophic organisms; nutritionally classified as saprophytes. According to the definition given by Chang and Miles, a mushroom is a "macrofungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with the naked eye and to be picked by hand" [1]. Oyster mushrooms are one of the most popular edible mushrooms and belong to the genus *Pleurotus* and the family *Pleurotaceae*. Many of the *Pleurotus* mushrooms are primary decomposers of hardwood trees and are found worldwide [2].

The oyster mushrooms can be cultivated successfully under semi-controlled conditions in a small space by using agricultural as well as industrial wastes and other refuse as substrate.

Nutritive food items are the basic requirement for human beings. Due to population explosion, the problem of protein hunger has become acute. Therefore, mushrooms being source of quality protein and is considered to be an alternative source to reduce protein malnutrition [3]. Nutritionally mushrooms have high contents of qualitatively good protein, crude fiber, minerals, vitamins, abundance of essential amino acids, mono and disaccharides, alcohols,

glycogen and chitin but are poor sources of lipids. Besides, they also rich sources of vitamins like thiamine, niacin, riboflavin, folic acid, ascorbic acid, pro-vitamin D and also has many mineral elements like K, P, Zn and Cu compared to vegetables [4].

A wide range of metabolites from mushrooms have found useful as antitumor, antioxidant, antihypertensive, ant platelet-aggregating, anti-hyperglycemic, antimicrobial and antiviral activities as been contributing in the popularity of mushrooms [5]. Besides, mushroom has been very useful for cholesterol reduction, immune enhancement, cancer fighting, anti-allergic activities, and cardiovascular treatment [6]. Furthermore, edible mushroom cultivation is a biotechnological process which aids in reducing and equally protecting the environment from excess solid waste [7].

However, the production and utilization of mushrooms in Ethiopia is neglected. As a result this country is not benefited from mushrooms as the rest of the world [8]. The Ethiopian population is on a continuous increase against a declining acreage of arable land consequently, the available arable land is being subdivided into smaller parcels which are intensively cultivated [9]. In order to encounter this problem, cultivation of edible mushrooms is one of the strategies that do not require large tracts of land.

Ethiopia has a favorable climate, labor and good water resources that create ample opportunities for production of mushrooms. Organic waste despite being a menace to the environment, they represent a potential bio resource for the production of various values added biomass based byproducts such as food in the form of mushroom, animal feed, bio energy, bio fertilizer, and other bio based products [10]. Bio refining process such as mushroom cultivation is the current economically viable biotechnology, which involves the production of protein-rich food (mushroom) from materials that would otherwise consider as “waste,” and being a means to overcome food insecurity, challenging issue in low and middle income countries [11]. Hence, mushroom cultivation technology is being a promising candidate to fight food insecurity along with the reduction of environmental pollution apart from their nutritional and medicinal value they have.

Due to this fact, mushroom cultivation technology regard as the most profitable business, environmentally friendly, and short biological process of food protein (mushroom) recovery utilizing the degrading capabilities of mushroom fungi [12]. Among mushroom fungi, *Pleurotus* species reveal high efficiency in degradation of a wide range of lignocelluloses residues such as wheat straw, cotton wastes, coffee pulp, corn cobs, sunflower seed hulls, wood chips and sawdust, peanut shells, vine pruning's, and others into mushroom protein, the productivity of the conversion being expressed by biological efficiency (BE) [13].

Straw is the dried, above ground, remains of physiologically mature plants from which seeds have been harvested [14]. As plants become physiologically mature, nutrient rich concentrates such as fat, starch, and protein are accumulated in the seeds [15]. Accordingly, less valuable nutrients like cellulose, hemicelluloses, and lignin remain in

the straw. Generally, straw is comprised of plant stem and leaf fractions. However, because of non-selective processing inherited in modern harvesting equipment, straw can contain other plant parts [9]. Millets play a major role in the food security and economy of many less developed countries in the world. They are commonly cultivated in India, Africa and China. It is thought to be one of the first grains cultivated by man [16]. Millets ranks as the sixth most important cereal and feeds one third of the total world population. Another attributes of millets that make them a preferred choice in areas where they are cultivated, are their short harvest period (45-65 days) [15].

Finger millet (*Elusine coracana*) is one of the important indigenous food crops of Ethiopia. It plays significant role both as food grain and animal feed in areas where production of other cereals are curtailed by marginal environments. According to the national statistical data some 400,000 44 hectares are put to production of finger millet each year mainly in the northern and north western parts of the country [17]. Crop straw burning in Africa is increasing and causes atmospheric pollution and soil degradation [18]. The use of agricultural wastes including finger millet straw for mushroom production instead of burning which will negatively affect the environment, could be considered a strategy of conversion of this wastes to use full products. Although, finger millet production has been increasing from time to time, the evaluation of the millet straw for the production of mushroom was not yet reported from Ethiopia. So, in this article we report the effect of millet straw with the supplement of different proportion of cotton seed waste on yield, yield related parameter and biological efficiency of oyster mushroom.

2. Materials and Methods

2.1. Organism and Culture Conditions

The fungal strain, *Pleurotus ostreatus* (Oyster mushroom) was obtained from the microbiology Laboratory, Department of Biology, Ambo University. The pure culture of *Pleurotus ostreatus* were transferred on to potato Dextrose Agar (PDA) Prepared in the laboratory using fresh potato 250g, glucose (Dextrone) 20g, agar 20 and Chloramphenicol 0.2g in 1000 ml of water. The medium was sterilized in autoclave at 121°C, 15psi, for 30 mites. The medium was poured in the Petri-dishes and allowed to cool under aseptic condition in a laminar flow chamber. The cooled and solidified medium was inoculated by 1x1cm agar block of the fungal strain and incubated at 28°C. The growth of the culture and presence of contamination were visually inspected at three day intervals.

2.2. Spawns Production

In this study, the spawn (Mushroom seed) of *Pleurotus ostreatus* was produced on yellow colored sorghum grain, wheat bran and calcium sulfate (gypsum) in the ratio of 88:10:2 respectively [19]. The required amount of sorghum grain was weighed and soaked overnight in sufficient amount

of water. The grain were washed and drained to remove the dead and floating seeds with water. After removing the excess water from the grain, the required amount of wheat bran and gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) were added and transferred to, 1000 ml glass bottles (75% level) leaving a head space over the grain and autoclaved at 121°C temperature for 45 min. After cooling, each bottle was inoculated with 20 agar blocks (1x1cm) of a 15 day old mushroom culture from the Petri dish and incubated for 21 days at 28°C until the substrate were fully colonized by the mycelia invasion and presence of contamination were inspected at five day intervals.

2.3. Substrate Collection and Preparation

Millet straw was collected from Horro Guduru Wellega Zone Horro District Didibe Kebele during elaboration experiment work done or two weak before substrate preparation and also calcium sulfate was obtained from Ambo University laboratory.

The substrates were prepared by using good quality of millet straw, and cotton seed. For the preparation of millet straw was chopped (2 cm size) and then it soaked in water for overnight till it achieved 65-70% of moisture content. The required amount of cotton seed waste was soaked in water separately. On the next day, water was squeezed from both substrates and mixed with required amount of wheat bran (10%) and calcium carbonate (1%) and filled in sterilizable yellow color polyethylene bags (Kurtu pestal).

The prepared millet straw together with cotton seed waste, wheat bran and calcium carbonate was sterilized at 15Psi pressure, 121°C temperatures for 1h in the sterilizable polyethylene bags. After sterilization the substrates were transferred into transparent polyethylene (cultivation bags) for easy supervision of the growth of the mycelia and presence of contamination. After the bags were cooled to room temperature it was spawned at 10% spawn (dry weight/wet weight basis) and the seeded polythene bags were then tightly tied with string made from polyester/cotton cloth. Pinholes were made through the bags ($1/100\text{cm}^2$) for drainage and aeration. The spawned substrates were kept in the room devoid of light for vegetative growth until the pin heads were formed. After primordial formation, large holes were made in the polythene bag to allow normal development of mushroom fruiting bodies.

Bags were transferred to mushroom house under prevailing conditions (room maintained at 85-90% humidity) by keeping water in open containers at different corners of the room. For the fruiting body development the cultivation bags were irrigated using tap water every morning and evening until all flushes of *Pleurotus ostreatus* fruiting bodies were harvested. Adequate ventilation was provided to prevent increased CO_2 concentration in the room by opening the door and windows of the room for half an hour in the morning and in the evening. The mushrooms were manually harvested at maturity which was indicated by upward curving of the edges of the cap. The different stages of mushroom production in these experiments are given below.

2.4. Experimental Design

The experiment was designed in a completely randomized Design (CRD) with three replications involving a 10×3 factorial arrangement for millet straw 10×3 with 30 treatments or preparations of growth substrates (millet straw) and selected mushroom species called *Pleurotus ostreatus* for 30 treatments and 1% of CaCO_3 was added in all of the treatments. The composition of different treatments of substrate mix is shown in table 1:

Table 1. Different substrate composition of the treatments of experiment I.

Treatment	Millet straw (gm)	Cotton seed waste (gm)	Total (gm)
T1	500	Control	500
T2	450	50	500
T3	400	100	500
T4	350	150	500
T5	300	200	500
T6	250	250	500
T7	200	300	500
T8	150	350	500
T9	100	400	500
T10	50	450	500

2.5. Mushroom Production and Product Running

The transparent polyethylene cultivation bags containing the substrate and substrate supplement were then transferred to mushroom house under normal environmental conditions until fruiting bodies were formed. After primordial formation, large holes were made in the polyethene bag to allow normal development of fruiting bodies. The cultivation bags were irrigated using tap water every morning and evening until all flushes of *Pulverous ostreatus* fruiting bodies were harvested. Adequate aeration were provided to prevent increased CO_2 concentration in the room by opening the door and windows of the room for half an hour in the morning and in the evening. The mushrooms were manually harvested at maturity which was indicated by upward curving of the edges of the cap.

Biological efficiency (B.E) were calculated and defined as the ratio of weight (gram) of fresh mushrooms was harvested to dry weight (gram) of the substrate. After 32 days of incubation, a fully matured mushroom on each substrate was collected and calculated using the formula below:-

$$\text{B.E} = \frac{\text{Weight of fresh mushrooms harvested}}{\text{dry weight of substrate}} \times 100 \text{ [20]}$$

2.6. Data analysis

The data were analyzed statically by comparing the mean weights and percentage of biological efficiency through one way ANOVA. The data groups were analyzed using a Statistical Package for Social Sciences (SPSS) for windows 21 version. Treatments means were compared using Least Significant Difference (LSD).

3. Results and Discussion

3.1. Culture Growth of Oyster Mushroom on Potato

Dextrose Agar (PDA)

In this experiment *P. ostreatus* was successfully grown on Potato dextrose agar (PDA) and the mushroom mycelia was completely covered the Petri-dishes within 9 days of incubation (Figure 1). Potato dextrose agar (PDA) is the simplest and the most popular medium for growing mycelia of most cultivated mushrooms [19]. The mycelium should be white and grow out from the block, if yellow, blue, green or grey mycelia on other places on the surface, then these are molds contaminant

3.2. Development of Spawn

The result revealed that the complete growth of spawn of oyster mushroom (*P. ostreatus*) on sorghum grain took on average 18 days to colonize the spawn substrate (Figure 2). The main advantage of grain is that it is very nutritious for fungi and form kernels easily. The kernels can easily be distributed in the substrate [21, 22].

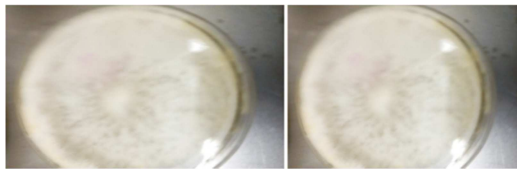


Figure 1. Culture growth of oyster mushroom on the surface of agar plate.



Figure 2. Spawn preparation of oyster mushroom.

3.3. Complete Mycelia Colonization, Primordial Formation and First maturation of Oyster Mushroom

The production bags that received different substrates mixture showed significant variation to complete mycelia colonization ($p \leq 0.05$). The fastest complete mycelia colonization date was recorded on T10 (10 days) while the slowest complete mycelia colonization date was recorded on T1 (20 days) (Table 2). Tekeste *et al.*, [23] colonization of the different substrates were completed from 15.66 to 20 days of inoculation. Slightly longer mycelia colonization was reported by Tsegaye [24], who indicated that complete mycelial invasion of the whole substrate with 20 ± 6 in the mixture of cotton waste and teff straw.

The day required for first primordial formation in the different treatment showed significant variation ($P \leq 0.05$). The fastest primordial was formed from T7 (14 days) while, T1 (24 days) showed slowest primordial formation (Table 2).

This may be due to humidity, aeration and the substrate itself. According to Gume *et al.*, [13] all the treatments they tested showed 3.73 to 5.13 days for primordial initiation after mycelia running. Tekeste *et al* [23] indicated that different substrates had valuable effects on duration to primordial induction ranging from 23.33 on cotton seed hull to 29 days for saw dust, which was longer than the results of this study.

There were significant ($P \leq 0.05$) differences in the first maturation (harvest) of oyster mushroom grown on different treatments. The fastest first harvest was recorded from T2 (16 days) while; T1 (29 days) shows the slowest harvest (Table 2). (The duration observed in the present study was longer when compared with the reported by Gume *et al.*, [13] which was 3.3 in the shortest and 6.0 in the longest. Studies indicated that environmental factor affects the incubation periods of oyster mushroom after primordial formation.

Table 2. Data on the duration growth parameters of Oyster mushroom.

Treatment number	Complete mycelia Extension	Primordial Formation	First maturation (harvesting)
T1	20	24	29
T2	12	15	16
T3	11	15	18
T4	14	19	24
T5	13	16	19
T6	11	15	18
T7	11	14	18
T8	11	15	19
T9	12	17	22
T10	10	15	23

3.4. Number of Bunches, Aborts and Fruiting Body Grown on Different Substrates

The number of bunches were significantly different ($P \leq 0.05$) among substrate types. The highest number of bunches was recorded on T3 (15) while, the fewer number of bunches were observed on T1, T7 and T9 (11 each) (Figure 3). This observation was in line with the results reported by Gume *et al.*, [13] who reported that substrates that gave higher yield also contained higher number of propagating fruit bodies per bunch and highest variability among different treatments on the mean number of mature fruit bodies and aborts. In majority of the substrates, the number of pinhead abortions exceeded number of matures.

The number of aborted showed significantly different ($P \leq 0.05$) variation. The highest number of aborts was recorded from T1 (46) while the least number of aborts was recorded from T7 (30) (Figure 3). The number of fruiting body formed were significantly different ($P \leq 0.05$) among substrate types. A higher number of aborts were recorded with treatments 60:40 and 30: 70 (105 each), while the treatment 80:20 showed lowest (80) number the substrate composed from waste paper and cotton seed waste. The highest number of fruiting body was recorded on T4 (80) while the least number of fruiting body was recorded on T1 (50) (Figure 3). This result was in line with the report of Keneni and Kebede, [25] who indicated that the highest number of fruiting bodies was collected from 80: 20

treatment followed by 90:10 and 60:40, producing 125 and 120 respectively and the rest of the treatment gave the least number of fruiting bodies

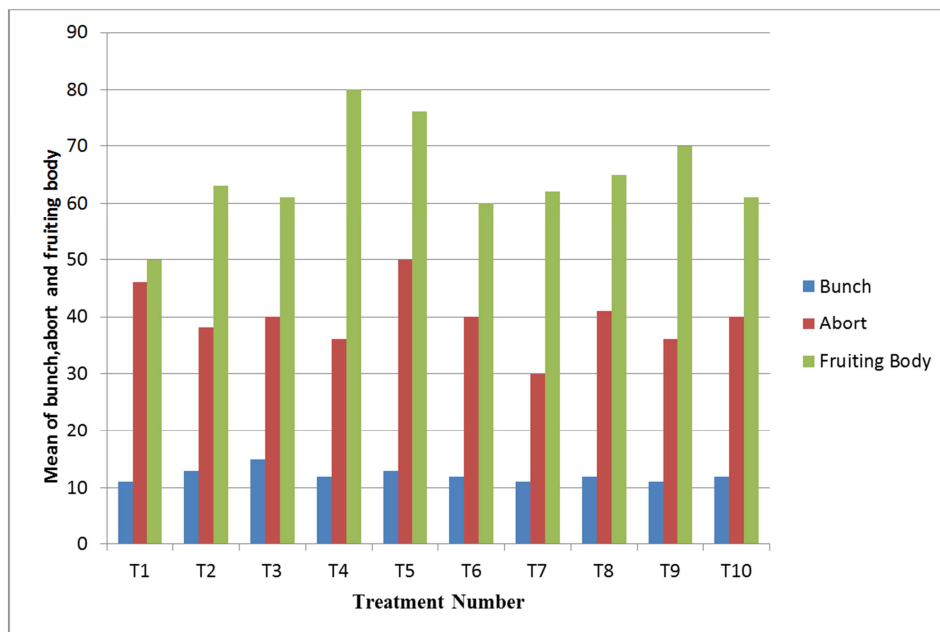


Figure 3. Number of bunch, Aborts and fruits of oyster mushroom grown on different substrates.

3.5. Stipe Length and Cap Diameter Grown on Oyster Mushroom

The stipe length was not significantly ($P \leq 0.05$) differs among substrate types. The highest stipe length was recorded from (T4 and T8) (1.9cm) while the smallest stipe lengths were recorded from T1 (1.5cm) (Figure 4). This result was smaller than the report of Oseni *et al.*, [26] who indicated that stipe length of Oyster mushrooms ranging from 39.4–59.5 mm (3.94–5.95cm) on fermented sawdust substrate supplemented with different wheat bran levels and highest stipe length (59.5 mm) (5.95 cm) was observed on

substratum supplemented with 15% wheat bran.

The cap diameter was not significantly ($P \leq 0.05$) differed among substrate types. The largest cap diameter was measured from T4 (7cm) while, (T1 and T7) (4cm) showed the smallest cap diameter (Figure 4). This result was similar with the reports of (Oseni *et al.*, [26] who reported highest mean cap diameter 57.9 to 62.3 mm on sawdust supplemented with different levels of wheat bran. The largest Cap diameter was obtained from sawdust substrate supplemented with 15% wheat bran (62.3 mm) and the smallest obtained on sawdust substrate supplemented with 5% wheat bran (57.9 mm).

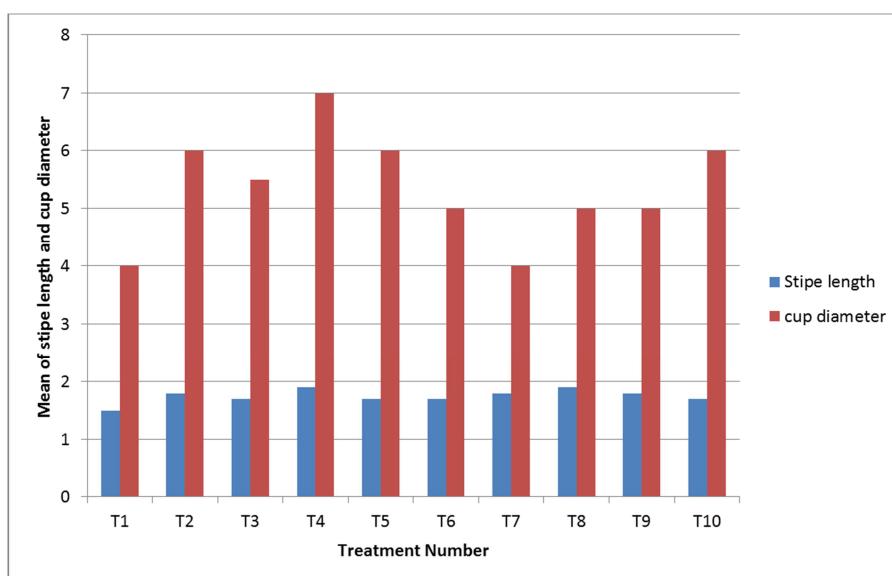


Figure 4. Stipe length and cap diameter of experiment I.

3.6. Yield of Fresh Oyster Mushroom

The fresh weight of mushroom within treatments showed significant variation ($P \leq 0.05$) among substrate types. The highest yield of fresh weight was recorded from T2 (720 gm) while T1 (350 gm) showed the least fresh weight. In the second flush the highest yield was obtained from T2 (520gm) while T10 (220 gm) was found to be the least. In third flush T3 (312 gm) gave the highest yield while T5 (155gm) was recorded as the least fresh weight. In the fourth flush, the highest fresh weight was recorded from T10 (210 gm) while the least fresh weight was recorded on T2 (69 gm). Relatively

as compared to all the flushes the lowest yield of mushroom was obtained in the 4th flush (Table 3 and Figure 5). According to Onyango *et al.*, [27] yields of mushroom in different substrates slightly declined from the first flush to the successive harvests. The crops of Oyster mushroom were harvested in four flushes and the maximum yield was obtained in the 1st than the 2nd, 3rd and 4th flushes, respectively as observed by Oseni, *et al.*, [26]. The highest total yield of fresh weight of mushrooms in grams was recorded on T3 (1618 gm) while the least total fresh weight of mushrooms in grams was recorded on T1 (871 gm) (Table 3).



Figure 5. Propagating pin heads and fruiting bodies of mushrooms: A, pin heads B, matured fruiting bodies of oyster mushroom.

The total fresh weight of mushroom recorded in this study was by far greater than the results reported in the literature. Nasir *et al.*, [28] indicated 403 g of fresh mushroom from date palm 25%+wheat straw and cotton waste 75%, which was the highest and date palm 100% gave 185.75g which was the least; Tekeste *et al* [23] reported the highest yields from cotton seed hull and the least from saw dust. The highest total yields of the fresh mushroom in this study may be due the different mixing ratio of the millet straw and cotton seed waste which optimized the availability of nutrients in the substrate mixture.

Table 3. The duration (day) between pinning to maturation under different treatments.

Treatments	1 st Flush	2 nd Flush	3 rd Flush	4 th Flush	Total
T1	350	260	161	100	871
T2	720	520	210	69.0	1,519
T3	670	510	312	126	1,618
T4	580	470	180	112	1,342
T5	600	320	155	105	1,180
T6	526	280	190	145	1,141
T7	460	261	212	180	1,113
T8	500	351	176	99	1,126
T9	426	269	218	127	1,040

T10	366	220	191	210	987
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3.7. Biological Efficiency

The effect of different treatments on biological efficiency of Oyster mushroom showed significant ($P \leq 0.05$) variations. The highest biological efficiency was recorded from T3 (302.4%) while, the least biological efficiency was recorded from T1 (138.25%) (Figure 6). Similar results was reported by (Pathmashini and Nigam, [29] that indicated significant differences in BE for *P. ostreatus* produced with sorghum (*Sorghum bicolor*), kurakkan (Eleusine coracana), maize (zea mays) and paddy (*Oryza sativa*) and separately fruited on sawdust of mango supplemented with rice bran, chalk and Epson. According to Gume *et al.*, [13] *Coridia africana* gave the least percentage of BE (29.07%) whereas combination from the four original substrates showed the highest B.E (77.38%). Pathania *et al.*, [30] reported considerable variation in yield of oyster mushroom grown on 0.5 apple pomace + 1.5 wheat straw 54.23%, with 0.25 kg of apple pomace and 1.75 kg of wheat straw 40.14% Tsegaye, [24] reported the bioconversion efficiency of cotton waste and coffee pulp 79.% and that coffee pulp and teff straw 63%. In

considering the biological efficiency of the oyster mushroom recorded in these investigations, it was found to be higher than most of the biological efficiency reported in the literature with different substrate mixtures. This may be due

to the fact that millet straw and cotton seed waste compositions makes available the macro and micro nutrients required for the growth of oyster mushroom.

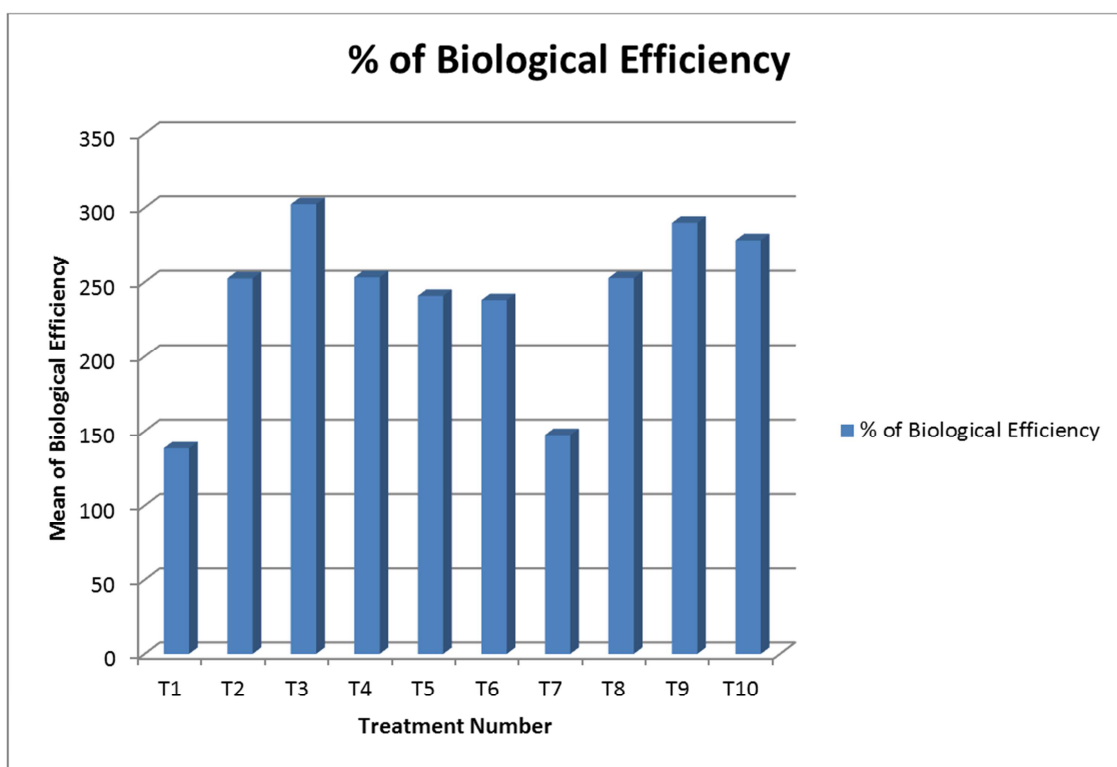


Figure 6. The percentage of Biological Efficiency.

4. Conclusions

Edible mushroom productions as one of microbial biotechnology have been advancing day by day. This is due to the fact that mushrooms are preferred for their tastes, flavor and organoleptic properties and also due they are rich in nutrients, vitamins and minerals. Furthermore mushrooms hold huge bioactive compounds which are found curative for many present day degenerative diseases. There are a number of agricultural and agro bio-processing by products which were evaluated for the usability of mushroom substrates. The evaluation of millet straw which was one of the agricultural by products with the supplement of different proportion of cotton seed waste was the first from our mushroom research report. The results observed in this study clearly showed that finger millet straw supplement with different proportions of cotton seed waste in all the treatments supported highest yield, yield related parameters and biological efficiency of more 100% even in the finger millet alone indicating that the oyster mushroom substrates based on the finger millet will be the potential candidate for small, pilot, farm or and industrial scale production of this mushroom.

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