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Cultivation of Oyster Mushroom (*Pleurotus ostreatus*) On Substrate Composed from Corn Stover Supplemented with Cotton Seed Waste in Ambo University

LELISA Urgesa*, ASEFA Keneni, ESAYAS Aga

Department of Biology, College of Computational and Natural Sciences, Ambo University, Ambo Ethiopia.

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****Corresponding Author***
Lelisa Urgesa
E-mail: *lelisaurgessa92 @gmail.com*

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Mushroom cultivation is one of the advancing microbial biotechnologies for supply of nutrition, medicinal application and for recycling of long lignocelluloses organic wastes. The main aim of this study was to assess the usability of corn Stover for the production of oyster mushroom (Pleurotus ostreatus) from December 2017 to March 1018 at mushroom house and biology laboratory of Ambo University. The mushroom culture was maintained on potatoes dextrose agar, and the spawn was prepared on yellow sorghum grain and substrate was collected from Ambo Woreda Kera kebele and processed, sterilized and inoculated with 10% of the spawn. There were ten different treatments (T1-T10) three triplication the major substrates. From all the different treatments T3 showed the fastest mycelia extension (0.35 cm/day) and T9 and T10 showed slowest mycelia extension (0.23and 0.21 cm/day) respectively. Treatment 1(T1), T2 and T3 showed shortest incubation periods (83days) and T10 had longer (95 days) for overall production. Treatment 4 showed shortest mean periods from pinning to maturation in the 2nd, 3rd and 4th harvests (8 to 5 days), while T1 took longer incubation periods 10 to 8 days in all the four harvests. Treatment 3 showed highest fresh weights in 1st flush (1000g) and T5 gave least fresh weight (150g). Maximum number (6cm) of bunches was recorded from T6 and the least from T1, T3, T4, T8, T9 and T10 equally (4cm). Pilus diameter was maximum from T10 (19.5 cm) and the (7cm) was noticed from T2, T6 and T7 of corn. The longest stipe length (6cm) recorded from T10 and shortest stipe length 2cm recorded from T8. Highest number of abort was recorded from T3 (24) and the lowest from T10 (1cm). The highest total fresh weight of fruit body and biological efficiency were recorded in T2, and T3 (2021–2181g) respectively and the least from T10 (1281g). The highest Spent Oyster Mushroom (SOMS) 76.07% was recorded from T1 and lowest (54.1%).

1. INTRODUCTION

1.1. Background

Mushrooms are fleshy, spore-bearing reproductive structures of macro-fungi grown on organic substrates and for a long time, have played an important role as a human food due to its nutritional and medicinal properties (Etich *et al.,* 2013). Mushrooms are types of fungi, which can play highly beneficial roles in forest ecosystems (Chang and Miles, 2004).

Edible mushroom are highly nutritious and can be compared with eggs, milk and meat (Oei, 2003). Edible mushrooms provide high quality of protein that can be produced with greater biological efficiency than animal protein and they are also, rich in fiber, minerals and vitamins and have low fat content, with high proportion of polyunsaturated fatty acids relative to total content of fatty acids (Marshall and Nair, 2009). The edible mushrooms are excellent foods that can be incorporated into well balanced diets due to their low content of fat, energy, high content of dietary fiber and functional compounds. Their benefits to health include antitumor, immune modulators and hypo-cholesterolemic effects (Bismita, 2011).

Edible mushrooms once called the "food of the gods" and still treated as a garnish or delicacy can be taken regularly as part of the human diet or be treated as healthy food or as functional food (Chang and Miles, 2004). Oyster mushrooms are one of the most popular edible mushrooms and belong to the genus *Pleurotus* and the family Pleurotaceae (Badshah *et al*., 1992).

In Ethiopia, mushroom cultivation is a very recent practice. Previously mushroom consumption was confined to rural inhabitants and picked from farmlands, forests and around waste dumpsites when environmental conditions particularly humidity favor their sporocarp formation. Mushrooms are now cultivated and marketed in urban centers (Kumela Dibaba, 2012). Dawit Abate (2008) reported that small scale mushroom farm was started in 1997 by the cultivation of the Oyster mushroom (*Pleurotus)* species. Later, the button (*Agaricus bisporus*) followed by Shiitake (*Lentinus edodes*) mushroom. The local demand for mushrooms is steadily growing to about 36 tons per year (button 50%, oyster 40% and Shiitake 10%) (Kumela Dibaba, 2012).

Mushrooms are a diverse group of saprotrophytic fungi belonging to the genus *Pleurotus* (Kang, 2004). The oyster mushroom *(Pleurotus ostreatus)* is one of the most easily cultivable mushrooms in the world. It is renowned for both its wide range of substrate compatibility and its mild, nutty, oyster-like flavour when cooked. Many different subspecies, varieties, and strains can be found within this species, but there are two major ecotypes: brown forms from North America and blue/brown forms from Europe (Stamets, 2005).

Plearatus ostreatus is both edible and delicious. It is commercially cultivated across the world. Nutritionally, these mushrooms are a good source of protein, potassium, fibber and carbohydrates. Additionally they contain low levels of many vitamins (niacin, vitamin C and D) and minerals (calcium and sodium) (Stamets, 2005).

Plearatus ostreatus has many potential medicinal uses. It naturally produces isomers of lovastatin, which are well-documented blood cholesterol reducing compounds (Chen *et al*., 2012). Ubiquity proteins have also been identified in these mushrooms that have antiviral and even anti-HIV properties. Studies conducted on rats have also shown oyster mushroom rich diets to inhibit tumour growth and protect from chemicals that induce colon cancer. Though very few people are allergic to these mushrooms when cooked, an estimated 10% of Americans and Europeans may be allergic to raw extracts (Stamets, 2005).

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 (Singh *et al*., 2005).

One of the limiting factors in the cultivation of mushrooms is that the availability of a good substrate which is essential in order to promote satisfactory yield of the mushrooms (Ueitele *et al*., 2014). A good substrate should consist of nitrogen supplement and carbohydrates in order to promote rapid growth of the mushroom (Ogundele *et al*., 2014). Performance of *Pleurotus ostreatus* mushroom grown on maize/corn stalk residues supplemented with various levels of maize/corn flour and Cotton seed waste the inclusion of maize additive shows an increase in both the nutritional value and productivity of mushrooms (Oei, 2005). In this line with this study was designed in the aim of evaluating the agricultural solid waste (Corn Stover) supplemented with cotton seed waste for the production of oyster mushroom (*P.ostreatus*).

MATERIALS AND METHODS

Organism and Culture Conditions

The fungal strain, Oyster (*Pleurotus ostreatus)* mushroom was obtained from the Laboratory of Department of Biology, Ambo University. The pure culture of *Pleurotus ostreatus* was transferred on to Potato Dextrose Agar (PDA) prepared in the laboratory using 40 g in 1000 ml of water and also Malt Extract Aga prepared (MEA) 50gm in 1000ml of water. The medium was poured into the Petri dishes and allowed to cool under aseptic condition in a laminar flow chamber. The cooled and solidified medium was inoculated with 1×1 cm agar block of the fungal strain and incubated at 28^{o}C in dark room. The growth of the culture and presence of contamination was visually inspected at three days intervals.

Source of Spawn

Spawn was prepared under laboratory condition by using yellow colour sorghum grains.

Grain Spawns Production

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 (Dawit Abate, 1998). The required amount of sorghum grain was weighed and soaked overnight in a sufficient amount of water. The grains was washed and drained to remove the dead and floating seeds with water. After removal, the excess water from the grain, the required amount of wheat bran and gypsum ($CaSO4 .2H_20$ (to adjust the pH of the grains and to remove excess moisture and the don't form Clumps)) was added and transferred to 1000 ml glass bottles (75% level) leaving a head space over the grain and autoclave at 121^0C temperature for 45 min. After cooled, each bottle was inoculated with 20 agar blocks (1 x 1 cm) of a 15 days old mushroom cultured from the Petri dish and incubated for 21 days at 28^oC until the yellow sorghum was fully colonized and the mycelia invasion was inspected at five days intervals.

3.4. Substrates Preparation

Corn Stover was collected from Ambo woreda Kera kebele and chopped by an axe from 1cm-3cm in estimation and also lime stone was obtained from Biology Laboratory. These substrates were then transported to Ambo University Biology Laboratory. Two different substrates and substrate combination preparations were made for the mushroom species (*Pleurotus ostreatus*) by mixing cotton seed waste with corn stover *and* cotton seed waste with corn cob in varying proportions. The 10 % of wheat bran and 1 % of $CaCO_3$ were added to substrates, Sterilized at a temperature of 121°C in an autoclave to avoid contamination. The sterilized substrates were kept in a clean room and allowed to cool down for two or more hours (Atikpo *et al*., 2008). After cooled, the substrates (cotton seed waste, corn stover and corn cob) were soaked transferred to transparent plastic bags were prepared corresponding to the substrate preparations in replicates were used (Diriba Muleta *et al*., 2013).

3.4.1. Substrate Inoculation

Substrates were inoculated with 5% of spawn under aseptic condition using sterile spoon. Then after, 50 g (which is equal to 10% of the weight of the substrate mixed) of the Oyster mushroom *(Pleurotus ostreatus)* spawns were added and thoroughly mixed with the substrate kept in the polyethylene bags using sterile spoons under the laminar flow hood. Then, rubber bands tied the open ends of the bags and nine small holes were made using sterile needle to allow air exchange of bags (Dawit Abate, 2008).

3.5. Incubation and Mycelia Growth

All inoculated bags were incubated at the room temperature and placed at 15cm apart in a completely randomized design in a cleaned and disinfected dark room with detoil (Dawit Abate, 1998)

3.6. Mushroom Production and Product Running

Fresh air exchange between the dark room and the outside environment was allowed by opening windows at night and closing during the daytime to enhance the quick colonization of the substrate. After full colonization the bags were transferred to the cropping room, whose environment was kept illuminated by sunlight through the improvised windows and a temperature and humidity of 29°C and 75-85%, respectively, were maintained by sprinkling the bags with water twice or more a day. The humidity and temperature ranges were maintained by spraying water to the walls and floors of the cropping room. Formations of complete mushrooms were occurred one week after the colonized substrates are transferred to the cropping room (Oei, 2005). After 32 days of incubation, fully matured mushroom species on each substrate was collected

3.7. Experimental Design

The experiment was designed in a Completely Randomized Design (CRD) with triplications involving 10 X 3 arrangements with 30 treatments or prepared of growth substrates (Corn Stover) and selected call *Pleurotus ostreatus.* For 30 treatments, 5% of spawns prepared, 1% of $CaCO_3$ and 10% of wheat bran were added into all of the treatments.

Table 1: The composition of different treatments of *corn stover*

Treatment	*Corn stover (gm)*	*Corn stover %*	*Cotton Seed Waste (gm)*	*Cotton seed waste %*	*Total (gm)*
CST1	*500g*	*100%*	____	___	*500*
CST2	*450g*	*90%*	*50g*	*10%*	*500*
CST3	*400g*	*80%*	*100g*	*20%*	*500*
CST4	*350g*	*70%*	*150g*	*30%*	*500*
CST5	*300g*	*60%*	*200g*	*40%*	*500*
CST6	*250g*	*50%*	*250g*	*50%*	*500*
CST7	*200g*	*40%*	*300g*	*60%*	*500*
CST8	*150g*	*30%*	*350g*	*70%*	*500*
CS T9	*100g*	*20%*	*400g*	*80%*	*500*
CST10	*50g*	*10%*	*450g*	*90%*	*500*

3.9. Determination of Biological Efficiency

The biological efficiency (BE) of the mushroom species was calculated using the formula recommended by Chang and Miles (1989) as follows:

$$\% \text{ Biological Efficiency} = \frac{\text{Fresh weight of fruiting}}{\text{Dry substrate}} \times 100$$

3.10. Determination Spent mushroom Substrates

The Spent Efficiency of Oyster mushroom substrates were calculated using following formula as follow:

% Spent Mushroom Substrate =

$$\frac{\textbf{Spent mushroom subsrate} \quad \textbf{X 100}}{\textbf{Dry substrate before ennoculation}}$$

3.11. Data Collection

The yield of *Pleurotus ostreatus* on the different substrates supplementation were determined by recording the number, weight and size of the fruit bodies after sprouting. The measurements from the various replicates were added and their mean values were calculated.

The following parameters of growth / yield were measured.

Number of fruit bodies: These were done by directly counting the number of fruit bodies on each substrate.

Diameter of the pilus: These were also measured in centimeters with ruler from one edge of the pilus across the stripe to the other edge.

Fresh weight of fruit bodies: This was done by weighing fresh mushroom using an electrical weighting balance.

3.12. Data Analysis

The data were analyzed 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 version 21.0. Treatments means were compared using LSD and DUNCAN A p- value of < 0.05 was considered to determine statistical significance using 95% confidence intervals

4. RESULTS AND DISCUSSION

4.1. Mycelia Extension of *P.ostreatus* Grown on Corn Stover

Mycelia growth is a preliminary step that creates suitable internal conditions for fruiting. Thus, outstanding growth of mycelium is a vital factor in mushroom cultivation (Pokhrel *et al*., 2009). The mean values of mushroom grown on different substrates composition showed highly significant ($P \leq 0.05$) differences in the mycelia extension. T3 showed the fastest mycelia extension followed by T2, while T9 and T10 exhibited slowest mycelia extension on 8^{th} and 16^{th} days of incubation periods (Table 2). There were significant ($P \leq 0.05$) differences in the days required for complete invasion of the substrates receiving different treatments. The time required for complete invasion of the substrates was significantly ($P \leq 0.05$) less for T1, T2 and T3 when compared to that of T9 and T10 (Table 2 and Fig 1). Total days required to complete the production cycle was shortest for T2 and T3 while it took more days for T10 (Table 2). Similar result was reported by Asefa and Geda, (2014 b); Gume *et al.*(2013);Mekonnen and Semira, (2014) wheat straw and rice straw on which much of research work has been done on this mushroom species. Ashraf *et al.* (2013) reported that all the treatments they tested showed 3.73 to 5.13 days for primordial initiation after mycelia running.

Fig 1: Mycelia extension: A) Substrate inoculation B) Mycelia extension

Table 2: Mycelia extension on different treatments measured at 8th and 16th days of incubation on Corn stover

Treatments	Mycelia extension in (cm)		Mean values (cm/day)	Number of days required for complete invasion	Total days required to complete the cycle
	8th days	16th days			
T1	2.3^{b}	4.5^{dc}	0.32^{ba}	28^{a}	83^{a}
T2	2.4b^{a}	4.6^{d}	0.34^{a}	28^{a}	83^{a}
T3	2.5^{a}	7.8^{a}	0.35^{a}	28^{a}	83^{a}
T4	1.95^{c}	6.5^{ba}	0.27^{d}	34^{g}	93^{d}
T5	2.15^{bc}	7.1^{a}	0.30^{b}	32^{e}	92^{c}
T6	1.85^{c}	5.5^{bc}	0.26^{e}	35^{h}	92^{c}
T7	2.0^{bc}	6.3^{b}	0.28^{c}	31^{d}	92^{c}
T8	2.22^{b}	6.52^{ba}	0.28^{c}	30^{c}	91^{b}
T9	1.5^{d}	4.98^{c}	0.23^{f}	29^{b}	91^{b}
T10	1.5^{d}	5.76^{bc}	0.21^{g}	33^{f}	94^{e}

Mean values within a column sharing the same superscript letter(s) are not significantly different by using DUNCAN test at p = 0.05.

4.2. Incubation Periods of Different Harvests of *P.ostreatus* Grown on Corn Stover

Mean incubation periods of mushroom flushes showed significant differences ($P \leq 0.05$). Treatment 3 (T3) (400 gm of Corn stover supplied with 100 gm cotton seed waste) showed shorter incubation periods 28 days, followed by T4 (29) days of 350 gm of Corn stover supplied with 150 gm cotton seed waste, while T10 (50 gm corn stover supplied with 450 gm of cotton seed waste) took relatively longer 45 days incubation to 1st flush. The incubation period taken from the 1st flush to the 2nd flushes shorter for the different treatments did not showed variation (Table 3). This study is similar to the results reported by different authors; Mekonnen and Semira, 2014 reported 17 days for cotton hulls and 35 days for saw dust as a substrate, while Asefa and Geda, (2014) reported that the mushroom grown on waste paper: cotton seed waste (80;20 and 70:30) took 42 days from incubation of 1st flush and 55 days for mushroom grown on waste paper: wheat bran (50:50).

Table 3: Incubation periods of different harvests for corn stover

Treatments	Incu -1st flush	1st–2nd flush	2nd–3rd flush	3rd–4th flush
T1	32^{d}	16^{b}	14^{c}	13^{c}
T2	31^{c}	15^{a}	13^{b}	12^{b}
T3	28^{a}	15^{a}	12^{a}	11^{a}
T4	29^{ba}	16^{b}	14.5^{d}	13^{c}
T5	30^{b}	17^{c}	14.5^{d}	13^{c}
T6	30^{b}	16^{b}	15^{e}	13^{c}
T7	31^{c}	15^{a}	14^{c}	12^{b}
T8	33^{e}	17^{c}	15^{e}	13^{c}
T9	36^{f}	17^{c}	14^{c}	12^{b}
T10	45^{g}	17^{c}	15.5^{f}	13^{c}

Mean values within a column sharing the same superscript letter(s) are not significantly different by using DUNCAN test at p = 0.05.

4.3. Pinning to Maturation of the Oyster Mushroom on substrate received Different Treatments

The mean periods taken from pinning to maturation of each treatment of *P.ostreatus* grown on Corn Stover showed significant (P≤0.05) variation. Treatment 8 (T8), T9 and T10 relatively took longer periods from pinning to maturation and T2, T3 and T5 took shorter periods from pinning to maturation in all flushes as compared to other treatments (Table 4). The mean periods taken from pinning to maturation of each treatment grown on Corn Stover showed significant (P≤0.05) variation. T1, T8 and T10 relatively took longer periods from pinning to maturation and T2, T3 and T4 took shorter periods from pinning to maturation in all flushes as compared to other treatments (Table 8). Similar results from pinning to maturation was reported by Asefa and Geda (2014), while shorter periods from pinning to maturation were reported by Gume *et al.*(2013).

Table 4: Pinning to maturation of the oyster mushroom under different treatments of corn stover

Mean duration (days)

Treatments	1st Flush	2nd Flush	3rd Flush	4th Flush
T1	10^{b}	9^{b}	8^{b}	6^{b}
T2	9^{a}	8^{a}	7^{a}	5^{a}
T3	9^{a}	8^{a}	7^{a}	5^{a}
T4	9^{a}	8^{a}	7^{a}	5^{a}
T5	11^{c}	10^{c}	8^{b}	6^{b}
T6	11^{c}	10^{c}	8^{b}	6^{b}
T7	11^{c}	10^{c}	8^{b}	6^{b}
T8	12^{d}	10^{c}	8^{b}	7^{c}
T9	12^{d}	11^{d}	9^{c}	7^{c}
T10	13^{e}	11^{d}	9^{c}	7^{c}

Mean values within a column sharing the same superscript letter(s) are not significantly different by using DUNCAN test at p = 0.05

4.4. Yield of Mushroom per Flushes

Yield of mushroom per flush (wet weight) showed significant variation between treatments (P≤0.05) (Fig 2) as well as between flushes. Treatment 2 (T2) showed the highest fresh weight of *P.ostreatus* grown on corn stover in grams in first flush followed by T3, T4, T5, T6 and T7 while T9 and T10 were found to be the least.

In the second flush, the highest yield was obtained from T2 followed by T3, T4, T5, T6, T7 and T8. Treatment 9 and T10 found to be the least. In third flush T2, T3, T4 and T1 gave the highest yield, followed by T6, T7 and T8; while the yield obtained from T9 and T10 were least. As compared to all other flushes the lowest yield of mushroom was obtained in the 4th flush (Fig 2). According to Mekonnen and Semira (2014) averagely the yields were highest in the first flush then declined gently in the second, third and fourth flush of all substrates. In this investigation similar results were observed.

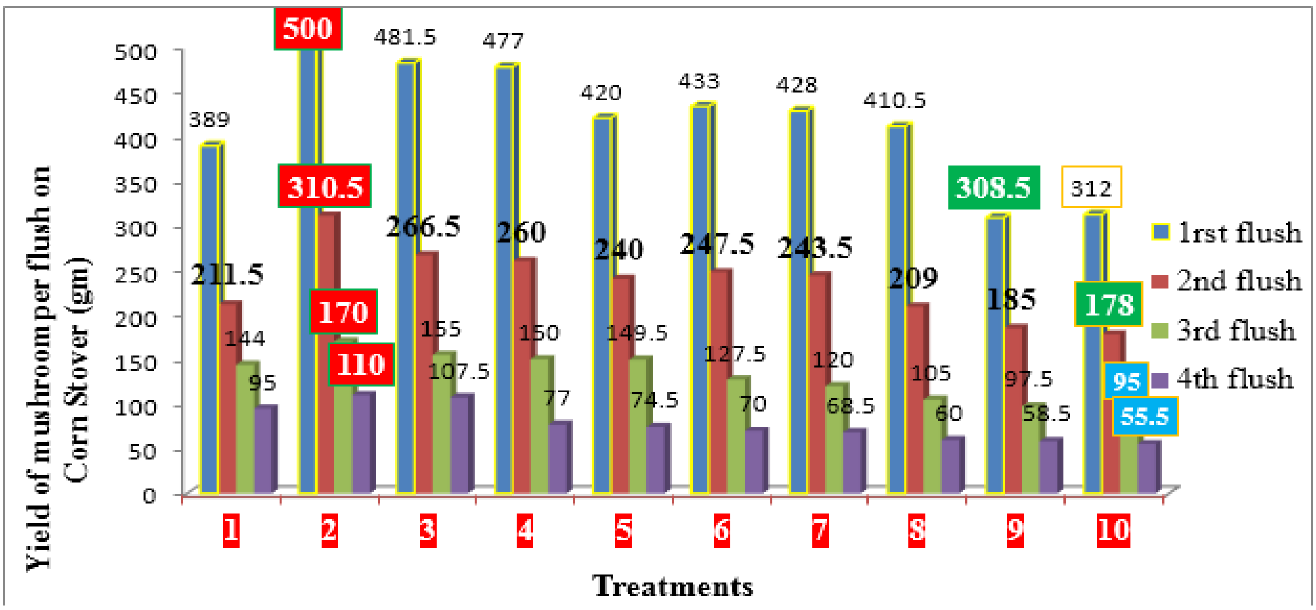

Fig 2: Yield of Oyster mushrooms (*P.ostreatus*) per Flushes grown on Corn stover

From Corn Stover substrate and its composition, a higher number of bunches were observed on T6, while the lowest were observed on T10 (2) while the least bunch number shown in T1 (3). Number of mature mushrooms, mean weights of mature mushrooms harvested and aborted pinheads was significantly ($p < 0.05$) varied among substrate types (Fig 3). This observation was agreement with the results reported by Gume *et al.,* (2013) 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. Kimenju *et al*. (2009) reported that more than 50% of pinheads emerged did not grow into marketable products. Gume *et al*. (2013) observed high rate of pinhead abortion from low-yield substrates.

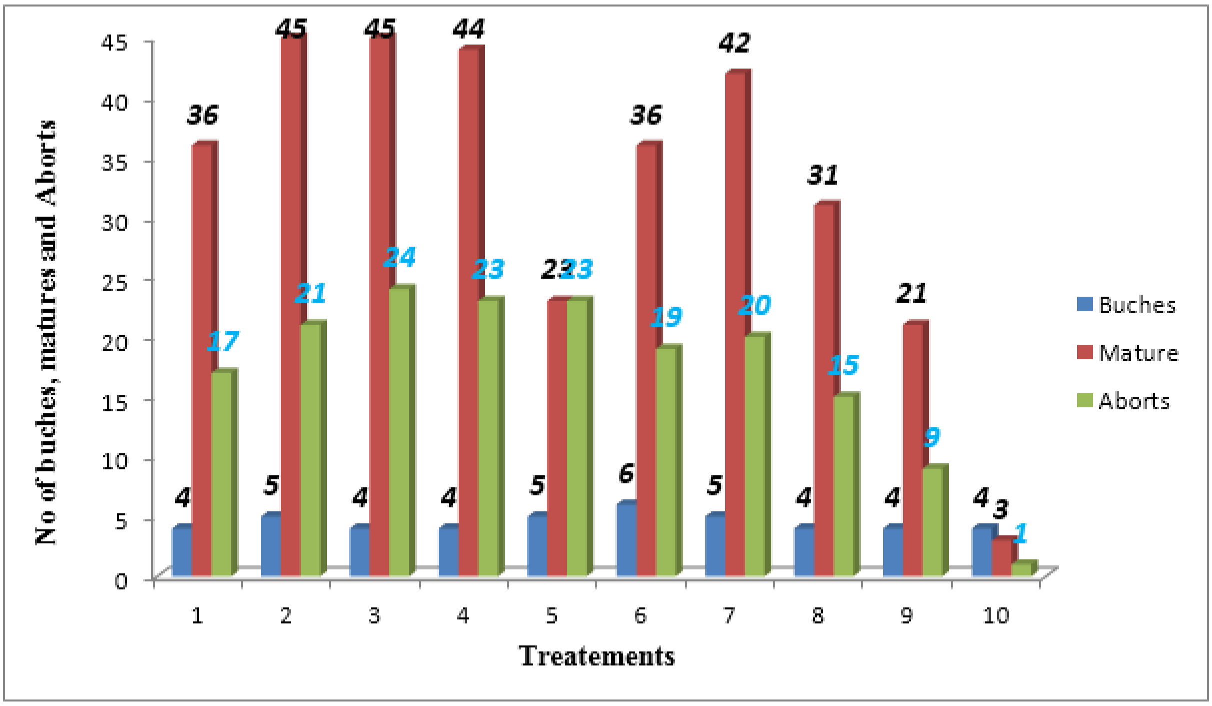

Fig 3: Number of bunches, Matures and aborts of *P.ostreatus* grown on corn stover

4.5.2 Pilus diameter and Stipes length

The highest mean cap diameter of *P. ostreatus* was observed in T10 (19.5) and T9 (15.5cm) whereas, the lowest mean cap diameter was T6 (7cm), T7 (7cm) and T2 (7cm) from Corn Stover (Fig 4). In this investigation greater values was recorded from all treatments except T2, T6, and T7 when compared with Islam *et al*. (2009) which recorded the largest (7.0 cm) pilus diameter from Mango sawdust. The authors obtained the shortest (1cm) cap diameter from coconut sawdust. However, the extreme greater values were recorded from T10 and T9 corn stover substrate due to favourable condition and highly adapted substrate composition. This may be because of less localized competition that existed in fewer fruit body containing bunches. Similarly, the stipe length of the samples showed in significant ($p<0.05$) variation with different treatments which was ranged from 2cm to 6cm, The stipe length of all the 10 treatments did vary significantly (2.5–6.0 cm), which is in not agreement with the results of Gume *et al*. (2013)(1.4–1.9 cm). But agreement with reported by Oseni *et al.(*2012) observed 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 highest stipe length 5.95 cm was observed on substratum supplemented with 15% wheat bran.

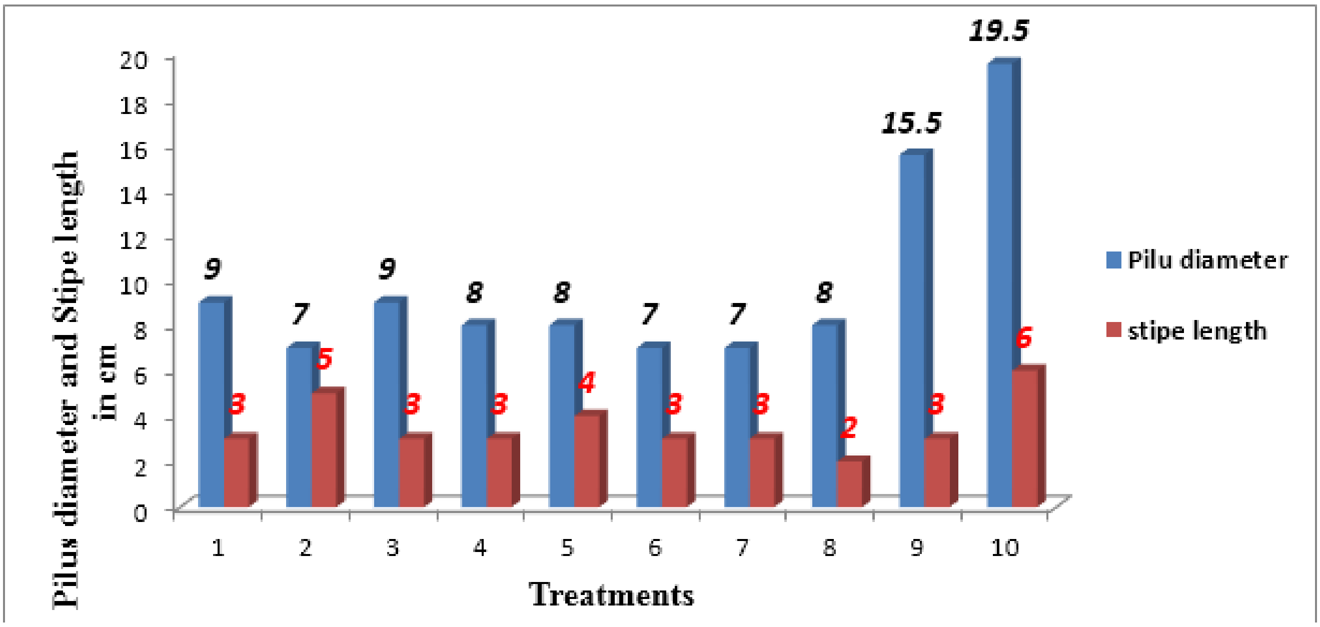

Fig 4: Pilus diameter and Stipes length of *P.ostreatus* grown on corn stover substrate

4.6. Biological Efficiency of Oyster Mushroom

In this investigation, the *Pleurotus ostreatus* were also evaluated for their biological efficiency (BE) and the results are provided below in table 10. The substrates composition significantly ($p<0.05$) affected the biological efficiencies (BE). High values 218.1% of BE were recorded from *Pleurotus ostreatus* grown on corn stover T2 (450 gm of corn stover supplied with 50 gm of cotton seed waste) followed by T3 (400 gm of corn stover supplied with 100 gm cotton seed waste) (202.1%). On the other hand, the least BE 128.1% was recorded from the *Pleurotus ostreatus* grown on corn stover T10 (50 gm of corn stover supplied with 450 gm of cotton seed waste) followed by values of *Pleurotus ostreatus* grown on corn stover T9 (100 gm of corn stover supplied with 400 gm of cotton seed waste) (Table 5).

This study was agreement with Mayfeb (2017) reported biological efficiency (BE) of *P.ostreatus* 74.6% to 205 % more values were recorded in this result from T2.

Table 5: Mean of Biological Efficiency of Oyster Mushroom Grown on Corn Stover

Treatments	Mean and Stdv of Corn Stover
1	163.9 ± 0.17^{e}
2	218.1 ± 0.1^{a}
3	202.1 ± 0.17^{ba}
4	192.6 ± 0.1^{ba}
5	176.8 ± 0.1^{b}
6	175.6 ± 0.2^{c}
7	172 ± 0^{d}
8	156.6 ± 0.44^{f}
9	129.9 ± 0.1^{g}
10	128.1 ± 0.1^{g}
Mean b/n	128.1±0.1-218.1±0.1

Mean values within a column sharing the same superscript letter(s) are not significantly different by using DUNCAN test at p = 0.05

4.7. Spent Efficiency Oyster Mushroom *(P.ostreatus)* Substrate Corn stover

At the end of several mushroom harvests, the growing material is considered spent. Spent Oyster mushroom (SOMS) contains enough digestible nutrition, primarily decomposed by mushroom, to be fed for livestock (Table 6). It will increase growers' income and protect environment to recycle SOMS for feeding livestock or soil for other plants.

In this investigation, the Spent *Oyster (P.ostreatus)* mushroom substrates were also evaluated the results are provided below in table 6. The Spent Oyster mushroom (*P.ostreatus*) substrates corn stover and corn cob were significantly at ($p<0.05$) level test. The highest values of SOMS of corn Stover was 76.07% recorded from SOMS of corn stover T10 (50gm of corn stover supplied with 450 gm of cotton seed waste) followed by T9 (100 gm of corn stover supplied with 400 gm cotton seed waste) (74.06%) and lower percent 23.93% and 25.94% changed to product respectively. On the other hand, the least SOMS corn stover 54.1% was observed from the corn stover T2 (450 gm of corn stover supplied with 50 gm of cotton seed waste) followed by SOMS values of 56.06% corn stover T3 (400gm of corn stover supplied with 100gm of cotton seed waste) and higher 55.9% and 53.94% gave product.

Table 6: Spent Oyster Mushroom Substrates of Corn Stover

Treatment	Mean and Stdv of SOMS of Corn stover
1	69.8±08^{d}
2	54.1±0.1^{j}
3	56.06±0.08^{i}
4	59.04±0.57^{h}
5	60.08±0.05^{g}
6	61.3±0.1^{f}
7	63±0.08^{e}
8	70.06±0.08^{c}
9	74.06±0.07^{b}
10	76.07±0.07^{a}
Mean b/n	54.1±0.1-76.07±0.07

Mean values within a column sharing the same superscript letter(s) are not significantly different by using DUNCAN test at p = 0.05.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusion

Production of edible mushroom has been considered as diversification of food production and also contribute in the struggle for food self sufficiency and mitigating issue of food insecurity particularly in the developing countries like Ethiopia. Testing the usability of Corn Stover with the supplement of different ratio of Cotton seed waste was not yet tried for mushroom production in Ethiopia. Over all this investigation yield enormous information on yield, yield related parameters, biological efficiency and nutritional composition of the mushroom biomass grown on different substrate mix ratio. So, based on the results of this study the following conclusions were made:

- The different treatments resulted in significant variation on growth, yield, and yield related parameters, and biological efficiency. From all the treatments T2 and T3 of corn stover substrate compositions were found to be highest yielding with all the parameters tested. While the rest of the treatments did not gave comparable yields and biological efficiencies..
- Over all, the results of this study showed that the possibility of mixing Corn stover with Cotton seed waste in different proportion which results in highest yield, biological efficiency, good nutritional contents and good quality mushroom fruiting bodies..

5.2. Recommendations

Based on the above conclusion the following recommendations were made:

- Cultivation of *Pleurotus ostreatus* on substrate based on Corn stover with the supplement of different proportion of cotton seed waste resulted in the higher yield, yield related parameters, biological efficiency and nutrient content of the mushroom fruit bodies. As a result , this technology (oyster mushroom cultivation) based on Corn stover should reach the community so that they will be able to convert low cost/no cost agricultural residues to the value added mushroom biomass which could be nutritionally rich, medically valuable and environmental friendly.
- Treatments 2 (T2) compositions were highest yielding with all the parameters tested, so that for the commercial cultivation of the oyster mushroom these treatment should be evaluated at the Pilate or commercial scale. Further detailed studies must be made order to evaluate the mineral composition of the oyster mushroom, which is not studded in this study.
- From all Treatments, T2 and T3 so as to identify the substrate composition which will give all rounded mushroom biomass for nutrition, medicine etc.

Since the Ethiopian communities have been not yet understood the nutritional, medical and the environmental value of mushroom production, awareness rising in the form of continues training should be organized and conducted.

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