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Nutrient Compositions and Optimization of Elephant Grass *(Pennisetum purpureum*) Stem to Cotton Seed Proportion for the Cultivation of Oyster Mushroom (*Pleurotus ostreatus*) at Ambo Western, Ethiopia.

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ABSTRACT

At present more emphasis has been given to mushroom cultivation for the nutrition and medicinal uses and Agricultural product recycling technology. The aim of this study was to investigate the usability of Pennisetum purpureum stem as major substrate for cultivation of oyster (Pleurotus ostreatus) mushroom with supplement of different proportion of cotton seed waste. The culture of the oyster mushroom was maintained on potato dextrose agar, and the spawn was prepared on yellow collared sorghum and sterilized substrate was inoculated with 10% of the spawn wet basis on dry basis of the substrate. The experimental design constitutes ten treatments on the stem of elephant grass with ratios of cotton seed wastes (T1-T10) in three replications. At T4 (70:30) the fastest mycelial grow on the stem of elephant grass and Slowest mycelia extension were observed on T7 (60:40). the highest fresh weight were observed on T4 (70:30) stem of elephant were recorded 1254 g / 500g dry substrate, highest number of fruits recorded on T5 (62) while largest cap diameter (11.5cm) was recorded on T2 of the stem of elephant grass the highest stipe length were recorded on T9 (3.67cm) . The highest biological efficiency observed on T4 (250.5%) on stem of elephant grass. the highest protein contents were recorded on T7 (36.17%) on stem of elephant grass with supplementation of cottonseed wastes and the least one were recorded on T9 (80:20) 16.87%, on the others treatments intermediate number of food contents were recorded. Even though on the stem of elephant grass and the proportion supplementation of cotton seed waste was varied for all the treatments except on T1 treatments make 100% Pennisetum purpureum dry stem were made. Over all, the results of the study showed that the Pennisetum purpureum stem supplemented with cotton seed wastes can be gave highest yield and yield related parameters of oyster mushroom. The results of the present study implies to carry out further research on the optimization of Pleurotus ostreatus production using two or more type of substrates tested in this experiment.

INTRODUCTION

Pleurotus species, commonly known as oyster mushrooms, are edible fungi cultivated worldwide especially in south East Asia, India, Europe and Africa (Mandeel *et al.,* 2005).

China produces 64 % of all edible mushrooms in the world and 85% of all oyster mushrooms all over the world (*Pleurotus* spp.) is also produced in China (Chang.,1999). Mushroom production in rural communities can alleviate poverty and improve the diversification of agricultural production (Godfrey *et al*., 2010).

Mushrooms cultivation offers benefit to market gardens when it is integrated into the existing production system by producing nutritious food at a profit, while using materials that would otherwise be considered "waste" (Beetz and Kustudia, 2004). This is because mushrooms contain many essential nutrients and they are found to solve dietary related health problems (Atikpo *et al.,* 2008)

It is a valuable mushroom with good marketability and is relatively easy to grow. It requires no arable land for production and the abundant agricultural waste found countrywide offers opportunity for production, which in turn provides a more economical and environmentally friendly disposal system (Stamets, 2009; Olfati and Peyvast, 2012; Philippoussis and Diamantopoulos, 2012).

There are many types of mushrooms that offer a long list of health benefits. Among them oyster mushroom occupies 14% of the global market and ranks third in the global trade. It tolerates a temperature of 7 - 37°C with an optimal range of 26 - 28°C and is rich in protein, fiber, iron, vitamins and minerals (Wani *et al.,* 2012).

According to (FAO, 2009), it is an enterprise for both men and women and it is especially an excellent enterprise for women since it does not demand much labor and energy for production. Mushroom production indirectly provides materials that are used to improve the soil structure for production of other crops. Mushroom cultivation is a useful method of environmental waste management and waste disposal. Mushrooms are also considered a good source of protein, considering protein content of dry mushroom, however, it is important to emphasize that the protein content of fresh mushroom is hardly higher than 4% (Silva *et al*., 2007; Bernardi *et al*., 2009). Mushroom cultivation has been reported as other effective way of alleviating poverty in developing countries (Masarirambi *et al.*, 2011).

However, there is no mushroom cultivation practice in the country to fill the demands of people interested in the mushroom consumption. Those very few mushroom farms in Ethiopia are restricted to the capital city. Some research based practices in some parts of the country are still at the stage of trials. The current study was, therefore, initiated to assess the suitability of differ-ent locally available substrates and their combinations for cultivation of *P. ostreatus* and to estimate yields of cultivated mushrooms on different locally available cheap substrates.

Elephant grass is very important forage in the tropics due to its fast productivity. It is particularly suited to feed cattle and buffaloes. Elephant grass is mainly used in cut-and-carry systems ("zero grazing") and fed in stalls, or made into silage or hay. Elephant grass can be grazed, provided it can be kept at the lush vegetative stage: livestock tend to feed only the younger leaves (FAO, 2015). Elephant grass, as implied by its name, is an important source of forage for elephants in Africa (*Cook et al.,* 2005). However the aimed through this research to reveal the use of inexpensive agricultural foods to grow mushrooms and evaluates suitability of Elephants grass (*Pennisetum purpureum)* for cultivation mushroom.

Statement of the problem

Mushroom cultivation could be a possible option to alleviate poverty and malnutrition in developing countries (Diriba *et al.,* 2013). The science of mushroom growing is now confined to a few producers in the country. A number of research activities have been carried out on the cultivation of oyster mushroom using different agricultural waste products worldwide. Several publication have focused on the utilization of different composition of agricultural waste products as substrate for mushroom production (Asefa and Geda 2014b) .However, the utilization of Elephant grass (*Pennisetum purpureum*) which is grown enormously in the swampy, waterlogged areas in our country has not been attempted so far as a substratum for growing mushroom species.

The Elephants grass (*Pennisetum purpureum*) is available at high amount in different localities such as agricultural centers, and spring areas in Ambo University. Inside the campus of Ambo University also there is plenty of this grass were available and left unused. In this context this research has been initiated in order to understand the possible utilization of this plant biomass along with cotton seed waste as substratum for growing the oyster mushroom (*P.ostreatus*). It is aimed through this research to reveal the use of inexpensive agricultural food to grow mushrooms and evaluate suitability of Elephants grass (*Pennisetum purpureum)* for cultivation mushroom.

Significance of the study

The practice of mushroom cultivation is not well known in Ethiopia even though a number of attempts have been made to make awareness among the community. Several studies have been conducted in the utilization of various plant parts as substratum for the cultivation of oyster mushroom. However, no research has been

conducted on Elephant grass as a substratum for mushroom cultivation in Ethiopia. Therefore, the present study was conducted to explore the possibility of using these plants for the cultivation of the oyster mushroom. Moreover, the results of this study would initiate more in depth research on the application of this plant for different species of mushrooms

MATERIALS AND METHODS

Description of Study Area

The research study was carried out at Ambo University, Ethiopia, in mushroom production center (MPC); the institution is located about 116 km away from the capital city of Ethiopia. Geographically Ambo University main campus, which was located at the altitude of this institution, is 2101 meter above sea level. The Ambo city was located at Latitude: 8° 58' 59.99" N and Longitude: 37° 50' 59.99" E. the selected area has good climate condition to cultivate mushroom production. Its temperature ranges from 19-29^{o}c.

Organism and culture conditions

The fungal strain, *Pleurotus ostreatus* (Oyster mushrooms) were obtained from Micro biology Laboratory, Department of Biology, Ambo University, Ambo, Ethiopia. The pure culture of *Pleurotus ostreatus* were transferred on to Potato Dextrose Agar (PDA) prepared in the laboratory and chloramphenicol 0.2 g in 1000 ml of water. The medium were poured into the Petri dishes and allowed to cool in under aseptic condition in laminar flow chamber. The cooled and solidified medium were inoculated by 1 cm×1 cm agar block of the fungal strain and incubated at 25^{o}C. The growth of the culture and presence of contamination were visually inspected at three days interval.

Source of spawn and Spawn production

In this study, the spawn (mushroom seed) of *Pleurotus ostreatus* was prepared on yellow colored sorghum grain, Wheat brain and calcium sulfate (gypsum) in the ratio of 88:10:2, respectively (Dawit Abebe, 1998). The required amount of sorghum grain was weighed and soaked overnight in sufficient amount of water. The grains were washed and drained to remove the dead and floating seeds with excess of water. After removing the excess water from the grain, the required amount of Wheat bran and gypsum ($CaSO_4.2H_2O$) 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 minutes.

After cooling, each bottle was inoculated with 20 agar blocks (1 cm × 1 cm) of 15day old mushroom culture from the Petri dish and incubated for 21 days at 28 ± 2$^{0}_{C}$ until the substrate were fully colonized and the mycelia invasion and contamination were inspected at five days interval. After 15 to 21 days the packet of the culture become white due to the completion of the mycelium running and then it was ready for inoculation on the substrates.

Substrate preparation and Processing for inoculation.

The substrates were prepared from Elephant grass, with the addition of cotton seed. Cotton seed were collected from Addis Abeba city markets and Elephant grass were collected from the Ambo University main campus. Stem of Elephant grass was chopped (2-3 cm) and dry these substrates were soaked in water over night to get wet and achieved 65-70 % of moisture content (Shown in Figure 3.1 below). The next day, all these wet substrates were separated from water and Excess water present in the substrates was drained thoroughly and mixed with required amount of calcium carbonate (1%) and filled in sterilizable yellow color polyethylene bags (Kurtu pestal).

The substrates were autoclaved at 15Psi pressure at 121$^{0}_{C}$ temperatures for 1h. After cooling the sterilization substrates were transferred to transparent polyethylene cultivation bags for easy supervision of the growth of the mycelia and presence of contamination. Each substrate (500 g) with 70% moisture was mixed with 10% spawn (dry weight/wet weight basis) and the inoculated polythene bags were then tightly tied with string made from polyester/cotton cloth. Pin holes were made through the bags (1/100 cm2) for drainage and aeration. Mycelium running rate on the substrates was observed after 7 days inoculation of spawn. It was kept in a spawn running room at room temperature in the dark until primordial were formed. After primordial formation, large holes were made in the polythene bag to allow normal development of fruiting bodies.

Bags were transferred to mushroom house under normal environmental conditions and relative humidity (the room maintained at 85-90%) by keeping water in open containers at different corners of the room. 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 fruiting bodies of mushrooms were manually harvested at maturity which was indicated by upward curving of the edges of the cap.

Elephant grass **stem of elephant grass**

Figure .1. Substrate preparations and processing

Experimental Design

Ten treatments (T1–T10) comprising different proportions Stem of Elephant grass and Cotton seed wastes, the combination of them are (500 g) along with 1% of lime stone (Calcium Carbonate) on dry weight basis were used. Experiment design is a completely randomized design with three replications per treatment, being each an experimental unit.

Table .1. Ratio of the Stem of elephant grass and cotton seed in the treatments

Treatment	(SE) Stem of Elephant grass(gram)	(CW) Cotton seed (gram)	Total	S: C Ratio (%)
T1	500	0	500	100:0 Control
T2	450	50	500	90:10
T3	400	100	500	80:20
T4	350	150	500	70:30
T5	300	200	500	60:40
T6	250	250	500	50:50
T7	200	300	500	40:60
T8	150	350	500	30:70
T9	100	400	500	20:80
T10 Control	0	500	500	0:100

Data collection

The performance and productivity of the mushroom were measured using the method outlined by Mkhize *et al*. (2016). The following parameters were measured in order to evaluate the performance and productivity of *P. ostreatus* mushroom. The number of days it took for the mycelia to fully colonise the substrate as also noted from the time it first inoculated the substrate up to a point where the mycelia full covered substrate. These included the biological efficiency, fresh mushroom yield, duration of mycelial growth rate, duration of primordial formation, duration of maturation of mushroom, time to fruiting bodies, Cup diameter, and stipe length of the mushroom were measured.

Biological Efficiency

The mushroom yield was calculated according to Morais, *et al*. (2000), using the equation: The main parameter used to evaluate mushroom yield is called biological efficiency (BE). It mainly depends on the characteristics of the material and the circumstances in which the growth process occurs. Biological efficiency was calculated and defined as the ratio of weight (g) of fresh mushrooms harvested to dry weight (g) of the substrate.

Biological Efficiency = Weight of fresh fruiting bodies (g) /Weight of substrate (g) × 100.

Nutrient analysis

Determination of Moisture

The moisture content is determined by measuring a material before & after the water removed by evaporation Moisture content was determined by following the formula

%Moisture =initial –dried/initial *100.

Where M initial and M dried are the mass of sample before and after drying respectively to obtain an accurate measurement of the moisture content of material evaporation method necessary to remove all water molecules.

Determination of total protein

To about 0.7 gram of sample in a digestion flask, 1 gram of Copper Sulphate, 10 gram of Potassium sulphate and 20 ml of Sulphuric acid was added. After complete digestion the content is transferred into a vessel. 25 ml of 0.2N Sulphuric acid was pipette out into beaker and distillation was started. The distillate was allowed to collect in Sulphuric acid for a known volume and time. The collected distillate is titrated against 0.2N Sodium Hydroxide using Methyl red as an indicator.

The percentage of Protein was calculated. Total nitrogen was estimated by following the standard Kjeldahl method (Chang, *et al*, 2003).

$$N\ (\%)\ \text{in the supplied sample} = \frac{(Va \times Na - Vb \times Nb) \times 1.401}{W}$$

Where, Va = mL HCl measured in the conical task in the distill (usually 20.00 mL)
Vb = mL NaOH used for titration of the content in the conical flask
Na = Normality of the HCl measured into the conical flask
Nb = Normality of the NaOH used for titration
W = g of mushroom powder used for the analysis

Crude protein content was obtained by multiplying the total nitrogen value by the conventional factor 6.25. (Chang *et al*, 2003). The percentage of protein in the sample was calculated by the following equation:
Crude Protein (%) = % N X 6.25

Determination of total lipid

Total lipid was determined by slight modified method of Folch, *et al.* (1957). Five gram of each sample was suspended in 50ml of chloroform: methanol (2:1) mixture then mixed thoroughly and let stand for 3 days. The solution was filtrated and centrifuged at 1000 rpm by a centrifuge machine. The upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by heating. The remaining was the crude lipid.

Determination of total ash

One gram of the sample was weighed accurately into a crucible. The crucible was placed and heated first over a low flame till all the material was completely charred, followed by heating in an oven for about 6 hours at 600^0C. It was then cooled and weighed. Then total ash was calculated as following equation (Raghuramalu *et al*., 2003).

Ash content (g/100g) = weight of ash *100/ weight of sample

Determination of Fibre Content

5 gm of mushroom sample was extracted using Petroleum ether. The fat free material was transferred in a beaker and 200 m1 of dilute sulphuric acid was added and boiled. Whole boiling acid in a flask is connected to reflux condenser and heated for 30 minutes. The flask

was removed and filtered and washed thoroughly with boiling water followed by washing in boiling Sodium Hydroxide and again refluxed for 30 minutes. The contents were filtered and washed with boiling water and finally washed the ethanol. The residues were dried and incinerated in muffle furnaces at 660 degree Celsius and the crucible along with ash was weighed and percentage of fiber was calculated.

$$\% \text{ of crude fiber} = \frac{100(\text{Wt of crucible with before ashing- Wt of crucible after ashing})}{\text{Weight of Sample}}$$

Determination of total carbohydrate

The content of the available carbohydrate were determined by the following equation (Raghuramalu *et al.,* 2003).
Carbohydrate (g/100g sample) = [100 – (Moisture + Fat + Protein + Ash + Crude Fiber)]

Data analysis

The data were analyzed by comparing the mean weights and percent biological efficiency through one way ANOVA. The data groups were analyzed using 21versions of Statistical Package for Social Sciences (SPSS) and the treatment mean will be compared using on (LSD).P≤ 0.05%.

RESULTS AND DISCUSSIONS

Culture of *Pleurotus ostreatus*

The Pleurotus ostreatus was cultured on malt extract agar and potatoes dextrose agar for 7 days at 28°C and mycelium covered the plate. It was fully grow on plates as shown in (figure below). PDA and MEA were the simplest and the most popular medium for growing mycelia of most cultivated mushrooms (Chang, 1999). *P. ostreatus* was successfully grown on PDA and MEA. The oyster completely covered the peteridishes after 7 days and its color and appearance looks like pure cotton. The mycelium should be white and grow out from the tissue. If yellow, blue, green or grey mycelia form on other places on the surface, then these are fungal contaminants (Oei and Nieuwenhuijzen, 2005). A creamy, shiny growth often indicates bacterial contamination (Oei and Nieuwenhuijzen, 2005). *P. ostreatus* is a slow grower when it is compared to molds and other fungi.

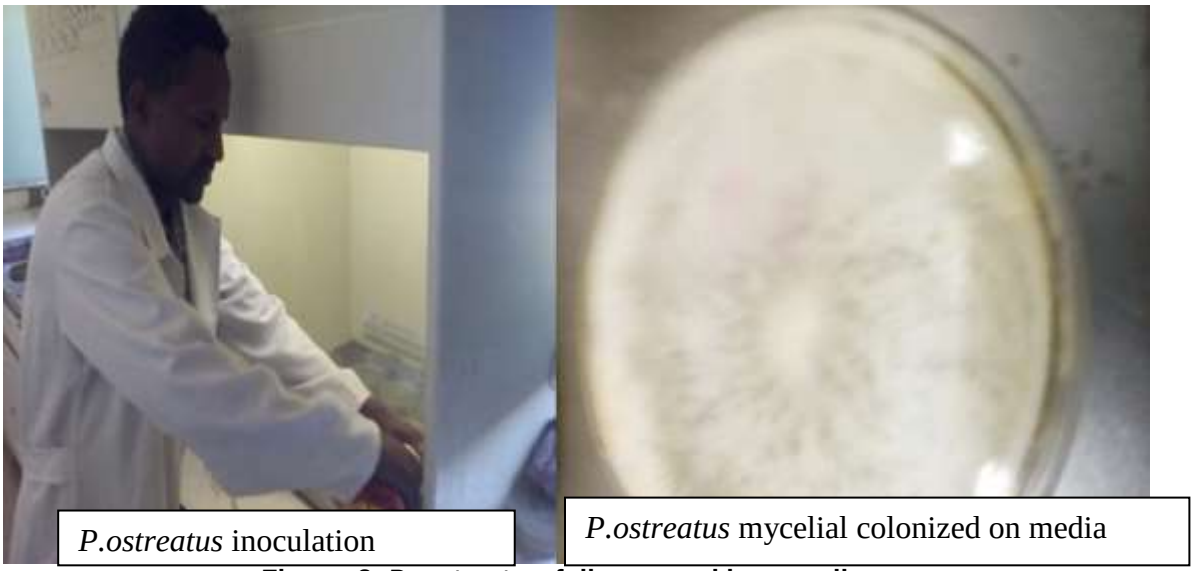

Figure .2. *P. ostreatus*, fully covered by mycelium

Spawn production

In this research or experiment, yellow Sorghum was inoculated by *P. ostreatus* for spawn production of oyster mushroom. Sorghum based spawn took 25 days to colonize the substrate completely (figure 3 below). The moisture content of the sterile moist sorghum (65-70%) was found to be suitable for growth of mycelium of oyster mushroom. The mycelium was completely colonizing sorghums and it was completely change the color to wheat. Those fully colonized by mycelium without any contamination of microorganisms and ready for inoculation of the substrates, Sara, (2007).

Figure. 3. *P. ostreatus* completely colonize the spawn

Day's taken mycelial extension on stem of elephant grass

The fastest grow on the stem of elephant grass, it takes 14^{th} to 15^{th} days on the T4 and T3 treatments and the lowest mycelial extension were recorded on T7 32 days .the other were required intermediate days to colonize mycelial extensions from days of inoculation stem of elephant grass. (Shown Table 2 below). There were significant (P≤0.05) differences in the days required for complete invasion mycelial on the substrates receiving for different treatments. The time required for complete colonization of the substrate by oyster mushroom was longer on stem of elephant grass supplemented with a cotton seed waste at ratios of (40:60). (Follow table 4 below).This result was also line to, the mean value of mycelia extension reported by Gume *et al*, (2013), Mekonnen and Semira, (2014). And Asefa and Geda, (2014 b). Gume *et al*., (2013) reported the highest mycelial running rate was observed in substrate composed from saw dust maize comb and coffee husk. In this study, there were slight differences on days required for complete colonization of the substrates that received different treatments.

Primordial formation on stem of elephant grass with addition of cottonseed wastes

Also the growth was observed on the different ratios of the substrate from stem of elephant grass and on different ratios of stem of elephant grasses supplemented with a cotton seed wastes. The first primordial appears after 14 days after inoculation depending upon types of substrate. The primordial formation and number of primordial per plastic bag (substrate) was affected by humidity, aeration and the substrate itself. Number of primordial was highly growth or appeared on the ratio of 70:30 (T4) of the substrates on stem of elephant grass. It indicates the growths was formed on the high ratios of treatments stem of elephant grass with addition of cotton seed wastes and on the other treatments at the intermediate duration of time number of primordial were produced. But when we compare leaf and stem of elephant grass with ratios of cotton seed wastes highly its growth on the stem of elephant grass than on the leaf of elephant grass. (Shown in figure 4 below).

Figure .4: Primordial formation on stem of elephant grass

Fruiting body development on stem of elephant grass with addition cotton seed wastes

The effect chopped stem of *Pennisetum purpureum* were evaluated on different treatment of the substrates supplemented with cotton seed wastes. The number of fruit body and size of fruit body were produced on the different treatments. The fruiting body of mushroom was highly growth on the all treatments of substrate on stem of elephant grass and mixed with the cotton seed wastes. The fruiting body formed on all treatments stem of elephant grass, the size and the numbers of fruiting body were different from substrates to substrates of the treatments. The large fruiting body were collected on the T5 (40:60), when the ratios of treatment of the substrates were more and on the others also T3 (80:20), on the high ratios of the substrates good quality and size large fruiting body were collected. Follow figure below.

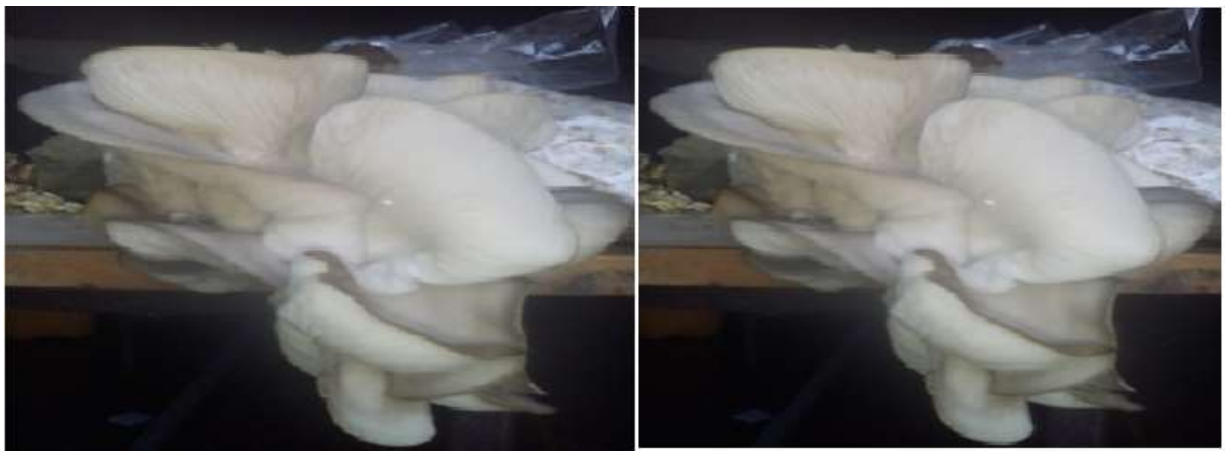

Figure .5: Fruiting body of Oyster Mushroom on stem of elephant grass

Duration of primordial formation on the stem of elephant grass mixed with the different ratio of cotton seed wastes

The number of primordial were first observed at the T4 (70:30) and T2 (90:10) and T3 (80:20) at the ratios of stem of the elephant grass were more and some of the slow primordial formation were T6 (50:50) and the other treatments were observed intermediate number days of primordial formation after mycelial colonization for all. There were significant (P≤0.05) differences in the primordial formation of oyster mushroom grown on stem of different Treatments. (It was shown in the table 2 below). These longer days of initiation of primordial formation after mycelia running may be due to slow releasing of nutrients from the both leaf and stem of the substrates as compared to other treatments, for example, wheat straw and rice straw on which much of research work has been done on this mushroom species. The observed result was near line to similar with Ashraf (2013). Ashraf *et al*., (2013) reported that all the treatments they tested showed 3.73 to 5.13 days for primordial initiation after mycelia running.

Duration for maturation mushroom and harvested on the stem of elephant grass with different ratios of cotton seed wastes

There were not significant (P≤0.05) differences in the maturation formation of oyster mushroom grown on stem different Treatments. On the second substrates stem of elephant grass more number of days taken on the T6 (50:50) 49th days taken to harvest and T7 (60:40) 47.5th days were recorded and the first one harvested were on the T4 or the shortest number days observed and others were required intermediate number of days to harvested the fruiting body of mushroom. But when we compare both of the treatments the second treatments stem of elephant grass with addition of cottonseed wastes take some number of days difference to harvested mushroom than the first treatments on leaf of elephant grass with addition of cotton seed wastes to harvested mushroom. (Shown on table 2 below).The period of primordial to maturation of mushrooms in this study, the shortest mean duration was 5 days and the longest was 10 day throughout the treatment substrates to the treatment of substrates. (Follow the table 4.1 and 4.2 bellow). This near agrees with the range of maturation period (3.29 to 4.33) of *Pleurotus species* reported by Islam *et al*., (2009).

Table. 2. Days for the emergence of the various growth parameters of *P. ostreatus* on Elephant grass stem substratum

Treatment	Mycelial extension	Primordial formation	Maturation of mushroom frist harvested
T1	18	21	28
T2	16	18	25.5
T3	15	18	24
T4	14	16	20.66
T5	18	22	31
T6	30	46	49
T7	32	37	47.5
T8	30	34	39
T9	19	24	33.33
T10	10	16	25.7
MEAN	19.89	24.9	28.67
STDEV	7.5	9.89	13.5

Number of bunches, Aborted and Fruits on the stem of elephant grass with ratios of cottonseed wastes

There were not significant (P≤0.05) differences on the Fruiting body of oyster mushroom grown on stem elephant grass with addition of cottonseed wastes. On the stem of elephant grass with the addition of cotton seed wastes the average number of fruiting body formed was averagely 39.4.The highest number of fruits were observed on the stem of elephant grass on the treatment T5 (60:40) counted 62, and the least number of fruits was observed on stem of elephant grass on the treatment one of substrate T1 (100:00), 27 were counted and High number of aborted were recorded on the T10 and least number of aborted were observed on T2 and T6. (Shown on figure 7 below). The results of the study were found less than the number of fruiting bodies with the previous findings of Bhuyan, (2008), and Sarker, (2004). According to those authors the highest average number of fruiting body/packet was observed in the treatment T3 (122.3) and the lowest average number of fruiting body /packet was in the treatment T2 (76.0).

Pilus Diameter and Stipe length on the stem of elephant grass supplemented with ratio of cotton seed wastes

There were not significant (P≤0.05) differences in the cup diameter and Stipe length of oyster mushroom grown on stem of different Treatments. The number of cup diameter observed from the different ratios of the substrates was different from treatment to treatments of substrates and the averagely mean were 8.2393 respectively on Stem of elephant grass supplanted with a cotton seed wastes**.** On this treatments on the stem of elephant grass with the ratios of cotton seed wastes the largest cup diameter were observed on the T2 (11.5cm), T10 (11.8cm) on control of the experiment, and the least cup diameter was measured on the T1 (7cm). (Shown on figure 6 below) .the others were measured intermediate to each other. This is much greater than the pilus diameter reported by (Gume *et al*., 2013). According to these authors, mean pileus diameter of mushrooms ranged from 3.8 to 5.2cm.

The highest Stipe length were recorded on the T4 (4cm) and the least one were recorded on the treatment T2 (2.5cm) on stem of elephant grass with a

ratios of cotton seed wastes. (Shown on figure 6 below). The result of this study were similar line with the study of 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. The result observed in this study similar to the result reported in literature by from (Mdconline, 2013). On average, the cup diameter ranges between 2-15 cm and stalk length is around 4 cm (Mdconline, 2013). When the number cup diameter was measured largest in number the length of the Stipe were less measured in number. The Stipe length of the samples collected from different treatments show significant variation.

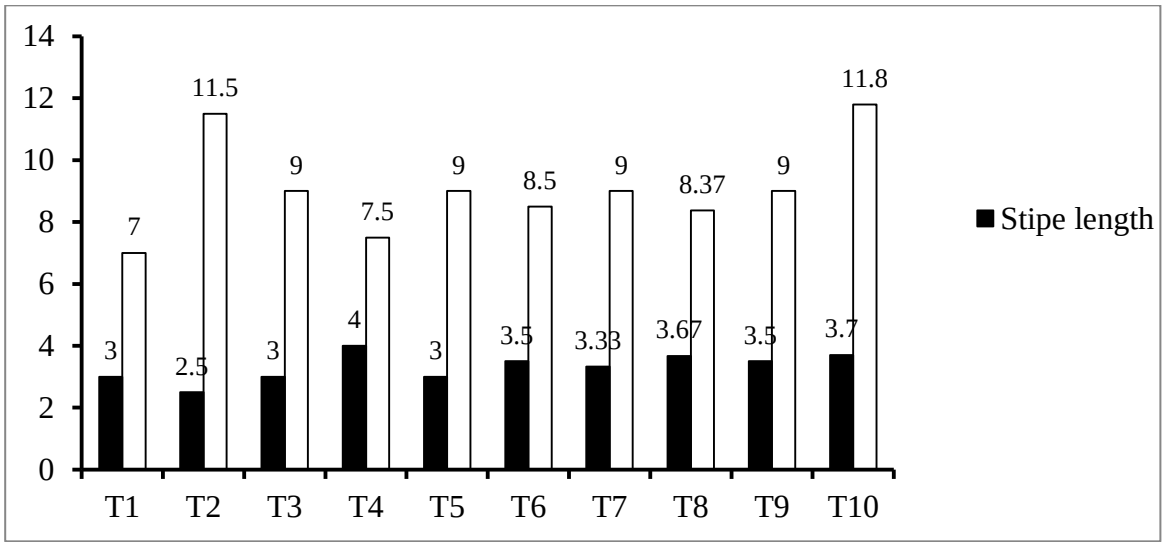

Figure. 6. Cup diameter and Stipe length of Oyster mushroom on different proportion of stem of elephant grass with cotton seed wastes

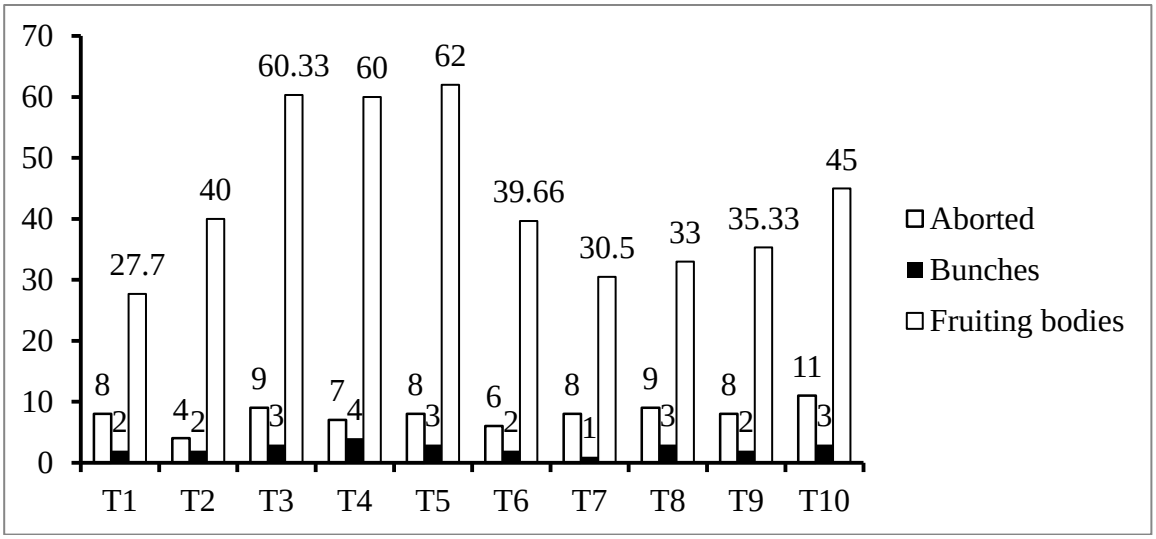

Figure. 7. Number of bunches, Fruits and Aborted of Oyster mushroom on different proportion of stem of elephant grass with cotton seed waste

Yield of mushroom per flush on stem of the substrates

The gram weight of flush on the stem of elephant grass with a ratios of cotton seed waste the highest one were observed on the T4(1254) and T1(580) was show least in gram when we compare with in a treatments. Also high yield were observed on the control T10 (1228). (Shown on table 3 below). The comparation between substrates stem of *Pennisetum purpureum* the yield observed in gram were almost similar to each other. In all treatments from the cycle one to 2nd to 3rd and to 4th the yield were reduced and the cycle were completed within 70 days. Kimenju *et al*. (2009) reported that yields of mushroom in different substrates slightly declined from the first flush to the successive harvests. Our

observation on the different harvest is in line with reports in the literature. Ashraf *et al.,* (2013) reported that the different treatments vary in the amount of mushroom yield harvest at different flushes and at each successive harvest, the amount of the yield declined.

Table. 3. Yield Fresh mushroom on different treatment stem of elephant grass

Treatments	1st Flush	2nd Flush	3rd Flush	4th Flush	Total
T1	205	196	99	80	580
T2	370	300	190	108	968
T3	450	330	270	180	1230
T4	500	380	204	170	1254
T5	420	260	210	121	1011
T6	370	245	199	175	989
T7	350	224	165	99	838
T8	340	255	203	185	983
T9	336	197	115	78	726
T10	530	308	210	180	1228

Biological efficiency of *Pleurotus ostreatus* grown on stem of different treatments

The effect of different treatments on biological efficiency of oyster mushroom showed significant (P≤0.05) differences on stem of elephant grass with addition of cotton seed wastes. The BE were recorded on this substrate treatments of stem of elephant grass supplemented with a cotton seed wastes the highest biological efficiency were recorded on T4 (250.8%) at the ratios of 70:30 and least one were recorded on T1 (116%) at the ratios of 100:00 stem of elephant grass. Intermediate numbers of biological efficiency were recorded on the others and the highest one were also recorded on control T10 (245.6%) at the ratios of 100% cotton seed wastes. (Figure below 8).biological efficiency obtained in this study were compared to the sawdust reported by Shah *et al*., (2004)reported that B.E. remained between 21.05-64.69% when it was cultivated on different substrates it was not related the finding of this study. Nunez and Mendoza (2002) it's reported the biological efficiency values were varying from 50.8 to 106.2 % in *Pleurotus ostreatus* on different substrates were less than the present studies. The observed results were not related to the finding of Patra and Pani, (1995), who reported the biological efficiency (50-75%) of *Pleurotus species* grown on most of agro industrial residues, namely; corncobs, various grasses and reed stems, vine shoots, cottonseed hulls and sugarcane baggase. In this finding, biological efficiency was indicated for comparison between treatments of the substrate in which, the most effective substrate in bioconversion to fresh fruiting bodies for cultivation of *Pleurotus species* was no more different number observed. The highest number of biological efficiency recorded on leaf of elephant grass followed by stem of elephant grass with addition of different ratios of cotton seed wastes. Both substrates have more cellulose were to supported the fast Mycelial growth during cultivation of *Pleurotus species.*

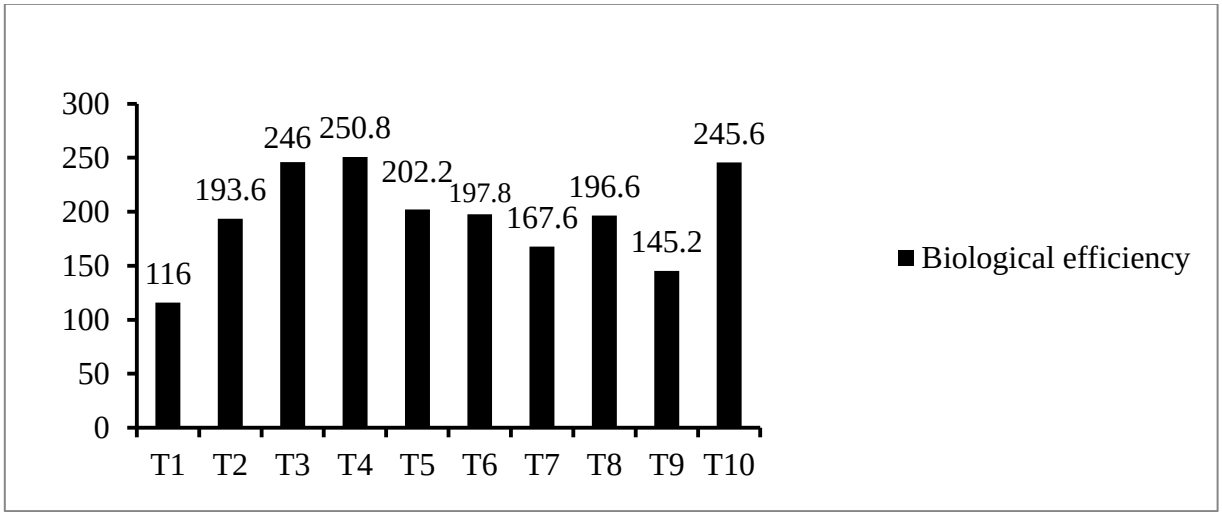

Figure .8.Biological efficiency on stemof elephant grass supplented with cottonseed wastes

Nutritional analysis of *P. ostreatus* mushroom

Moisture content on stem of elephant grass

Also the moisture contents of the second treatments on the stem of elephant grass with the addition of cotton seed wastes no more difference from the first treatments on the leaf of elephant grasses. The moisture contents of stem of elephant grass were between 87%-92%. The highest number of moisture was observed on the treatment T6 and least in number of moisture was observed on the T3, at the ratios of 80:20. The result was almost near similar line to Alam; *et al.,* (2007) reported 87–87.5% moisture for existing *Pleurotus spp.* in Bangladesh and according to Moni*, et al,* (2004). Its similar result was also observed .The moisture percentage of *Oyster Mushrooms* grown on different substrates observed in this study are supported by earlier studies by Moni *et al.,* (88.15-91.64%) and Alam *et al* (87- 87.5%). Generally, fresh *Pleurotus* mushroom contain 85-95% moisture (Khan, 2010). The moisture content all different composition of the substrates were followed in the table below (Table.4 below.).The interaction between the types of growth substrate or substrate combinations and their types had significant (p≤0.05) difference on moisture contents of mushrooms on stem of elephant grass supplemented with a cotton seed wastes

Crude protein (CP) on stem of elephant grass

Pleurotus ostreatus grown on the stem of elephant grass on different treatments were significant (p≤0.05) different from one another. The content of protein observed on the stem of elephant grass with addition of different ratios of cotton seed wastes were tested, the highest protein contents were observed on the treatment T7, (36.17%) when the ratios of 40:60 that means at the highest ratios of cotton seed wastes and the least one were observed at the treatment T9,(16.87%). the contents of the others treatment were inter mediate numbers on the stem of elephant grass and it's also recorded on control of the treatments T10 ,(23.35%) (Shown in Table 4. below).The result of the present study similar line with the studies of Chang *et al.*, (1981) who reported that the fruit bodies of Oyster Mushrooms contained 26.6-34.1% protein. The crude protein results of this study is close to the finding of Hassan and Medany, (2014), who reported 26.83% of crude protein for *P. ostreatus.* The results are all most similar to Breene, (1990) who reported values of crude protein content ranging from 19-39%. Protein content of mushrooms depends on the composition of the substratum, size of pileus, harvest time and species of mushrooms (Bano and Rajarathnam, 1982). Protein content of the mushrooms has also been reported to vary from flush to flush (Crisan and Sands, 1978). Haddad and Hayes, (1978) indicated that protein in *A. bisporus* mycelium ranged from 32 to 42% on the dry weight basis. Ogundele *et al*., (2017) observed that the protein content varied when culture on different substrate which related to the nutrient composition of the substrates used.

Crude Carbohydrate (CC) on stem of elephant grass

The content of carbohydrate observed on the stem of elephant grass with addition of different ratios of cotton seed wastes were tested, the highest protein contents were observed on the treatment T7, (57.78%) when the ratios of 40:60 that means at the highest ratios of cotton seed wastes and the one least contents carbohydrates were observed at the treatment T9,(42.9%). the carbohydrate contents of the others were followed on the stem of elephant grass .the inter mediate numbers were observed on the others and T10 on the control of the experiment,(45.18%) of carbohydrate were observed. (Shown in Table 4. below). There were significant (P≤0.05) differences on the carbohydrate contents of oyster mushroom grown on stem of different Treatments. The carbohydrate content is in agreement with the report that Carbohydrates constitute the prevailing component of mushroom dry matter; usually about 50-60% (Deepalakshmi and Mirunalini, 2014).The carbohydrate contents of both of them were no more different from each other. The results observed were near line similar to the result reported by (Deepalakshmi and Mirunalini, 2014).Also the results of this study were match to the study of Alam *et al.*, (2007) who found 39.82-42.83% of carbohydrates in *Pleurotus spp*.

Crude Fat (CF) on stem of elephant grass

The content of fat observed on the stem of elephant grass with addition of different ratios of cotton seed wastes were also tested, the highest fat contents were observed on the treatment T9, (4.31%) when the ratios of 80:20 that means at the highest ratios of cotton seed wastes and the 2nd were observed at the treatment T3 (4.26%) on the ratios of 80:20 and least one were recorded on T7, (2.75%) and T10 (3.84%) on control experiment. (Shown in Table 4. below). The fat contents of both of them were no more different from each other. The result of this study was similar result With Alam *e t al.*, (2007), its reported *Pleurotus* mushroom ranging between (4.30-4.41). The result observed were similar line to (Khan, 2010). Its reported *Pleurotus* mushroom contain 0.5-5% of fats. The results obtained in this study were close to that obtained by (Reguła and Siwulski, 2007), who reported 2.66% crude fat for dried oyster mushroom. There were not significant (P≤0.05) differences on the fat contents of oyster mushroom grown on stem different Treatments.

Crude fiber (CF) on stem of elephant grass

The content of Crude fiber observed on the stem of elephant grass with addition of different ratios of cotton seed wastes were tested, the highest Crude fiber contents were observed on the treatment T6, (20.67%) when the ratios of 50:50% that means on the equal ratios of the substrates. and the least one were observed on the T8, (17.5%).On the control experiment T10, (18.31%) were recorded. (Shown in Table 4. below).There were significant (P≤0.05) differences on the fiber contents of oyster mushroom grown on stem different Treatments. The crude fiber contents of both of them were no more different from each other. According to Teklit (2015) has compared the nutritional composition of cultivated mushrooms in Ethiopia and found that the crude fiber content varies from 18.23-29.02% .it also similar result observed in these studies. The results observed were near the result gained by (Kalac. *et al.,)* having reported that about 4-9% and 22-30% for soluble and insoluble fiber, respectively (Kalac. *et al*., 2009).

Crude Ash (CA) content on stem of elephant grass

The content of ash observed on the stem of elephant grass with addition of different ratios of cotton seed wastes were tested, the highest ash contents were observed on the treatment T5, (15.92%) when the ratios of 60:40 that means at the highest ratios of stem of elephant grass and the least one were observed on T6, (11.73%) and T10 (8.41%) on the control experiments. (Shown in Table 4 below).There were significant (P≤0.05) differences on the Ash contents of oyster mushroom grown on stem different Treatments. The findings of the study were supported by the study of Khlood-Ananbeh, *et al.*, (2005). Who reported ash contents were moderate in the fruiting bodies. Alam *et al.*, (2007).Reported 8.28 - 9.02% of ash in *Pleurotus spp* its less than the present study. In *Pleurotus Florida*, Teklit, (2015) observed 9.41% ash content which less than the present study on those grown on elephant grass substratum. The ash contents of both of them were different from each other the present observed on stem of elephant grass with addition of cotton seed waste was higher than the leaf of elephant grasses.

Table. 4.The effect of additives or proportion of stem substrates on mushroom production

T/t.2.	Moisture (db)	Crude Protein (db)	Crude fat (db)	Crude Fiber (db)	Crude Ash(db)	Carbohydrates (db)
T1	89.25	26.78	4.19	19.533	14	53.753
T2	88.41	25.1	3.35	18.88	12.25	47.99
T3	87	21.65	4.26	17.53	13.27	43.71
T4	89.833	18.66	3.34	19.83	12.41	44.07
T5	89.66	24.08	3.84	17.65	15.92	51.15
T6	90.27	21.01	3.31	20.65	11.73	46.97
T7	88	36.17	2.73	18.68	12.2	57.78
T8	90.5	25.05	2.79	17.5	12.32	48.16
T9	90.5	16.87	4.31	18.31	12.91	42.9
T10	92	23.28	3.19	18.31	8.41	45.18

There were significant (P≤0.05) differences on the nutrient contents of oyster mushroom grown on stem of different Treatments

CONCLUSIONS

Cultivation of edible mushroom has been considered as an additional practice of food production and contributes in the struggle for food security and solving the problem of malnutrition in developing countries. The use of *Pennisetum purpureum* stem as a major substrate with the supplementation of cotton seed waste has not yet been tested in the production of oyster mushroom in Ethiopia. The present study reveals the usability of *Pennisetum purpureum stem* and cotton seed wastes with respect to gave the highest yield, yield para meter, biological efficiency and nutrient of the oyster mushroom. Based on the results of the study the following conclusion was made: The highest total yield was collected on T4 (1254g/500g) of dry substrates on the stem of elephant grass and Highest biological efficiency was recorded on T4 (250.5) stem of elephant grass and on all the other treatments intermediate results were recorded. The highest stipe length (4.5cm) on T6. And highest number of fruits (62) was recorded on T5 While the largest cup diameter (11.5cm) on T2 and the highest stipe length (3.67cm) on T9 of the stem of elephant grasses the major substratum. The highest protein contents were recorded on T7 (60:40) 36.17% and the protein contents were recorded on T9 (80:20) 16.87%.

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