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Growth and Yield of Oyster Mushroom (Pleurotus ostreatus) on Substrate Composed of Maize (Zea mays L.) Stem and Cotton (Gossypium spp) Seed Waste

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Abstract
Oyster mushroom (*Pleurotus* ostreatus) is known to utilize a wide range of agricultural by -products and organic wastes from agro-processing industries. This study was carried out in order to evaluate the usability of maize stem along with different proportion of cotton seed waste for the growth, yield and yield related parameters of oyster mushroom. From all the different treatments tested, those composed the maize stem: cotton seed waste in the ratio of (60:40 and30:70) showed fastest mycelial run, 3.6 and 7.2 cm respectively, on 7th and 14th days of incubation. Maize stem: cotton seed waste (60:40 and 80:20) took shortest time from incubation to 1st flush 30 days; while maize stem: cotton seed waste (90:10 and 80:20) showed shortest pinning to maturation throughout the flushes. Maize stem: cotton seed waste (30:70) observed to have higher number of aborts 105, higher number of fruiting bodies 125 and maize stem: cotton seed waste (30:70) showed larger pilus diameter 9.2cm, higher fresh weight of matures 795g and highest biological efficiency 159%. Although the total yields of the mushroom biomass, as well as the biological efficiency were greatly affected by the different substrate compositions, all combination of the substrate gave more than 100% biological efficiency which makes maize stem together with cotton seed waste a good option for mass production of oyster mushroom which can produce good quality mushroom fruit bodies.
Keywords: Cotton seed waste, growth, maize stem, oyster mushroom, yield

Introduction

Mushroom is fruiting bodies of fungus growing on damp rotten log of wood, decaying organic matter and soil rich in organic substances. Edible mushrooms are highly nutritious and can be compared with eggs, milk and meat (Caglarimak, 2007; Stamets, 2005). Edible mushrooms on dry basis contain about 19-40% protein; that is its protein content is twice that of vegetable and four times that of oranges, and they are rich with vitamins, minerals, less percent of unsaturated fatty acid and carbohydrate which makes it so ideal for diabetic and the obesity patient (Ogundana and Fagede, 1982). Most mushrooms have exceptional medicinal potentials and properties; curative and prophylactic especially in diseases such as high blood pressure, asthma, respiratory tract infections, anemia, hepatitis, cancer, tumor, etc (Wasser, 2002, 2008). Mushrooms are also important for cholesterol reduction, immune enhancement, cancer, anti allergic activities, antimicrobial and cardiovascular treatment. They also have a long history of use as traditional medicine in China. Their legendary effects on promoting good health and increasing adaptive abilities have been also been demonstrated (Wasser, 2002). Mushroom cultivation also serves as the most efficient and economically-viable biotechnology for the conversion of ligno-cellulosic waste materials into high-quality protein food for revenue generation (Ortega et al, 1992;Berch et al., 2007).However, the cultivation of mushroom is still at its infancy in some parts of Africa, especially in Ethiopia. The major problem associated with the transfer of technology for mushroom cultivation is the lack of technical know-how for its cultivation (Dawit, 1998).

In Ethiopia more than 80% of the population is engaged in agricultural activities and huge amount of residues are produced as by products during the harvest season. Maize production has been wide spread in low high land areas through cultivation of improved varieties. Four different by products are produced from maize production including: stems, leaves, maize stover and comb. Each one of the residues has been observed to be greater than the produces. The agricultural residues are generally burnt in the fields leading to emission of green house gases and the environmental degradation. The best way of minimizing these wastes is recycling through utilizing these products as substratum for growing mushroom. The mushrooms can use the plant derivatives as source of carbon and other nutrients and convert the waste biomass in to value added mushroom fruit bodies besides, contributing to the environmental sustainability. Mushrooms of the genus *Pleurotus* belonging the family *Tricholomataceae* are commonly known as oyster mushrooms which occupy the second position among cultivated edible mushrooms worldwide due to their nutritional and medicinal values (Khan et al., 2008). The present paper was designed following the above objective in order to understand the effect of different proportion of maize stem together with cotton seed waste on growth, yield and yield related parameters of the oyster mushroom *Pleurotus ostreatus*.

Materials and Methods

The Oyster mushroom strain, *Pleurotus ostreatus* was obtained from the Mycology Laboratory, Department of Biology, Addis Ababa University, Ethiopia. The pure spawn culture of *Pleurotus ostreatus* was transferred onto Potato Dextrose Agar (PDA) and propagated in the laboratory at 25°C. The growth of the culture and presence of contamination were visually inspected at three day intervals.

Grain Spawn production

In this study, the spawn (mushroom seed) of *Pleurotus ostreatus* was produced on yellow colored sorghum, wheat bran and calcium sulfate (gypsum) in the ratio of 88:10:2 respectively (Dawit, 1998). The grains were soaked overnight, washed and drained to remove the dead and floating seeds. After removing the excess water from the grain, the required amount of wheat bran and gypsum ($CaSO_4$ $2H_20$) were added and transferred to 1000 ml glass bottles (75%level) leaving a head space over the grain and autoclaved at 121^0C temperature for 45 minutes. After cooling, each bottle was inoculated with 20 agar blocks (1 cm x 1 cm) of a 15day old mushroom culture from the Petri dish and incubated for 21 days at 28±2°C until the substrate were fully colonized and the mycelia invasion and contamination were inspected at five day intervals.

Mushroom substrates

Maize stems were processed as described below to use as substrate in the oyster mushroom cultivation.

Treatments

Nine treatments comprising different proportions of the substrates (maize stems and cotton seed (500g) along with wheat bran (50g) and lime stone (Calcium Carbonate 5g) on dry weight basis were used as shown in Table 1.

Table1: Composition of the different treatments

Proportion of Maize stem and cotton seed waste	Maize stem (g)	Cotton seed waste (g)	Total (g)
90:10	450	50	500
80:20	400	100	500
70:30	350	150	500
60:40	300	200	500
50:50	250	250	500
10:90	50	450	500
20:80	100	400	500
30:70	150	350	500
40:60	200	300	500

Preparation of the substrate and sterilization

For each treatment the required amount of cotton seed waste was weighed and soaked in water overnight. The maize stems were shredded into small pieces approximately (3-5 cm), and the required amount was weighed and soaked in water over night. On the next day, excess water present in the substrates was drained thoroughly and mixed with 10 % wheat bran and one percent calcium carbonate and filled in sterilizable yellow colored polyethylene bags (Kurtu pestal). The substrates were autoclaved at 15Psi pressure at 121°C temperatures for 1h. After sterilization the 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 content 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. The inoculated bags were kept in a spawn running room at room temperature in the dark until primordial were formed. After primordial formation the bags were transferred in mushroom production house and large holes were made in the polythene bag to allow normal development of fruiting bodies. In the mushroom house the bags were kept under normal environmental conditions and relative humidity of the room was 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 increase in carbon dioxide 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 up ward curving of the edges of the cap.

Biological efficiency was calculated and defined as the ratio of weight (g) of fresh mushrooms harvested to dry weight (g) of the substrate.

$$\text{Biological Efficiency} = \frac{\text{Weight of fresh fruiting bodies (g)}}{\text{Weight of dry substrate (g)}} \times 100$$

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 16.0.Treatments means were compared using LSD.

Results

Vegetative growth of the mushroom on substrates (Mycelia extension)

There were significant (P≤0.05) differences in the mycelial extension of oyster mushroom grown on different proportion of substrates. Maize stem: cotton seed waste (60:40 and 20:80) showed the fastest mycelial extension followed by (70:30 and 10:90) while, (90:10, 50: 50 and 30:70) exhibited slowest mycelial extension on 7th and 14th days of incubation periods (Table 2). The mean values of mycelia extension cm/day was found to be proportional to the mycelia extension. There were significant (P<0.05) differences in the time(day) required for complete colonization of the mushroom on the different treatments(substrates). The time required for complete colonization of the substrate were significantly (P≤0.05) less (18days) for all the treatments except 90:10 and 80:20 combinations (Table 2) of maize stem and cotton seed waste.

Table 2: Mycelial extension on the substrates on 7th and 14th days of incubation

Maize stem: cotton seed waste	Mycelia extension (cm)		Mean value (cm/day)	Total days required for complete colonization
	7th day	14th day		
90:10	**3.20^{c}**	**6.40^{d}**	**0.40^{b}**	**22^{a}**
80:20	**3.40^{b}**	**6.80^{c}**	**0.43^{ab}**	**21^{ab}**
70:30	**3.50^{ab}**	**7.00^{b}**	**0.44^{a}**	**18^{c}**
60:40	**3.60^{a}**	**7.20^{a}**	**0.45^{a}**	**18^{c}**
50:50	**3.20^{c}**	**6.40^{d}**	**0.40^{b}**	**18^{c}**
10:90	**3.50^{ab}**	**7.00^{b}**	**0.44^{a}**	**18^{c}**
20:80	**3.60^{a}**	**7.20^{a}**	**0.45^{a}**	**18^{c}**
30:70	**3.20^{c}**	**6.40^{d}**	**0.40^{b}**	**18^{c}**
40:60	**3.40^{b}**	**6.80^{c}**	**0.43^{ab}**	**18^{c}**

Mean values within a column sharing the same superscript letter(s) are not significantly different by using LSD test at (P≤0.05)

Growth rate of mushroom (Flushes)

Mean incubation periods of mushroom flushes showed highly significant differences (P≤0.05). Maize stem: cotton seed waste (60:40 and 30:70) showed relatively shorter incubation periods, followed by (50:50); while (90:10 and 80:20) took longer incubation to 1st flush. All the rest of the treatments took incubation to 1st flush between the shortest and longest . The incubation period taken from the 1st flush to the 2nd flushes also shorter for (60:40 and 30:70) while it took longer incubation from 1st to 2nd flushes for (90:10 followed by (80:20 and 40:60). Similar trends were observed for 2nd to 3rd and from 3rd to 4th flushes (Table 3).

Table.3.The incubation period(days) [1]for the various treatments at different flushes

Maize stem: cotton seed waste	Treatments	Inoculation–1st flush	1st-2nd flush	2nd--3rd flush	3rd--4th flush
90:10	**T1**	**45^{a}**	**18^{a}**	**16^{a}**	**15^{a}**
80:20	**T2**	**42^{b}**	**17^{ab}**	**15^{ab}**	**14^{ab}**
70:30	**T3**	**35^{d}**	**16^{bc}**	**15^{ab}**	**14^{ab}**
60:40	**T4**	**30^{f}**	**15^{c}**	**14^{b}**	**13^{b}**
50:50	**T5**	**32^{e}**	**16^{bc}**	**15^{ab}**	**14^{ab}**
10:90	**T6**	**37^{c}**	**16^{bc}**	**14^{b}**	**13^{b}**
20:80	**T7**	**36^{cd}**	**16^{bc}**	**15^{ab}**	**14^{ab}**
30:70	**T8**	**30^{f}**	**15^{c}**	**14^{b}**	**13^{b}**
40:60	**T9**	**32^{e}**	**17^{ab}**	**15^{ab}**	**14^{ab}**

Mean values with in a column sharing the same superscript letter(s) are not significantly different by using LSD test at P≤0.05

Pinning to maturation duration of oyster mushroom

The data (mean)on the duration from pinning to maturation of the mushroom under each treatment showed

[1] Check and insert

significant variation (P≤0.05). The treatments (90:10 and 80:20) showed shortest mean periods from pinning to maturation in all the flushes when compared to other treatments. While the treatments 50:50, 10:90 and 30:70 took longer mean periods (Table 4).

Table 4: The duration [1](day)between Pinning to maturation under different treatments regimes

Maize stem: cotton seed waste	1st flush	2nd flush	3rd flush	4th flush
90:10	10^{b}	9^{b}	8^{b}	7^{b}
80:20	10^{b}	9^{b}	8^{b}	7^{b}
70:30	11^{ab}	10^{ab}	9^{ab}	8^{ab}
60:40	11^{ab}	10^{ab}	9^{ab}	8^{ab}
50:50	12^{a}	11^{a}	10^{a}	9^{a}
10:90	12^{a}	11^{a}	10^{a}	9^{a}
20:80	11^{ab}	10^{ab}	9^{ab}	8^{ab}
30:70	12^{a}	11^{a}	10^{a}	9^{a}
40:60	11^{ab}	10^{ab}	9^{ab}	8^{ab}

Mean values within a column sharing the same superscript letter(s) are not significantly different by using LSD test at P≤0.05

Yield of mushroom per flushes

Yield of mushroom per flush (wet/fresh weight) showed significant variation between treatments (P≤0.05)(Table 5) as well as between flushes. The treatment 30:70 (Maize stem: cotton seed waste) showed the highest fresh weight in grams in first flush followed by (10:90) , while the treatments 90:10 and 80:20 produced the least amount. In the second flush the highest yield was obtained from (70:30) followed by (10:90 and 30:70) combination. In the third flush the substrate formed of maize stem: cotton seed waste 50:50 treatment gave the highest yield, followed by 80:20,20:80 and10:90 combinations. Relatively lowest yield of mushroom was obtained in the 4th flush (Table 5) in all the treatments.

Table5: Mean yield per flush and total wet/fresh weight(gm) of the mushroom

Maize stem: cotton seed waste	1st flush	2nd flush	3rd flush	4th flush	Total
90:10	250^{i}	130^{d}	100^{c}	70^{a}	550^{i}
80:20	280^{h}	150^{c}	110^{b}	90^{a}	630^{g}
70:30	320^{f}	250^{a}	100^{c}	100^{a}	770^{d}
60:40	340^{e}	150^{c}	100^{c}	100^{a}	690^{f}
50:50	375^{c}	150^{c}	150^{a}	100^{a}	775^{c}
10:90	415^{b}	160^{b}	110^{b}	100^{a}	785^{b}
20:80	300^{g}	100^{e}	110^{b}	110^{a}	620^{h}
30:70	445^{a}	160^{b}	100^{c}	100^{a}	795^{a}
40:60	355^{d}	150^{c}	100^{c}	100^{a}	705^{e}

Yield parameters of oyster mushroom

Different yield parameters considered in the experiment showed significant (P≤0.05) variation among the treatments. A higher number of bunches were recorded on treatment having maize stem: cotton seed waste in the ratio of 90:10 and 20:80(Fig1) while the lowest were recorded on (70:30). The highest number of fruiting bodies were collected from 80:20substrate combination followed by 90:10 and 60:40treatment. Maize stem: cotton seed waste 50:50 and 40:60 produced the least number of fruiting bodies. A higher number of aborts were recorded with 60:40 and 30:70maize and cotton seed waste treatments and the 80:20combination showed least number. Pilus diameter was found to be maximum for the samples collected from 30:70combination and minimum from 20:90maize and cotton seed waste substratum (Fig2). The stipe length of the samples did not show significant variation among different treatments and ranged only 2.0-3.5cm. The highest total wet/fresh weight of mature mushroom was recorded in maize stem: cotton seed waste combination 30:70 (795g) followed by 10:90 (785g). The least total fresh/wet weight (550g) was recorded in 90:10 treatment (Table 5).

[1] Is it days

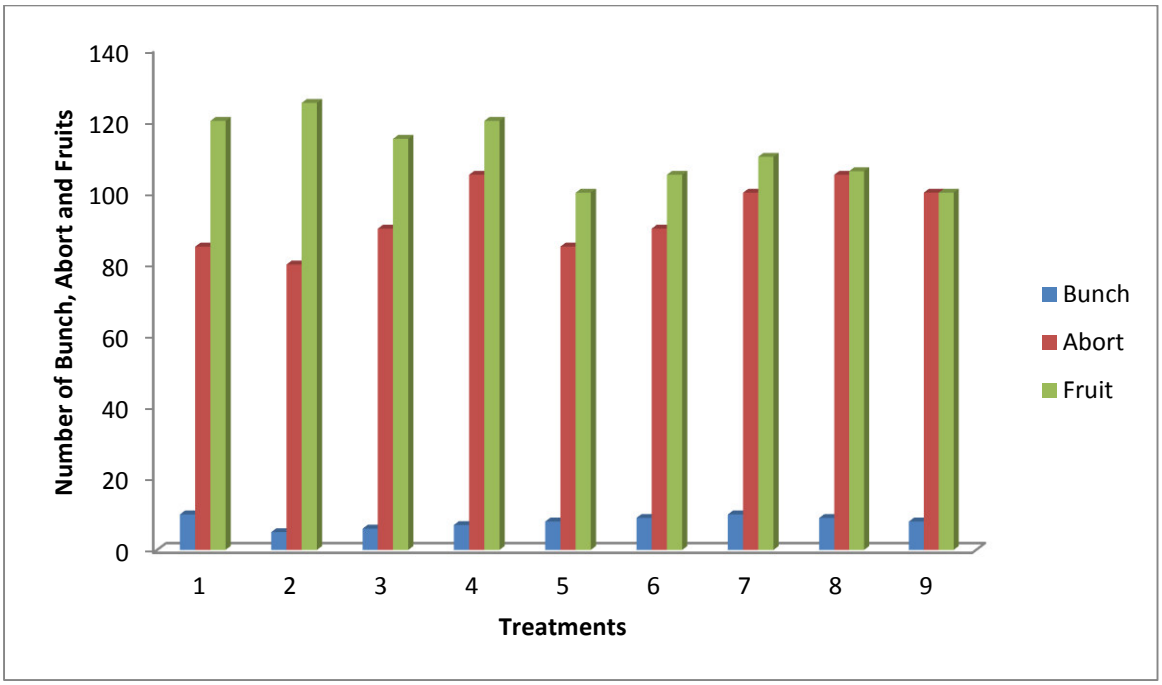

Fig 1: Number of bunchs , aborts and fruits of oyster mushroom grown on the substrate composed from different proportion of maize stem: cotton seed waste: 1(90:10), 2 (80:20), 3(70:30), 4(60:40), 5(50:50), 6(10:90) , 7(20:80), 8(30:70), 9(40:60)

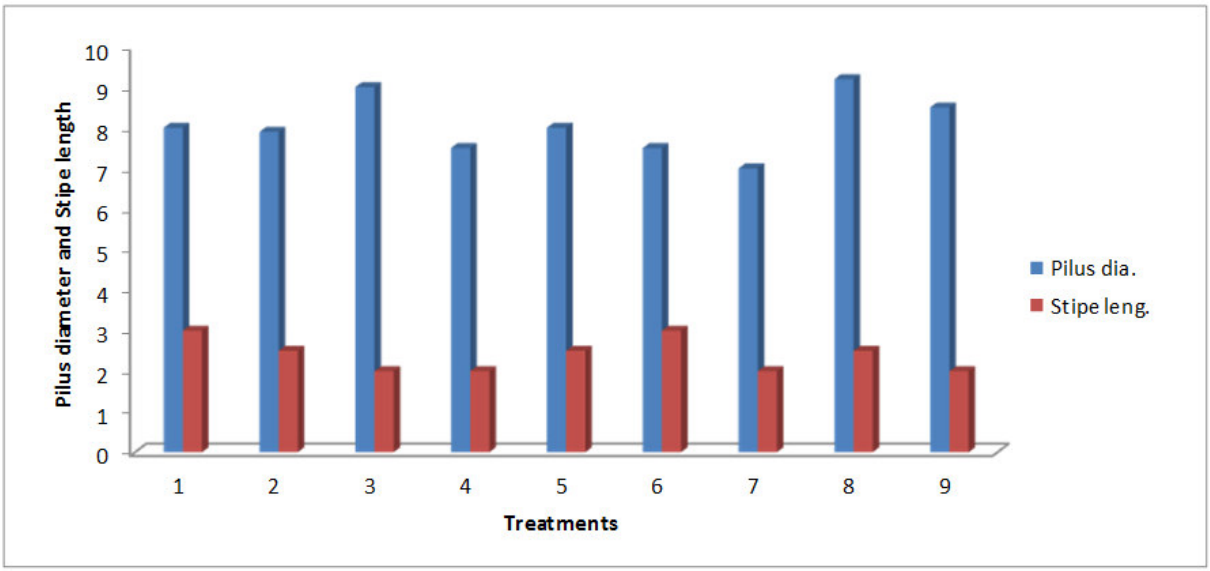

Fig2: Pilus diameter and stipe length of the oyster mushroom grown on substrate composed from different proportion of maize stem: cotton seed waste : 1(90:10), 2 (80:20), 3(70:30), 4(60:40), 5(50:50), 6(10:90) , 7(20:80), 8(30:70), 9(40:60)

Biological efficiency of oyster mushroom grown on different ratios of substrates

The effect of different treatments on biological efficiency of the oyster mushroom varied significantly ($P \leq 0.05$). The highest biological efficiency was recorded with 30:70(159%) treatment followed by 10:90(157%). The least was recorded with the treatment 90:10 (110%) (Fig 3).

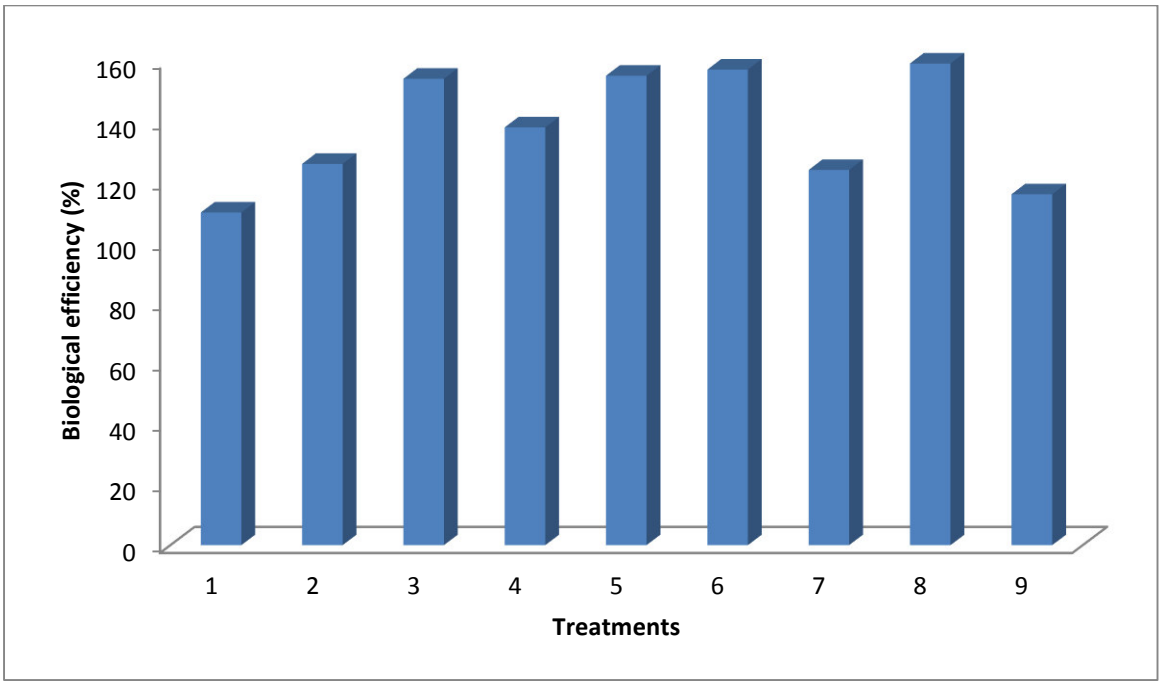

Fig3: Biological efficency of the oyster mushroom gorwn on different susbtrate composed from maizestem: cotton seed waste: 1(90:10), 2 (80:20), 3(70:30), 4(60:40), 5(50:50), 6(10:90) , 7(20:80), 8(30:70), 9(40:60)

Figure 4: Stages in the development of mushroom: (A) Pure culture of *P. ostreatus* grown on the surface of agar medium , (B) Oyster mushroom spawn developed on sorghum grain and ready for use; (C) maize stem collected to be used as a major substrate (d) dried and sheered maize stem in to smaller size (E) substrate inoculated with mushroom spawn and spawn run in the production plastic bag; (f) Primordial formation and elongation of pin heads to differentiate to mushroom and (5) mature oyster mushroom ready for harvest.

Discussion

Mushroom cultivation technology is one of the forthcoming technologies that are suitable for waste recycling, reducing pollutants and contributing in the environmental sustainability through production of mushroom fruit bodies which are nutritious and have medicinal properties. In this investigation, the efficiency of maize stem, one of most abundant agricultural residues, as a major substrate for oyster mushroom production along with cotton seed waste were evaluated. The substrate treatments composed of maize stem and cotton seed waste at the com position of 60: 40, and 30:70 showed uniform and fastest mycelial extension on 7th and 14th days of incubation periods. These observations, was in line with the results reported by (Asefa and Geda, 2014 a& b); who observed the fastest mycelial run on the substrate composed from paper waste: cotton seed waste (90:10) than waste paper and wheat bran (50:50).In this study, the mean values of mycelial growth rate (cm/day) were found to be proportional to the mycelial/ extension on 7th and 14th days. This result was also similar to, the mean value of mycelia extension reported by Gume et al. (2013) and Asefa and Geda (2014 a& b). Gume et al (2013) reported the highest mycelial running rate was observed in substrate composed from saw dust maize comb and coffee husk (sdZcCh) (0.69 cm/day) and the lowest mycelial running rate (0.17 cm/day) was in coffee husk. In this study, there were slight differences on days required for complete colonization of the mushroom the substrates that received different treatments. Similar result was reported by (Asefa and Geda, 2014 b; Gume et al 2013; and Mekonnen and Semira, 2014). In general, fastest mycelia run and shortest time taken for complete colonization of the substrate, may be due to the maize stem containing sugars which are easily assimilable carbohydrates together with starch as reserve food.

In this investigation maize stem: cotton seed waste substrates (60:40) and (30: 70) showed relatively shorter incubation (30 days) to1st flush. The variation in the period for first flushing is not constant and depends on factors like substrate composition(Mekonnen and Semira, 2014; Asefa and Geda , 2014 a&b) ranging from 17 to 55days. In this study, the incubation period between flushes was almost similar for the various combinations tested. According to Mekonnen and Semira, (2014) oyster mushroom grown on cotton hull substrates took 4 and 7 days for first and second harvesting time respectively than the saw dust substrate. The combination of substrate with less amount cotton seed waste showed shortest mean periods from pinning to maturation from first to fourth flushing in the order 10, 9, 8, and 7days respectively as compared to other treatments.

Similar short pinning to maturation period of 4 days was reported by Gume et al.(2013) while cultivating on sawdust and coffee waste. In this study maize stem: cotton seed waste (30:70) showed the highest fresh weight 445g per 500g substrate in wet/dry mass basis in first flush followed by (10: 90) 415g; while (90:10 and 80:20) gave 250g and 280g respectively, were found to be the least. In the second flush the highest yield was obtained from (70:30) 250g followed by (10:90) and (30:70) 160g each, while (20:80) 100g was found to be the least in the second flush. In third flush (50:50), gave the highest yield, followed by (80:20 and 20:80); whereas, the yield obtained from the remaining treatments were least. In this investigation, in all the treatments the lowest yield of mushroom was obtained in 4th flush. This observation was in line with, (Asefa and Geda 2014 ab; Gume et al.2014). According to Mekonnen and Semira , 2014 averagely the yields were highest in the first flush then declined gently in the second and third flush of all substrates. These authors did not collected mushroom fruits in fourth flushes from other substrates and no product was recorded from teff straw after the first flush.

In this investigation, higher number of bunches were recorded on maize stem: cotton seed waste (90:10 and 20:80) 10 each per 500g dry substrate; while the lowest were bunches recorded on (80:20) 5 per 500g of dry substrate. The highest number of fruiting bodies were 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. 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. Pilus diameter was found to be maximum 9.2 cm for the samples collected from 30:70 combination substrates and minimum 7.5 cm each from 60:40 and 10:90 treatments. This is much greater than the pilus diameter reported by Gume et al.(2013who observed mean pileus diameter ranging from 3.8 to 5.2 cm. The stipe length of the different treatments did not show significant variation. The highest total wet/fresh weight of mature mushroom recorded was 795g in 30:70 treatment followed by 10:90 substrate combination yielding 785g. The least total wet / fresh weight (550g) was recorded in the 90:10 treatments. The highest biological efficiency 159% was recorded with 30:70 substrate combination followed by157% with 10:90 treatment. The least biological efficiency 110%. was recorded with the treatment 90:10. Fekadu (2014) observed considerable variation in yield of oyster mushroom grown on *Grevillea robusta* leaves with different supplements and reported the maximum biological yield of 55g/3.5 kg with supplements of 18% of cow dung on substrate which give 15.86% of biological efficiency, this figure is by far lower than any of the present treatments. Gume et al. (2013) reported that substrate composed of Sawdust of *C. africana* (sd1*C*) gave the least percentage of BE (29.07%) than the four original substrates (sd*Z*c*C*h) showed the highest BE (77.38%).

Conclusion

This study was carried out in order to evaluate the usability of maize stem together with different proportion of

cotton seed waste on growth, yield and yield related parameters of oyster mushroom. The maize stem together with the different proportion of the cotton seed waste affects the vegetative or the mycelium growth, the reproductive growth, yield and yield related parameters as well as the time taken to complete the cultivation cycle of the oyster mushroom. The total yield of the mushroom biomass, as well as the biological efficiency were affected by the different treatments. In general, the use of maize stem as a major substrate for mushroom production could be considered as a good option for minimizing the amount of agricultural residues in the environment, which other wise pollute the environment through the emission of the amount of green house gases when burnt. The acceptability of the mushroom grown on maize stem in the market could also be increased since the maize is the staple cereal food in many parts of the world as well it also directly used as animal feed .

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