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Full Length Research Paper

Bioconversion of coffee husk for oyster mushroom (*Pleurotus ostereatus*) cultivation in Jimma

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At present, coffee production is dramatically increasing in the world. Consequently, coffee husk are also increasing at the same time. In order to minimize this hazardous husk, the current study was initiated to evaluate the suitability of coffee husk for cultivation of commercial oyster mushroom species (*Pleurotus ostreatus*) after compositing with different main substrate combinations. Composting of coffee waste (husk) was conducted with cow dung, poultry manure and bone meal in the ratio of 3:1. During cultivation of mushroom, some biological efficient and other parameters were conducted. Data were analyzed using SPSS version 16. The substrate combinations of coffee husk showed a significant variation ($p<0.05$). The highest yield (192.3 g) and biological efficiency (21.37%) was obtained from combination A on 20 days composting. Nevertheless, the lowest biological efficience was obtained from combination of A + B (7.32%) on the first 30 days of composting. No variation was observed between combination A and CH ($P>0.05$) in terms of yield on the first 5 and 10 days of composting. But, great variations ($p<0.05$) were generally detected between combinations A+B and A, and A+B and CH. Therefore, better yield of oyster mushroom was obtained after bioconversion of this cost-effective and cheap agro-waste of coffee husk.

Key words: Coffee husk, compost, microorganism, oyster mushroom, substrate combination.

INTRODUCTION

Coffee is one of the most essential beverages of the world and its annual production was reported to be about one million tons in more than 50 countries (Fan et al., 2006). Studies (Mekuria et al., 2004; Petit, 2007) revealed that Arabica coffee (Coffea arabica) has for centuries played an important role in the Ethiopian economy and represents the main cash crop cultivated by small-scale farmers for social, economic, political and ecological sustainability. However, only about 9.5% of the weight of the fresh material is used for the preparation of the beverage and about 90.5% is left as residue during the industrial coffee production (Soccol, 1996).

The main end-product (pulp and husk) of a coffee which is about one million ton, remain a serious problem to the environment owning to having undesirable organic acid such as phenol (monomers), caffeine, tannins (polyphenols) (Martinez-Carrera et al., 2000). Coffee wastes such as pulp, husk and effluent are the main byproducts generated by the coffee processing units and are disposed into farming lands and surface waters (Preethu et al., 2007). Preethu et al. (2007) further remarked that the presence of toxic substances (organic acids) in these byproducts have the capacity to affect the soil, water quality and other plant growth.

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However, the utilization of these coffee husks for mushroom cultivation has been at the infancy stage in Ethiopia. Nevertheless, some countries cultivate mushrooms on coffee waste (husk and pulp) after bioconversion and with different treatments. Fan et al. (2005) observed that in Brazil, coffee residue (husk) was used for shiitake mushroom cultivation after bioremediation with *Pleurotus* species. However, Baje (2010) reported the unsuitability of unconverted coffee husk for cultivation of mushroom. Therefore, to fill this gap of knowledge, this study was initiated to evaluate the suitability of coffee husk for mushroom cultivation after bioconversion of these wastes.

MATERIALS AND METHODS

Description of the study site

The study was conducted at the Applied Microbiology Research Laboratory, Department of Biology, Jimma University Ethiopia from October to November 2010/2011. Jimma town is located 350 km southwest of the capital city, Addis Ababa. The area receives adequate amount of rain fall with the total mean annual rain fall of about 1529 mm. The maximum and minimum temperatures are 26.3 and 11.6°C, respectively. The altitude of the study site ranges from 1554 to 2149 m above sea level with longitude of 38° 7' 0" E and 7° 18' 0" N latitude (Paulos and Teketel, 2000).

Types, sources and collection of main substrates

Four different types of substrates namely, coffee husk, bone meal, cow dung and poultry manure were used. A 1% gypsum was used as a substrate additive in order to act as buffer and help to maintain proper pH level of the substarate. The bone meal was obtained from Addis Ababa abaiter. Both cow dung and poultry manure were collected from Jimma town whereas coffee husk was collected from coffee mills within Jimma town.

Compositing of coffee husk with other substrates

Substrate combination, compost preparation and turning compost piles

The coffee husk was moistened for three days before piles formation. 75% (v/v) of coffee husk was composted with different substrates (25% v/v each) such as cow dung, poultry manure and bone meal. Combinations were made with each of substrate and three compost piles were made on the pure area under the shade (Table 1). A 0.5% of a chopped grass was added and composted A 0.5% of a chopped grass was added and composted together with each combination in order to attach each A 0.5% of a chopped grass was added and composted together with each combination in order to attach each eachcombination together. Constant moisture level was maintained during every five days of turning compost by spraying water two times a day. The compost piles were turned every five days of composting. It was watered two times each day based on the conditions. The mixed main substrate combination was covered with plastic sheets. Finally, composting was run for one month.

Source of mushroom culture and culture transfer

Pure culture (slants, plates and mother spawn) of oyster mushroom (*Pleurotus ostreatus*) was obtained from Mycology Laboratory, Addis Ababa University. Pure cultures were transferred into sterile potato dextrose agar (PDA) plates and slants. The plates and slants were stored at ambient temperature (22±2°C) for 7 days. After this, the slant was kept in refrigerator at 4±2°C. Non-contaminated plate cultures were directly used for the spawn preparation.

Spawn preparation and inoculation

Clean red sorghum was washed in clean water five times to remove chaff, dust and other particles. The grains (10.08 kg) were soaked in water for 24 h and drained. It was mixed with 1% calcium carbonate ($CaCO_3$) (1.008 kg) and 2.52 kg (dry wt.) shredded wheat (2.5%) supplements. It was filled into the bottle and autoclaved at 121°C for 1.5 h. The moisture was adjusted to 50-60% following the methods of Chen et al. (2012). After autoclaving at 121°C for 1.5 h, the spawn medium (red sorghum) was inoculated with bits of mycelia of strain growing vigorously on PDA slants. It was then incubated at room temperature in the dark room at 24-29°C according to Bernabe-Gonzalez and Cayetano-Catarino (2009). The spawn in the bottle was ready for inoculation to the main substrate after 29 days growth when the mixture turned totally white (Leifa et al., 2001).

$$\text{Moisture contents} = \frac{\text{Wet weight} - \text{dry weight} \times 100\%}{\text{Wet weight}}$$

Filling plastic bags with substrate combination and spawn running

A 4.2 kg substrate combination was taken from each pile, that is, coffee husk + cow dung (A), coffee husk + chicken manure (B) and coffee husk + bone meal (C) every five days of turning compost. A 1.8 kg of coffee husk (CH) only was also taken and filled into plastic bag for spawn running every five days of turning compost.

Table 1. Combinations of coffee husk with different substrates used for spawn running.

S/N	Main substrate combination	Supplements
1	CH only	-
2	A	0.5% Chopped grass
3	B	“
4	C	“
5	A + B	“
6	A + C	“
7	B + C	“
8	A + B + C	“

Note: A = Coffee husk (75%) + cow dung (25%) (v/v), B = Coffee husk (75%) (v/v) + Poultry manure (25%). C = Coffee husk (75%) (v/v) + Bone meal (25%) (v/v), A+B = Coffee husk + cow dung (50%) (v/v) and Coffee husk + poultry manure (50%) (v/v), A+C = Coffee husk + cow dung (50%) (v/v) and Coffee husk + bone meal (50%) (v/v), B+C = Coffee husk + poultry manure (50%) (v/v) and coffee husk + bone meal (50%) (v/v) and A + B + C = Coffee husk + cow dung (33%) (v/v) and Coffee husk + poultry manure (33%) (v/v) and coffee husk + bone meal (34%) (v/v).

Both substrate combinations and CH only were mixed with 1% of calcium carbonate ($CaCO_3$). A combination was taken and placed in the plastic bowl. A little water was added onto substrate combination and adjusted to 50-60% moisture content. Each of the three main substrate combinations that were taken from every pile and CH were filled into heat resistant polyethylene bags in duplicate (that is, two bags) according to the combinations shown in Table 1. A total of 16 bags (with a dimension of 20cm × 35 cm) of substrate combinations were taken every five days of turning compost piles for seven times (one month) (Table 1).

For combinations of A + B, A + C and B + C, half of a combination of A, B and C (450 g each of them) was taken and filled into polyethylene bags. For combination of A + B + C, 300 g sample from main substrate combinations were taken and filled into the bags. Totally, 112 bags were filled with substrate combination for spawn running. Each bag contained 900 g dry weight of substrate combinations. Generally, combinations were made according to Table 1. The bags were labeled according to the substrate combination made. They were autoclaved at 121°C for 1.5 h and were kept within a refrigerator for cooling. Later, a 90 g red-sorghum spawn of *Pleurotus ostreatus* (10% of substrates combination of spawn) was inoculated and mixed thoroughly with each bags of the main substrate combination using spoon under laminar hood in a layer manner. The mouths of bags were well tied using thread. Inoculated bags were incubated at room temperature (25°C) in the dark. Mycelia development in the bag was observed and noted every 7th and 14th days.

Incubation

Incubation for mushroom cultivation and humidity were conducted following the methods of Oei (2005). All treatment bags were examined after three days to look for the possible contaminants. The contaminated bags were automatically removed and sterilized. After 20 days of incubation, close observation of the bags was made to recognize the complete spawn run and primordial formations.

Mycelia growth measurements

Mycelial extension throughout the substrate combination in the localized bags was measured on weekly basis using the transparent graduated ruler following the method used by Rajapakse et al. (2007).

Mushroom growing room and induction of fruiting

A 3 m × 3 m aseptic room was adjusted at Research and Postgraduate Laboratory, Biology Department, Jimma University. The wall and roof of the room were washed using the surfactant (savilon). The shelves and a straight long log of trees were set in the room. Long logs of trees were kept on the shelves in a horizontal position which were used for hanging of plastic bags with the inoculated substrate combination. Windows were opened at the day time for air exchange and closed at the night time so as to create a dark environment for incubation of oyster mushroom. Induction of fruiting during mushroom cultivation was conducted according to the methods of Leifa et al. (2001), Kivaisi (2007), and Mshandete and

Cuff (2008).

Harvesting and mushroom yield

Matured mushroom harvesting and meauremnts of Biological efficiency (B.E) were conducted following the methods of Harkonen et al. (2003) and Akyuz and Yildiz (2008). Mycelial ramification length (MRL), primordial formation and dates of each harvest were also recorded. Total matured and aborted caps per each bag were recorded. Total number of flushes (flush number) produced per each bag was also noted. Duration of time from inoculation to final harvest was calculated. The yield of oyster mushroom per bag was recorded. Cropping periods (sum of incubation and fruiting periods) were also determined.

$$\text{Biological efficiency (B.E) \%} = \frac{\text{Weight of fresh mushroom fruiting bodies} \times 100}{\text{Weight of dry substrate used for mushroom cultivation}}$$

Data analysis

The data were entered and managed in MS Excel. During mushroom cultivation, parameters such as cropping period, mushroom yield and biological efficiency (B.E) were statistically analyzed for each combination using Tukey's Honestly Significantly Different post-hoc test at $p<0.05$ after a one-way analysis of variance (ANOVA) using SPSS version 16.

RESULTS AND DISCUSSION

Mycelial ramification length (MRL)

Mycelial ramification lengths (MRL) occurred in different substrate combinations with different length. The lowest and highest mean of mycelial length per a day were detected for A+B+C (0.29 cm/day) and A (0.61 cm/day) substrate combinations, respectively (Table 2). For combinations B and A+B+C, the mean values of mycelial length were detected at the beginning time but gradually the ramifications of mycelia were ceased (Table 2).

However, there was no entirely mycelial ramification inthe substrate combinations C, A + C and B + C even at the very beginning of spawn running (Table 2). The shortest mean days of complete mycelium ramification in the entire substrate combination bags was observed for combination A (22.57±1.28 days) followed by CH (24±0.55 days) (Table 2). However, the longest mean day was observed for combination A+B (26.21±0.89 days). Nevertheless, for C, A+C, B+C and A+B+C, there was no detection of mycelia ramification (Table 2).
Contaminations due to *Penicillium sp.* and green mould were observed in the bags of substrate combination C.

Islam et al. (2009) recorded the fastest mycelium ramification rate (0.765 cm/day) for Mahogany sawdust, and the lowest rate (0.695 cm/day) was recorded for coconut saw dust. However, in the present study, there is no entirely mycelial ramification for the substrate combination C, A + C and B + C even at the very beginning of spawn running (Table 2). This may be due to inhibitory effect of constituents of bone meal for mushrooms mycelia and/or absence of suitable nutrient content in the bone meals. Ockerman and Hansen (2000) stated that bone meal contain high amount of ossein, an organic bases of calcium (32.5%), phosphorus (15.1%), heavy metals and very few of protein and fibers. Bone meals are not suitable as a fertilizer for plants due to shortage of nitrogen and phosphorus. Therefore, in the present study, these organic bases of bone meals (ossein) may inhibit the mycelia growth of oyster mushroom (Ockerman and Hansen, 2000).

The result revealed that mycelial extension rate was detected on different substrate at a different time. In this study, the shorter the mean days for mycelial extension, the higher the yield was obtained from combination A. This could be attributed to nutritional quality of combination A. There was also a significant variation between CH and combination A (p=00) and combination A and A+B (p=0.00) for mycelial extension in the entire bags. A study conducted by Islam et al. (2009) also revealed a comparable result that the time required for completing of mycelia extension in spawn packet ranged between 25-26 days and varied remarkably on different substrate types used.
Similar results were recorded by Kumara and Edirimanna (2009) that all strains of *Pleurotus* species colonized the sterile compost bags with mixture of sawdust, rice bran, mung bean powder and sufficient amount of water within 24-26 days after spawn running. However, Iqbal et al. (2005) obtained minimum days of completion of spawn running from sugarcane baggas substrate, in which they were 14.0, 16.0 and 20.0 days for *Pleurotus ostreatus* (Local), *Pleurotus ostreatus* (exotic) *Pleurotus sajarcaju*, respectively. In line with thepresent study, Dundar and Yildiz (2008) findings showed that the mycelium growing time ranged between 10.2 to 18.8 days based on the quality of N contents in the material used. They further noted that the shortest (10.2 days) and longest (18.8 days) days of mycelium extension time were observed from soybean stalk and cotton stalk substrate, respectively. The results of this study and others suggest extensive trials using diverse substrate combinations for the possible shortest period for mycelia extension.

Incubation period of primordial formation time (PFT) (days) for different substrate combinations

The mean value of incubation period of primordial

Table 2. Mycelial ramification length (MRL) for different substrate combination.

Substrate combination	Mycelia ramification length (cm)		Mean value per a day (cm)	Mean value of complete mycelium intension (in days)
	7th	14th		
CH	4.8	(7.76)[b]	0.55	(24±0.55)[b]
A	5.79	(8.5)[a]	0.61	(22.57±1.28)[c]
B	2.49	(5.64)[d]	0.40	**
C	ng	ng	ng	ng
A + B	3.3	(7.09)[c]	0.51	(26.21±0.89)[a]
A + C	ng	ng	ng	ng
B + C	ng	ng	ng	ng
A + B + C	2.96	(3.99)[e]	0.29	**

** Ceased of MRL; ng: No growth.
Mean values of substrate combinations within the same column with the same letters are not significantly different at 95% confidence interval by using LSD test (P>0.05).

Table 3. Mean value of incubation period of primordial formation time (PFT) for substrate combinations.

Substrate combination	Day-1	Day-5	Day-10	Day-15	Day-20	Day-25	Day-30
CH	(26.5±0.0)[ab]	(27.5±0.71)[b]	(26.5±0.00)[c]	(27.5±0.0)[b]	(27.5±0.71)[b]	(27.5±0.0)[b]	(27.5±1.41)[b]
A	(27±1.41)[a]	(27±0.00)[c]	(27±0.71)[b]	(25±0.0)[bc]	(25±0.71)[c]	(26.5±0.71)[c]	(27±0.71)[c]
B	ng	ng	ng	ng	ng	ng	ng
C	ng	ng	ng	ng	ng	ng	ng
A + B	ng	(32±0.71)[a]	(32.5±0.71)[a]	(31±0.0)[a]	(31±0.71)[a]	(32.5±0.0)[a]	(33.5±0.00)[a]
A + C	ng	ng	ng	ng	ng	ng	ng
B + C	ng	ng	ng	ng	ng	ng	ng
A + B + C	ng	ng	ng	ng	ng	ng	ng

Mean values of substrate combinations within the same column with the same letters are not significantly different at 95% confidence interval by using LSD test (P>0.05); ng: no growth.

formation time (PFT) from spawn running time varied (Table 3). It ranged between 25-33.5 days. Time required for PFT of A+B combination was the longest. For substrates obtained from Day-1, there was no primordial formation for combination A+B. But, on 15 and 20 days of composting, the PFT was shortest (Table 3). PFT and Biological efficiency (BE) have positive correlations (R= 0.866, R^2=0.750, p=0.00) with each other (Figure 1). No variations of PFT was observed for all combinations between days of composting (p=1.00) for the same combination. In the mushroom cultivation processes, primordial formation time (PFT) is somewhat longer. The longest time (31±0.0 to 33.5±0.00 days) of PFT was recorded for combination A+B (Day-30). However, the shortest (25±0.0 to 27±1.41 days) was recorded for combination A (Table 3). In this study, the shortest PFT was recorded on 15, 20 and 25 days of composting which probably linked to suitable time for mycelial extension (Table 3). The study that was conducted by Dundar and Yildiz (2008) demonstrated comparable results that the primordial formation ranged from 20 to 34.2 days. The same authors further noted that the first primordial formation time was detected on the pure soybean stalk on 20 days and the last was observed on cotton stalk and lentil straw combination on 34.2 days. In addition, Islam et al. (2009) reported a comparable result that the time required for primordial formation ranged between 28.61-38 days. Islam et al. (2009) further reported that the longest time require for primordial formation was recorded for coconut sawdust (38 days) and the least however, recorded for Kadom sawdust (29.3 days). The observed difference could be attributed to variability in inherent chemical composition of the substrates utilized for mushrooms cultivation.

Numbers of primordial formation (NPF) per a single bag

The largest mean value of number of primordial formation

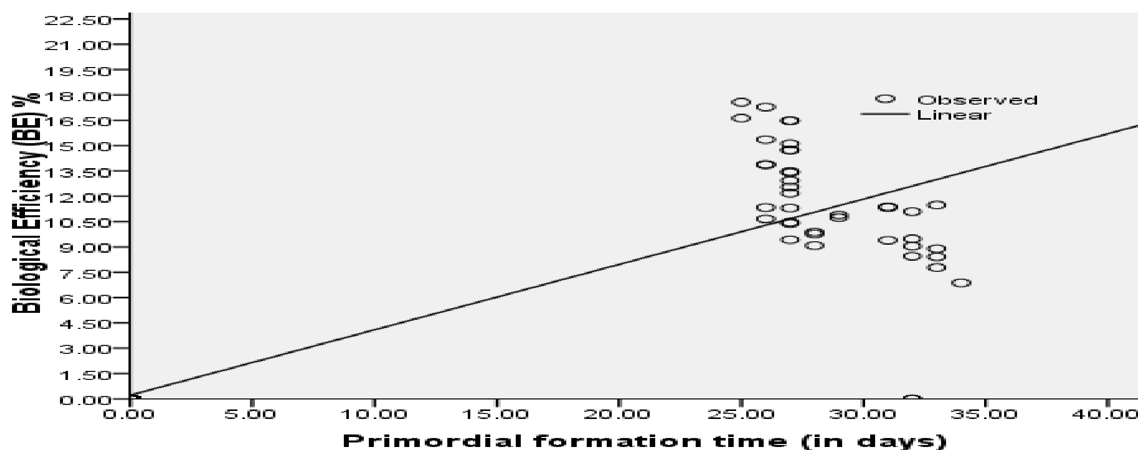

Figure 1. Correlation between biological efficiency and primordial formation time.

Table 4. Mean values of number of primordial (NPF) per 900 g substrate combinations.

	No. of primordial						
Substrate combination	Day-1	Day-5	Day-10	Day-15	Day-20	Day-25	Day-30
CH	(6.5±0.71)[a]	(7.5±0.71)[a]	(6.5±0.71)[b]	(7.5±0.71)[ab]	(7.5±2.12)[ab]	(8±1.41)[a]	(7.7±0.71)[a]
A	(5.5±0.71)[ab]	(7.5±0.71)[a]	(7.5±0.71)[a]	(8±1.41)[a]	(8±1.41)[a]	(7±1.41)[ab]	(5.5±0.71)[b]
B	ng	ng	ng	ng	ng	ng	ng
C	ng	ng	ng	ng	ng	ng	ng
A + B	ng	(4.5±0.71)[b]	(6.0±0.00)[bc]	(6.5±0.71)[abc]	(4.0±0.00)[b]	(3.5±0.71)[b]	(3±0.00)[c]
A + C	ng	ng	ng	ng	ng	ng	ng
B + C	ng	ng	ng	ng	ng	ng	ng
A + B + C	ng	ng	ng	ng	ng	ng	ng

Mean values of substrate combinations within the same column with the same letters are not significantly different at 95% confidence interval; ng: no growth.

(NPF) was observed for substrate combination A on Day-15 and Day-20 (Table 4). However, the least was recorded for combination A+B (3±0.00). Mean value of NPF was slightly few in number on the first 1, 5, 10, 25 and 30 days of composting for all combinations except on day 15 and 20 of composting (Table 4). Significant variations were observed between combination A and CH on the sampling days of 10 (p=0.02) and 30 (p=0.00) of composting. However, no significant variations was observed between combination A and CH on the sampling days of 1 (p=.431), 5 (p=1.00), 15 (p=0.438), 20 (p= 0.594) and 25 (0.219) of composting (Table 4). However, on the first 15 days of composting, variation was observed between combination A+B and CH (p>0.05) (Table 4). Different variation was observed between combination A and A+B (P>0.05) during all sampling days (Table 4). Greatest positive correlation was observed between BE and NPF (R=0.937, R^2=0.878, p=0.00) for all combination and CH (Table 4) that gave yield. This may indicate that BE % increase with increasing number of primordial formation (NPF) (Figure 2). The number of primordial formation (NPF) is associated with the quality of substrate used for mushroom cultivation (Islam et al., 2009; Hasan et al., 2010). In this study, some substrate combinations showed high number of primordial, whereas few showed low number of primordial.

The result indicated that the highest (8 NPF) was recorded for combination A on the first 20 days of composting (Table 4). There is also a positive correlation (R^2=0.878, p=0.00) between NPF and BE. The lowest (3 NPF) was however, recorded on combination A+B. In contrary to the present study, Hasan et al. (2010) recorded the highest number of primordial (99.33)

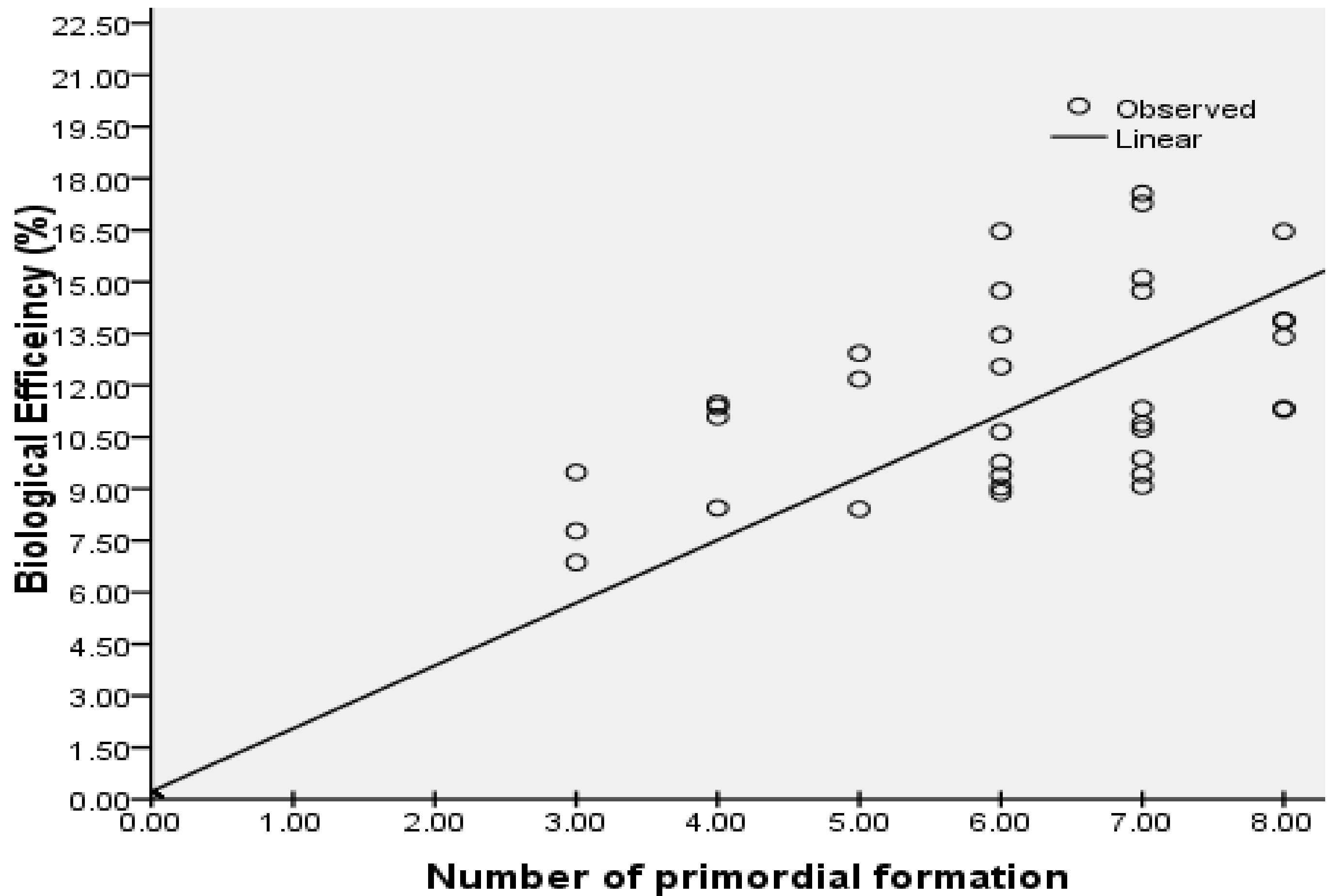

Figure 2. Correlation between biological efficiency and number of primordia formation (NPF).

initiation from rice straw and the least (7.67) from mehegoni leaves. In addition, research conducted by Islam et al. (2009) showed that the highest number of primordial (44) per packet was recorded in Mahogony sawdust and the lowest (32.6 days) in coconut sawdust.

The reported variations with regard to NPF can be related to the quality of the substrates and the amount of substrate combinations per bags used. For instance, Hasan et al. (2010) reported the lowest NPF from mehegoni leaves which might happen due to absence of glucose, fructose and trehalose in the substrate.

Incubation period of spawn running to harvesting

Time taken for incubation period of oyster mushroom from spawn running to harvesting varied. It was mainly based on the type of substrate combination used and duration of its decomposition (Table 5). On Day 30, the longest mean days of incubation period (47.5 days) of spawn running to maturation for first flush was detected for substrate combination of A and A+B. This may be due to depletion of nutritional quality of waste as composting proceeded. However, as age of compost increases, the time taken for incubation period was also increased, especially after 20 days on ward (Table 5). Great variations were observed between combination A+B and CH, combination A+B and A (P=0.00). Statistically, there was no difference between combination A and CH on the sampling days of 1 (p=0.195) and 5 (p=0.094) of composting in terms of incubation period (Table 5). However, significant variations were observed between combination A and CH for the rest days of composting ($p<0.05$).

Incubation period for the five days of composting (Day-5) was also longer (39.5) and for the same combination

Table 5. Mean value of time (days) taken from spawn running to harvesting period.

	Incubation period of spawn running to harvesting (in days)						
Substrate combination	**Day-1**	**Day-5**	**Day-10**	**Day-15**	**Day-20**	**Day-25**	**Day-30**
CH	(32.5)[a]	(32.5)[b]	(35)[b]	(33.5)[b]	(36)[b]	(36.5)[b]	(34.5)[c]
A	(32)[ab]	(32)[bc]	(29.5)[c]	(30)[c]	(29)[c]	(34.5)[c]	(36.5)[b]
B	ng	ng	ng	ng	ng	ng	ng
C	ng	ng	ng	ng	ng	ng	ng
A + B	ng	(39.5)[a]	(41.5)[a]	(38)[a]	(38)[a]	(41.5)[a]	(47.5)[a]
A + C	ng	ng	ng	ng	ng	ng	ng
B + C	ng	ng	ng	ng	ng	ng	ng
A + B + C	ng	ng	ng	ng	ng	ng	ng

Mean values of substrate combinations within the same column with the same letters are not significantly different at 95% confidence interval; ng: no growth.

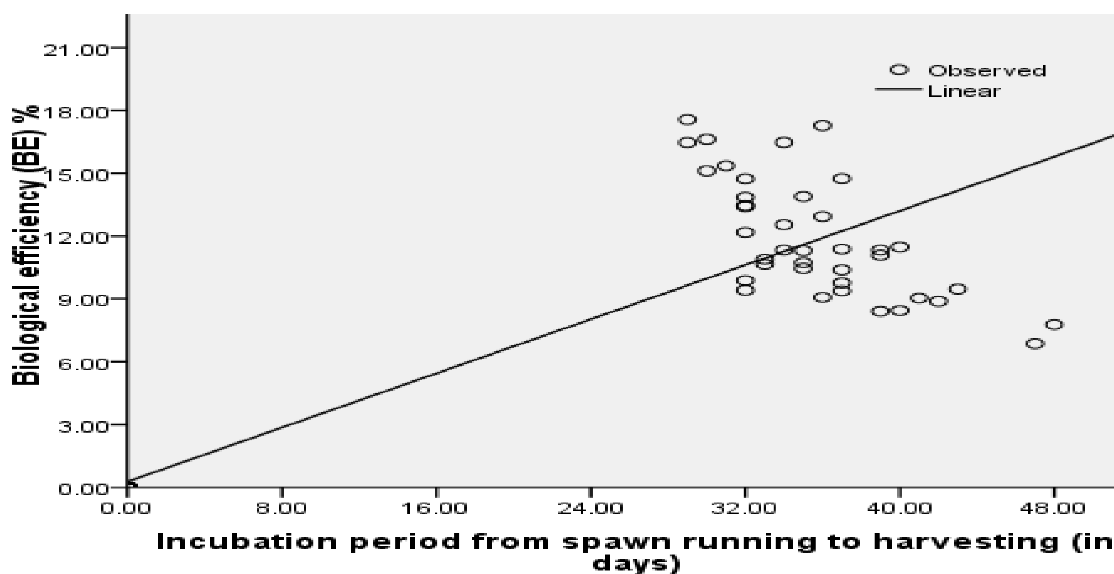

Figure 3. Correlation between incubation periods of spawn running to harvesting and BE for oyster mushroom.

the longest (47.5) incubation period was recorded on the first thirty days of composting (Table 5). The shortest (29) mean day was, however, observed for combination A (Table 5). Incubation periods of spawn running to harvesting are positively correlated (R= 0.892, R^2=0.795, p=0.00) with biological efficiency (BE) (Figure 3). Research conducted by Iqbal et al. (2005) revealed that the maximum time required for cropping period was 48.3, 53.3 and 50.7 days on the wheat straw based substrate. In addition to this, Iqbal et al. (2005) demonstrated that the minimum number of days needed for maturity of mushroom growth on the sugarcane baggase was 20.3, 22 and 37 days. They also recorded 33.3, 38 and 37.7 incubation days for cotton waste which might have been due to suitability of substrate used. Islam et al. (2009) reported that the shortest and longest cropping periods of oyster mushroom were recorded for kadom sawdust (31.01 days) and coconut sawdust (38.78 days), respectively.

Maturation period of fruiting bodies from pin-head appearance to harvesting time

The mean days of maturation period of fruit body showed

Table 6. Time (days) taken for maturation of fruit bodies of oyster mushroom on different substrate combinations.

	Maturation period of fruiting			
Substrate combination	**First Flush**	**Second Flush**	**Third Flush**	**Fourth Flush**
CH	$(6.64\pm1.00)^{b}$	$(5.5\pm1.00)^{b}$	$(5.0\pm0.78)^{a}$	ng
A	$(5.5\pm1.29)^{c}$	$(4.6\pm0.79)^{c}$	$(4.64\pm0.84)^{bc}$	(4.29±0.33)
B	ng	ng	ng	ng
C	ng	ng	ng	ng
A + B	$(7.5\pm0.85)^{a}$	$(6.0\pm0.74)^{a}$	$(4.7\pm2.13)^{b}$	ng
A + C	ng	ng	ng	ng
B + C	ng	ng	ng	ng
A + B + C	ng	ng	ng	ng

Mean values of substrate combinations within the same column with the same letters are not significantly different at 95% confidence interval; ng: no growth.

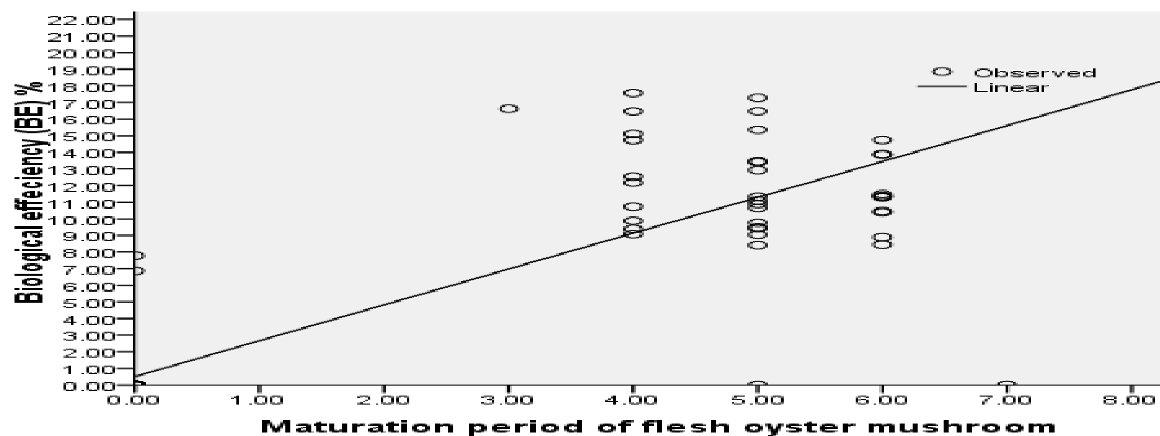

Figure 4. Correlation between biological efficiency and maturation period of oyster mushroom.

variations for all substrate combinations and among all flushes ($p<0.05$) except between combination A and A+B ($p=0.23$) on the third flush (Table 6). The shortest mean value (5.5 days) of pin-head formation to maturation of fruit body was observed for combination A from the first flush. However, the longest (7.5 days) was recorded for combination A+B followed by CH (6.64) (Table 6). However, significant positive correlations were observedbetween maturation period and BE (R= 0.850, $R^2=0.723$, $p=0.00$) for all combination that gave yield (Figure 4).

In this study, the shortest (4.29±0.33 days) mean days of maturation period of fruit body was observed for combination A in the fourth flush. However, the longest (7.5±0.85 days) was recorded for combination A+B in the first flush. Significant variations ($p<0.05$) were also observed among CH, A and A+B combination during maturation period of fruit body in the 1st, 2nd and 3rd flushes based on the type of substrate used (Table 6). Hasan et al. (2010) have reported that the shortest time for maturation period was recorded for banana leaf mid ribs (1 day) and the longest time was recorded for mehegoni leaves (5 days) at four flush rounding which is comparable with that of the present study.

Time required between observed flushes

Incubation period of consecutive flushes amongst substrate combinations varied (Table 7). The longest mean value of incubation period was recorded between

Table 7. Time taken between flushes of oyster mushroom.

Mean incubation period for time interval between flushes (Days)				
Substrate combination	**First Flush**	**1st-2nd Flush**	**2nd-3rd Flush**	**3rd-4th Flush**
CH	$(32.5)^{b}$	$(17.57 \pm 0.20)^{b}$	$(14.07 \pm 0.30)^{b}$	ng
A	$(32.5)^{b}$	$(18.71 \pm 0.81)^{b}$	$(12.79 \pm 0.30)^{c}$	(9.86±0.20)
B	ng	ng	ng	ng
C	ng	ng	ng	ng
A + B	$(35.14 \pm 0.0)^{a}$	$(19.93 \pm 0.30)^{a}$	$(14.57 \pm 0.00)^{a}$	ng
A + C	ng	ng	ng	ng
B + C	ng	ng	ng	ng
A + B + C	ng	ng	ng	ng

Mean values of substrate combinations within the same column with the same letters are not significantly different at 95% confidence interval; ng: growth.

1st - 2nd flush for all productive substrate combinations. However, for the next consecutive flushes, the time taken between flush collapsed (that is, between 2nd - 3rd and 3rd - 4th flushes). Growth was not detected between 3rd - 4th flushes for CH and A+B substrates after 90 days of observation (Table 7). However, growth for combination A was noticed between 3rd - 4th flushes (9.86±0.20 days) (Table 7). Time taken between consecutive flushes among the substrate combinations showed different patterns (Table 7).

The longest mean of incubation period was recorded between 1st - 2nd flush for all substrates gave the highest yield which was similar to the result reported by Shah et al. (2004). The yield of mushroom was decreased from flush to flush and inverse relationship with the next consecutive flushes which probably related to exhaustion of nutritional source in the substrate. Studies conducted by Iqbal et al. (2005) have reported 3-4 flushes of oyster mushroom from sugarcane baggas substrate. Faster growth was obtained from coffee husk alone. Delayed oyster mushroom was detected on the substrate combination of A+B. No growth was observed at all on the treated substrate combination of B, C, A+C, A+B and A+B+C. Essential steps taken for oyster mushroom development are presented in Figure 5.

Yield of mushroom and its Biological efficiency (BE) %

Yield of mushroom

The length of fresh oyster mushroom cap (pileus) diameter varied (Table 8). It was based on the type of combination and their number of flush. Generally, in the first flush, the diameter of pileus for all substrate combinations was the largest (Table 8). However, for the next flush (that is, 2nd, 3rd and 4th), the diameters became smaller and smaller (Table 8). In this observation, the largest mean value of cap (pileus) diameter was 6.5±0.22 cm for combination A+B. The lowest was measured for that of combination of A (6.28±0.11cm) in the first flush (Table 8). Positive correlation was observed between yield of mushroom and cap (pileus) diameter for all combination that gave yield (R=867, R^2=0.751, p=0.00) (Figure 6). The pileus diameters of mushroom varied on the bases of flushes, since consecutive flushes (that is, 2nd, 3rd and 4th) gave smaller diameters of mushroom caps from the same substrate combination. Islam et al. (2009) recorded the largest (7.0 cm) pileus diameter from Mango sawdust which was comparable to the current finding. The authors obtained the shortest (1) cap diameter from coconut sawdust.

Effect of different combination of coffee husk on stalk (stipe) length among importance combination

The mean values of stalk length ranged from 5.31±0.09 - 6.31±0.28 cm in the first flush for combination that gave yield (Table 9). However, the length of stalk became shorter and shorter as incubation time extended. The shortest mean of stalk length was detected for substrate combination of A+B (5.31±0.09 cm) and the largest was measured for substrate combination A (6.31±0.28 cm) (Table 9). Statistically, it was proved that greatly positive correlation (R=0.898, R^2= 0.806, p=0.00) was observed between stalk lengths and BE of cultivated mushroom for all combination and CH that gave yield. Stalk length and yield of *Pleurotus ostreatus* mushroom statistically have

Figure 5. Osyter mushroom growth steps: (A) Pure culture of slant growth of *Pleurotus ostreatus*, (B) Pure culture of plate, (C) Red-sorghum mother/grain spawn that was ready for spawn running, (D) Complete mycelial invasions of bags with *Pleurotus ostreatus* after spawn running, (E) Primordial appearance within a bag, (F) Pinhead formation from primordial appearance, (G) Immature branch of fresh mushroom caps, and (H) Matured oyster mushroom (ready for harvesting).

Table 8. Mean value of cap diameter (cm) of each flush for treated substrate combination.

Length of the largest Cap (pilus) diameter				
Substrate combination	First Flush	Second Flush	Third Flush	Fourth Flush
CH	(6.42±0.08)[ab]	(4.69±0.00)[a]	(3.42±0.31)[b]	ng
A	(6.28±0.11)[b]	(4.58±0.23)[ab]	(4.1±0.18)[a]	(1.97±0.04)
B	ng	ng	ng	ng
C	ng	ng	ng	ng
A + B	(6.5±0.22)[a]	(4.23±0.05)[b]	(2.44±0.02)[c]	ng
A + C	ng	ng	ng	ng
B + C	ng	ng	ng	ng
A + B + C	ng	ng	ng	ng

Mean values of substrate combinations in the same column with the same letters are not significantly different at 95% confidence interval; ng: no growth.

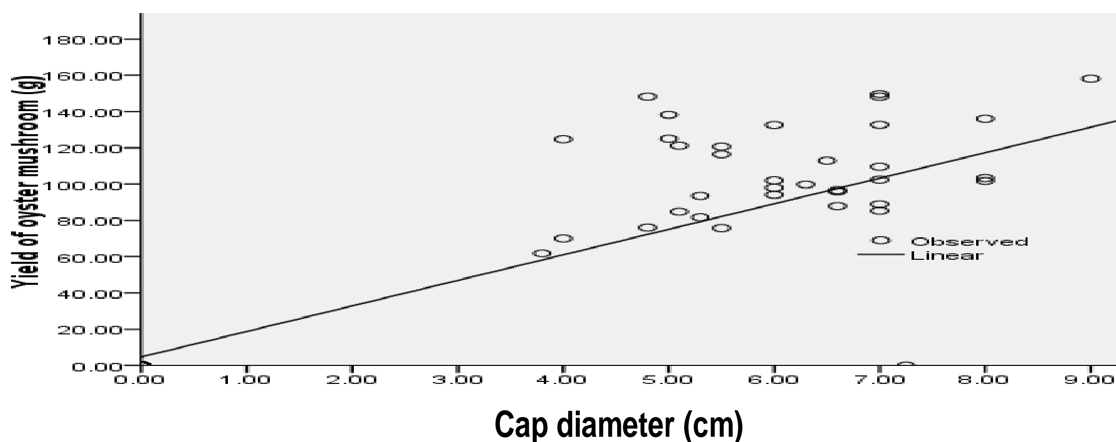

Figure 6. Correlation between yield and cap diameter of oyster maturation.

Table 9. Mean values of stalk length for substrate combination and untreated coffee husk.

Length of the largest stalk (Stipe) diameter				
Substrate combination	First Flush	Second Flush	Third Flush	Fourth Flush
CH	(6.22±0.05)[b]	(4.75±0.27)[b]	(2.95±0.04)[b]	ng
A	(6.31±0.28)[a]	(5.89±0.81)[a]	(4.09±0.33)[a]	(1.89±0.33)
B	ng	ng	ng	ng
C	ng	ng	ng	ng
A+B	(5.31±0.09)[bc]	(4.15±0.13)[bc]	(1.77±0.02)[c]	ng
A + C	ng	ng	ng	ng
B + C	ng	ng	ng	ng
A + B + C	ng	ng	ng	ng

Mean values of substrate combinations within the same column with the same letters are not significantly different at 95% confidence interval; ng: no growth.

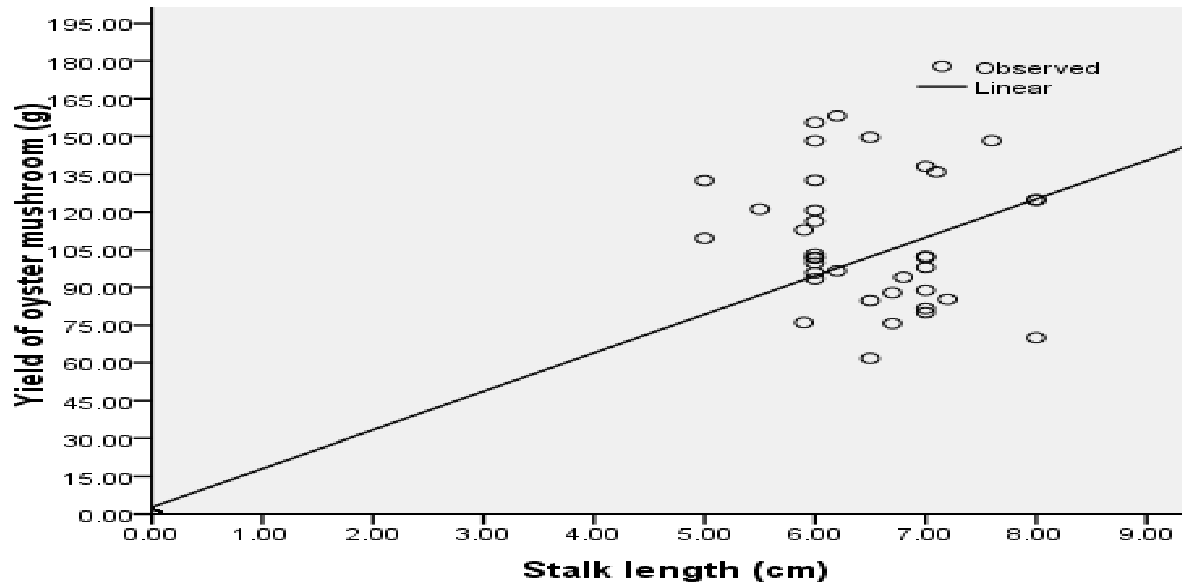

Figure 7. Correlation between stalk length and yield of oyster maturation.

Table 10. Mean value of total number of matured cap oyster mushroom.

Total no. of matured fresh mushroom				
Substrate combination	**First Flush**	**Second Flush**	**Third Flush**	**Fourth Flush**
CH	$(7.07±0.51)^{ba}$	$(5.93±0.10)^{b}$	$(4.50±0.10)^{b}$	ng
A	$(14.5±2.12)^{a}$	$(8.86±0.40)^{a}$	$(5.71±0.40)^{a}$	(3.36±0.51)
B	ng	ng	ng	ng
C	ng	ng	ng	ng
A + B	$(5.7±0.81)^{b}$	$(3.14±0.40)^{c}$	$(2.0±0.00)^{c}$	ng
A + C	ng	ng	ng	ng
B + C	ng	ng	ng	ng
A + B + C	ng	ng	ng	ng

Mean values of substrate combinations within the same column with the same letters are not significantly different at 95% confidence interval; ng: no growth.

positive relationship (R=0.898, R^2= 0.806, p=0.00) with each other in terms of composting days (Figure 7).

Caps yield of oyster (Pleurotus ostreatus) mushroom

Matured caps were the most essential edible in the mushroom parts. During mushroom cultivation, some caps of fresh oyster mushrooms were matured and the others are aborted (Table 10). During the first flush, about 7.07±0.51, 14.5±2.12 and 5.7±0.81 mean caps of fresh *Pleurotus ostreatus* mushroom were collected for A+B, A and CH, respectively.

Generally, the number of matured caps was decreasing from 1st to 4th flush. In all observed flushes, the highest numbers of matured mushrooms were recorded for combination A. However, the lowest was recorded for combination A+B (Table 10). Both matured caps and BE of combination that gave yield have a weak positive correlation with each other (R=0.567, R^2=0.321, p=0.00) which indicates that as mature cap increased, the obtained BE% was also increased. About 32.1% BE of *Pleurotus ostreatus* affected by cap yield during oyster

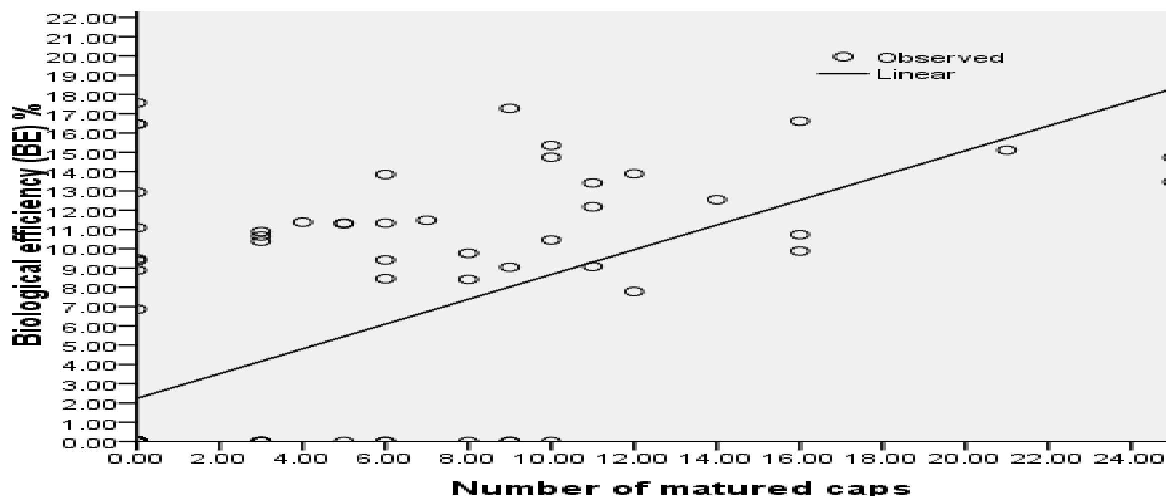

Figure 8. Correlation between biological efficiency and number of matured caps.

Table 11. Yield of oyster mushroom (*Pleurotus ostreatus*) (g).

	Yield of oyster mushroom per 900g substrate						
Substrate combination	**Day-1**	**Day-5**	**Day-10**	**Day-15**	**Day-20**	**Day-25**	**Day-30**
CH	$(90.35)^{b}$	$(111.35)^{bc}$	$(134.2)^{bc}$	$(95.3)^{bc}$	$(91)^{c}$	$(87.6)^{b}$	$(99.3)^{b}$
A	$(115.4)^{a}$	$(126.65)^{a}$	$(142.1)^{a}$	$(148.15)^{a}$	$(192.3)^{a}$	$(136.62)^{a}$	$(124.55)^{a}$
B	ng	ng	ng	ng	ng	ng	ng
C	ng	ng	ng	ng	ng	ng	ng
A + B	ng	$(75.87)^{b}$	$(80.7)^{b}$	$(93.25)^{b}$	$(101.1)^{b}$	$(94.3)^{bc}$	$(65.9)^{c}$
A + C	ng	ng	ng	ng	ng	ng	ng
B + C	ng	ng	ng	ng	ng	ng	ng
A + B + C	ng	ng	ng	ng	ng	ng	ng

Mean values of substrate combinations within the same column with the same letters are not significantly different at 95% confidence interval; ng: no growth.

mushroom cultivation (Figure 8). A comparable result was obtained by Islam et al. (2009) that the matured number of fruiting body on substrate of Mahogony sawdust produced 34 fruiting-bodies on average followed by Shiris sawdust that produced 32. Research conducted by Hasan et al. (2010) indicated that the number of effective (matured caps) of fruiting body showed a variation among varieties of oyster mushroom. In this result, the lowest number of matured cap (1) was recorded on rice straw. However, the highest was obtained from banana leaf midrids substrate (9.72). The average number of fruiting bodies of (Kumar and Edirimanna, 2009) hybridized strain of *Pleurotus* on composted mixture of sawdust and rice ranged from 7 - 26 which was a comparable result to this finding.

Yield of oyster mushroom per 900 g

The fresh yield of oyster mushroom was based on the kind of substrate combinations and duration of compost. On the first days of composting (Day-1), the yield was small for combination A and no yield at all for A+B combination (Table 11). The weight of mushroom yield was tested several times for CH but was nearly the same. The weight of mushroom yield was ranged between 90.35 - 134.2 g (Table 11). As duration of compost extended, they showed variation in weight. The highest yield was obtained on Days-15, 20 and 25 for combinations A and A+B. However, on Day-30, the yields decreased (Table 11).

There was significant variation (p=0.00) between

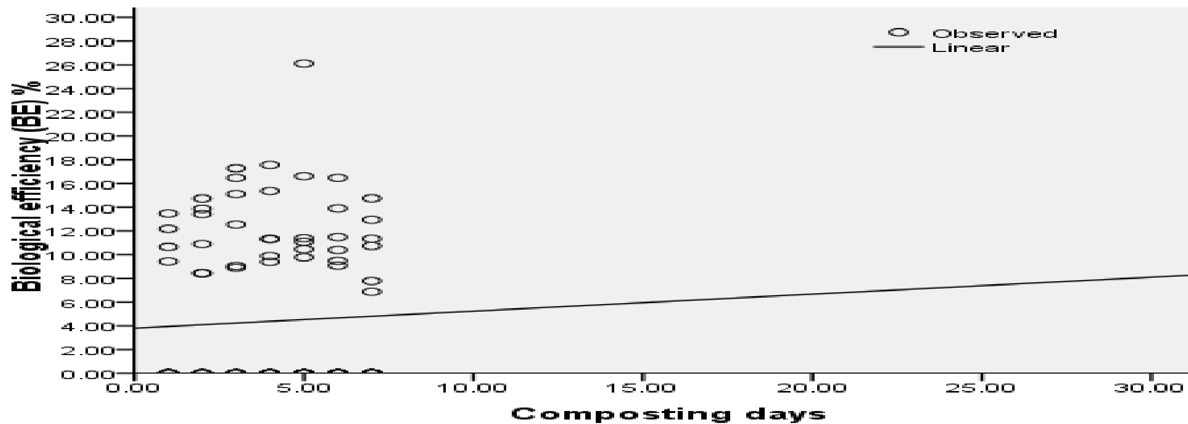

Figure 9. Correlation between biological efficiency and composting days.

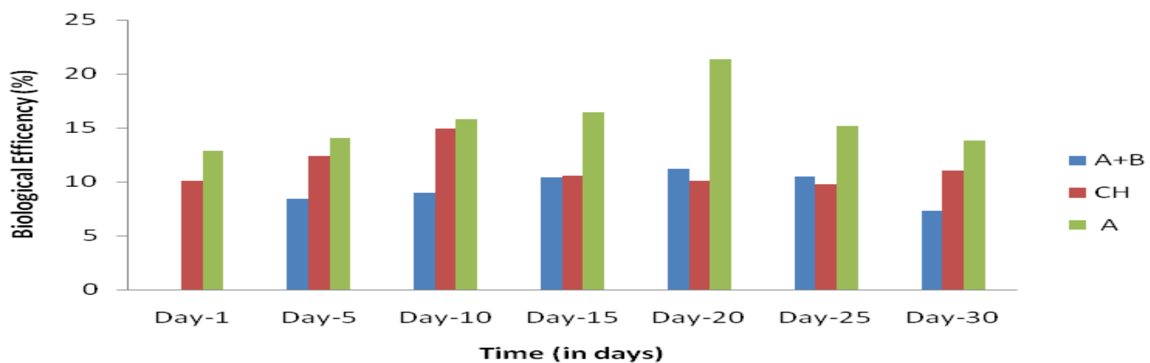

Figure 10. Biological efficiency (BE) % for substrate combinations per 900 g.

combination A and CH, and A and A+B (Table 11). Weak positive correlation was also observed between yield and duration of composting for combination that gave yield. Only about 2% yield of *Pleurotus ostreatus* mushroom was affected by days of composting (R^2=0.002, R=0.046, p=0.631). The same is true for BE and duration of compost (R=0.046, R^2=0.002, p=0.631). Statistically, there is also no variation between sampling days of composting for both yield and BE (p=0.960) even though slight differences are observed (Figure 9). A considerable yield of oyster mushroom was obtained from combination of coffee husk that was supplemented with different organic wastes. Type of substrate used and day of composting have also an effect on the yield of mushroom. The highest (192.3 g) yield of oyster mushroom was recorded for combination A on the first 20 days of composting. However, combination A+B gave the lowest (65.9 gm) yield on the first thirty days of composting. *Pleurotus sajor-caju* that grew on the mushroom spent of *Volvariella volvacea* produced a higher mean yield (254 g) (Villaceran et al., 2006) than composted rice straw that had a mean yield of 180 g per 1000 g of substrate. Iqbal et al. (2005) recorded that the maximum yield of *Pleurotus ostreatus* (Local), *Pleurotus ostreatus* (exotic) and *Pleurotus sajarcaju* fresh mushroom were 62.7, 66.7 and 47.0 g per 1500 g treated substrate of chickpea straw, respectively. They further observed that the minimum yield of *Pleurotus ostreatus* (Local) and *Pleurotus ostreatus* (exotic) were 48.3 and 57.0 g from agricultural waste products of wheat straw, respectively.

Biological efficiency (BE)

Biological efficiency (BE) of mushroom cultivation was

affected by different substrate combinations used (Figure 10). The lowest BE (7.32%) among substrate combinations was recorded for combination A+B on 30 composting days. Nevertheless, the highest BE (21.37%) was obtained from substrate combination A on Day-20 followed by the same substrate combination (16.46%) on Day-15 (Figure 10). There was great significant variation (p=0.00) among substrate combinations of coffee husk (CH), coffee husk and cow dung (A), and coffee husk and cow dung (A) + coffee husk and chicken manure (B) (A+B). Biological efficiency (BE) is one of the most imperative parameters of mushroom cultivation. The present study showed that the mean for BE of substrate combination A ranged between 12.82 and 21.37%. The obtained results were lower than that of the research conducted by Baje (2010) in which the mean ranged between 29.07 and 77.38% on sawdust substrate combination. Study conducted by Frimpong-Manso et al. (2011) indicated that the BE of oyster mushroom obtained from composted sawdust that was supplemented with different percentage of rice husk ranged between 7.8 and 75.3%. Another study (Hasan et al., 2010) demonstrated that the highest biological yield (119.0%) was obtained for varieties of *Pleurotus ostreatus* when they grew on rice straw + poultry litter (10%) + lime (1%) per 5000 gm per packet which is probably due to quality of substrate used. However, they obtained the lowest (11.67 g/packet) biological yield for the same varieties when grown on mehegoni leaves + urea (0.5%) + lime (1%).

A considerable amount of mushroom yield was also obtained from CH (9.73-14.91%) (Figure 10). Mushroom hyphae (mycelium) produced a wide range of extracellular enzymes that have the capacity to degrade complex organic wastes (Martinez-Carrera, 2002). Many white rot fungi are versatile and strong organisms having enormous potential for oxidative bioremediation of a variety of a low and high molecular weight which is a toxic chemical pollutant (that is, phenols) present in olive mill waste water (Thassitou and Arvanitoyannis, 2001). *Pleurotus* sp. are more effective for degradation of different wastes due to their ability to produce lignolytic enzymes such as lignin peroxidases, manganese peroxidases and laccases (Giannoutsou et al., 2004; Sampedro et al., 2007). Furthermore, Laconi et al. (2007) stated that some aerobic edible *Pleurotus*, when employed at large scale in olive mill waste water bioremediation, have the ability to detoxify phenolic compound by using lignolytic enzymes. The biological efficiency of *Pleurotus ostreatus* (Martinez-Carrera et al., 2000) on pasteurized coffee waste (pulp) was 159.9% which is higher than the current findings.

The implication of this study was able to minimize these agro-wastes (coffee husk) which are toxic and hazardous to the environments by using these wastes as a source of mushroom cultivation. Cultivations of edible oyster mushroom on these wastes are also a solution of food insecurity for household after bioconversion by composting with some organic supplements such as cow dung.

Conclusions

In this study, the higher yield of oyster mushroom for all productive substrate was obtained on the first 20 days of composting which is followed by 15 days of composting. But, as the composting process proceeded, finally, the obtained yield was considerably low. In this study, except combinations B, B+C, A+C and A+B+C, some combination of agricultural waste (coffee husk) with different supplement gave yields of oyster mushroom. This was probably due to potential/nutritional quality of combination of coffee husk as a good substrate for cultivation of oyster mushroom. The yield quality of mushroom was based on the duration of composting and type of substrate used. Combination A, A+B and CH gave the yield of mushroom. Generally, the better yield was obtained from combination A.

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