

## Full Length Research Paper

# Evaluation of locally available substrates for cultivation of oyster mushroom (*Pleurotus ostreatus*) in Jimma, Ethiopia

Beje Gume<sup>1</sup>, Diriba Muleta<sup>1\*</sup> and Dawit Abate<sup>2</sup>

<sup>1</sup>Department of Biology, College of Natural Sciences, Jimma University, P. O. Box 378, Jimma, Ethiopia

<sup>2</sup>Department of Biology, Faculty of Science, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia

Accepted 2 July, 2012

A total of eight locally available substrates and substrate combinations were tested for their productivity and biological efficiency (BE) for cultivation of commercial mushroom strain (*Pleurotus ostreatus*). Spawn preparation and running were carried out following standard methods. Main substrates were steam sterilized for 1 h and were inoculated with 10% spawn. The inoculated main substrates were arranged in a completely randomized design on shelves in the mushroom growing room and incubated at ambient temperature ( $22 \pm 2^\circ\text{C}$ ). Relative humidity of the mushroom growing room was controlled by manually spraying water on the walls and placing open containers filled with water in the corners of the room. Data were analyzed using SPSS version 14. The substrate types had significant ( $p < 0.05$ ) effects on the rate of mycelial extension, mean incubation period and yield at 2<sup>nd</sup> and 4<sup>th</sup> flushes, number of matured fruit bodies, aborted pinheads, weight of matured fruit bodies and BE. The fastest mean value (0.69 cm/day) of mycelial extension was recorded from sdZcCh (combination of sawdust of *Cordia africana* and *Pouteria adolfi-friederici*, corncobs and coffee bean husks). However, mycelial growth in coffee bean husks was completely ceased after 15 days. The first pinning took  $29 \pm 2.3$  days followed by  $12 \pm 6.3$ ,  $7.6 \pm 2.3$ , and  $6.4 \pm 2.5$  between the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> flushes, respectively. Mean durations from pinning to maturation ranged from 3 to 5 days. The highest BE was obtained from sdZcCh (77.38%). The present findings indicate the potential of agricultural wastes for cultivation of oyster mushrooms. All the substrates that gave over 40% BE could be recommended for oyster mushrooms cultivation.

**Key words:** Biological efficiency, edible mushrooms, *Pleurotus ostreatus*, spawn.

## INTRODUCTION

One of the world's biggest challenges is food insecurity. This problem is largely common in low- and middle-income countries which mainly have poor food production system and hence, suffer from serious malnutrition. Such countries must find ways of improving food production so as to feed vastly increasing human population. Mushroom cultivation could be a possible option to alleviate poverty and develop the life style of the vulnerable people

(Imtiaj and Rahman, 2008).

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.,

\*Corresponding author. E-mail: dmuleta@gmail.com.

2008).

Studies (Manzi et al., 1999) have demonstrated that oyster mushrooms are healthy foods, low in calories and in fat, rich in protein, chitin, vitamins and minerals. The authors further noted that *Pleurotus* mushrooms also contain high amounts of  $\gamma$ -amino butyric acid (GABA) and ornithine. GABA is a non-essential amino acid that functions as a neurotransmitter, whereas, ornithine is a precursor in the synthesis of arginine emphasizing the medicinal value of oyster mushrooms. For instance, an extract of *Pleurotus ostreatus* was able to alleviate the hepatotoxicity induced by  $\text{CCl}_4$  in animal model (Jayakumar et al., 2006). Similarly, an extract from *P. ostreatus* appeared to protect major organs such as the liver, heart, and brain of aged rats against oxidative stress (Jayakumar et al., 2007). In general, mushrooms are eaten as meat substitutes and flavoring agents (Pathmashini et al., 2008). In addition to the nutritional and medical values, mushrooms cultivation practices have paramount importance in food self-sufficiency attempts (Beetz and Kustudia, 2004), specially for low-income countries like Ethiopia. Mushrooms can generate additional employment and income through local, regional and national trade offering opportunities through processing enterprises (FAO, 2009).

Since mushroom cultivation practice does not always require access to land (that is, space conserving) and any significant capital investment, it is a viable and attractive activity for rural, peri-urban and urban dwellers. Mushroom cultivation is suitable for all job seeking groups including elders, disabled and youngsters. Although mushroom cultivation is labor intensive, this may not be a problem of tropical regions (Chang, 2007; FAO, 2009). In addition, mushroom cultivation drives towards full use of all materials in which nothing left as waste, without any adverse impacts on the environment through sustainable utilization of lignocellulosic wastes available in abundance everywhere, usually as by-products from agriculture, forestry and households (Chang, 2007). Currently, mushrooms are regarded as the most profitable and environment-friendly method for recycling of the vast lignocellulosic waste substrates which could otherwise dropped into the environment and cause pollution (Rai and Ahlawat, 2002).

The agriculture-based Ethiopian economy is highly dependent on *Coffea arabica* and releases huge amount of coffee wastes to the environment from dry processing methods (Gole et al., 2002). Moreover, *Cordia africana* and *Pouteria adolfi-friederici* are the most over exploited important timber trees with excess production of sawdusts at each wood processing firm in the country (FAO, 1968). Similarly, there are approximately 35 million cattle and 32.2 million chickens (FAO, 1999) in Ethiopia that can potentially discharge significant amount of organic wastes (manures) to the environment. Voluminous number of literature has reported the possibility of using these agro-wastes for a range of purposes (that is,

mushrooms cultivation) in order to generate value added products and reduce environmental pollution.

Despite the high diversity of wild edible mushrooms in Africa including Ethiopia, very little of it is known. Cultivation and production of mushrooms has not been practiced on commercial scales in most developing countries which has consequently affected commercial mushroom marketing which is yet to be embraced by most farmers (Abate, 1998; Olumide, 2007). In developing countries, governmental and non-governmental organizations have not given due attention to mushrooms as an important crop that can fetch farmers a substantial income to alleviate poverty (Olumide, 2007). Similarly, it is well accepted that mushrooms are not a luxury food but a national necessity to combat poverty and malnutrition (Chang, 2007). 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 different locally available substrates and their combinations for cultivation of *P. ostreatus* and to estimate yields of cultivated mushrooms on different locally available cheap substrates.

## MATERIALS AND METHODS

### The study area

The study was conducted at Jimma University. Jimma is located at 350 km south-west of Addis Ababa. It is found in Oromia Regional State (Figure 1) which lies within 7°15'N to 8°45'N latitude and 35°30'E to 37°30'E longitude. The altitude of the area ranges from 1300 to 2100 m. The mean annual rainfall of the area is between 1800 to 2300 mm with maximum rainfall between months of June and September. The annual mean temperature of the area is between 15 and 22°C.

Main substrate collection was carried out from Bonga district, Kaffa zone, South Nations Nationalities and Peoples Regional State (SNNPRS), which is located at about 120 km from Jimma and 455 km southwest from the capital of the country. Generally, southwest Ethiopia is characterized by receiving high amount of annual rainfall, high humidity and having relatively large area vegetation coverage (Ersado, 2001; Tuno, 2001).

### Culture source

Plate and slant pure cultures of *P. ostreatus* were obtained from Mycology Laboratory, Department of Biology, Addis Ababa University (AAU).

### Pure culture maintenance

Pure cultures from AAU were transferred into sterile potato dextrose agar (PDA) plates and slants. The plate and slant cultures were stored at ambient temperature ( $22 \pm 2^\circ\text{C}$ ) for 7 days; after which the slants were kept in a refrigerator at  $4 \pm 2^\circ\text{C}$ .



Figure 1. Map of the study area.

### Spawn preparation

Clean sorghum and wheat grains were obtained from local markets. Sorghum (95%) and shredded wheat (5%) dry weight were used for the spawn preparation. The dry sorghum was weighed and soaked in water for 36 h. After the grain imbibed and reached at its 60% moisture, it was mixed with 5% shredded wheat and 1% gypsum, and packaged (leaving air space) in different glass jars of 300 to 500 ml volume. Each jar was filled  $\frac{3}{4}$  of its volume with spawn substrate. The packaged content was then sterilized in steam (autoclave) at 15 psi for 1 h. The jars containing sterile sorghum grains were inoculated aseptically by transferring approximately 1 cm<sup>2</sup> blocks of fungal cultures sliced to pieces using sterile scalpels. Thereafter, the jars were kept at ambient temperature ( $22 \pm 2^\circ\text{C}$ ) and inspected until the grains had been fully colonized by mycelia and the spawns turn white (cottony) in the jars. The jars were shaken on the 3<sup>rd</sup> and 8<sup>th</sup> days to hasten through invasion of the grains by the mushroom mycelia.

### Mushroom growing room

A 3 × 3 m aseptic room was prepared at Research and Post Graduate Laboratory, Department of Biology, Jimma University ahead of time by washing the walls, the sealing and the floor by detergent (savlon). Shelves were set in the room that accommodates 24 treatment bags. Windows were closed and covered with

thick cartoons to create dark environment for incubation of mushroom bags.

### Main substrates

#### Types, sources and collection of main substrates

Three different types of main substrates namely, sawdust, coffee bean husks, and corncobs were used. Sawdusts from two types of trees such as *C. africana* 'Wanza' (sd<sup>1</sup>C) and *P. adolfi-friederici* 'Kerero' (sd<sup>2</sup>A), were collected from private wood workshops. Coffee bean husks (Ch) were collected from coffee mill (a small private industry in the area) and corncobs (Zc) from resident farmers around Bonga.

#### Substrate preparation and combinations

Sawdust and coffee bean husks were used directly while the corncobs were mechanically broken down into small pieces (approximately 1 cm<sup>3</sup>) with local mortar and pestle. *C. africana* (sawdust), *P. adolfi-friederici* (sawdust), coffee bean husks and corncobs were used separately as growth substrates. Combinations of *C. africana* sawdust and coffee bean husks (sd<sup>1</sup>Ch), *P. adolfi-friederici* sawdust and coffee bean husks (sd<sup>2</sup>Ch), and corncobs and coffee bean husks (ZcCh); all 50 to 50% were prepared. In addition, 25% composite of each of the four original substrates (sd<sup>1</sup>C, sd<sup>2</sup>A, Ch

was supplemented in the main substrate and and Zc). In total, there were eight treatments. Shredded wheat (5%) 1% gypsum was also used as additives.

After 24 h of soaking in water, the excess water was removed from the moist substrate by decanting and manually squeezing by hand. Following the method of Atikpo et al. (2008), 1.2 kg (dry weight) of each of the eight main substrates were mixed in clean separate plastic containers with 60 g (dry weight) of shredded wheat and 12 g of gypsum. The substrates were weighed to test their appropriate moisture content (70 to 80%). Then, each of the eight main substrates was divided into three heat resistant polypropylene bags – a total of 24 bags with the dimension of 20 × 30 cm. Each bag contained 400 g dry weight of the main substrate. The bags were labeled according to substrate they contained and autoclaved at 15 psi for 1 h and kept in a clean room for 12 h until they cooled down.

### Spawning and spawn running

After the substrates in the polypropylene bags were cooled, the content in each of the bags was transferred into another 24 (triplicates of the eight substrates) ultraviolet ray (UV) sterilized transparent polyethylene bags. Thereafter, 40 g (10% of the substrate) of *P. ostreatus* spawn was added into the polyethylene bags using sterile spoons under laminar flow hood. The 1<sup>st</sup> and 2<sup>nd</sup> replicate bags were spawned by localized spawning at the open end and at three locations (top, middle and bottom), respectively.

The 3<sup>rd</sup> replicate bag was spawned by throughout mixing of the spawn into the substrate. Then, the open end of the bags was tied by rubber bands and numbers of small holes were made using sterile needle to allow air exchange of bags. Finally, all the bags were incubated at ambient temperature (22 ± 2°C) on shelves in a completely randomized design.

### Incubation and control of the environment

All cultivated bags were incubated in a complete darkness. Fresh air exchange between the dark room and the outside environment was allowed by opening windows at night. Plastic containers were filled with water and placed open at every corner of the dark room to maintain appropriate humidity in the room for the mycelial growth. In addition, water was sprayed on the walls. This condition can maintain approximately 70 to 80% humidity as suggested by Oei (2005). All treatment bags were inspected on the basis of 3 days to observe contaminations that could happen. After 20 days of incubation, close observation of the bags was made to recognize complete spawn run and primordia formations.

### Mycelial growth measurement

Mycelial extension through the substrates in the localized treatment bags was measured in weekly basis using transparent graduated ruler following the method used by Rajapakse et al. (2007) and the results were recorded.

### Induction of fruiting

Following the method described by Kivaishi (2007), after the incubation period, when the bags were completely colonized by the mycelium, they were opened to induce formation of fruit bodies. Holes (approximately 2 cm in diameter) were made in the sides of bags to allow pinheads to emerge. The bags were watered three times a day, and higher relative humidity was maintained by spraying water on the walls of the room on daily basis.

### Harvesting and yield measures

Mature mushrooms were picked by hand without harming the substrate when they started to wrinkle-ripe. This was done for three consecutive flushes. For incubation between flushes, the bags were not watered for 5 days. Following the method of Iqbal et al. (2005), the yield parameters were recorded with respect to time (days) taken for completion of spawn running, time taken for the first appearance of pinhead formation, time taken for maturity of fruit bodies, number of flushes, time interval between flushes and yield of flushes on the treatment substrates. The pileus diameter and the stipe length were measured with graduated transparent ruler. Mature mushrooms were weighed with analytical balance to determine the biological efficiency (BE) of mushrooms produced from substrates. The average bioconversion efficiency of harvests was computed according to the following formula (Peng et al., 2000):

$$\% \text{ Yield} = \frac{\text{Weight of fresh mushroom harvested}}{\text{Dry weight of dry substrate before inoculation}} \times 100$$

### Data analysis

Data were analyzed statistically on the basis of substrates and yield parameters per flush using SPSS window version 15.0, SPSS Inc, Chicago, IL, USA. Analysis of variance (ANOVA) was used to indicate significant mean differences at 95% confidence interval.

## RESULTS

### Mycelial invasion

Replicate bags showed mycelial extension originating from the spawn grains in the substrate just after 24 h of spawning. Complete invasion of the whole substrate was seen within 29 ± 2.3 days (Table 1). The mean rate of mycelial running among substrate types used was varied markedly (CV > 10%). The highest running rate was observed in sdZcCh (0.69 cm/day) followed by ZcCh (0.64 cm/day), sd<sup>2</sup>A (0.61 cm/day), sd<sup>1</sup>Ch and sd<sup>2</sup>Ch (0.60 cm/day), Zc (0.57 cm/day), and sd<sup>1</sup>C (0.44 cm/day). The lowest mycelial running rate (0.17 cm/day) was observed in Ch (Table 1).

### Changes after spawning (cultivation)

Except for Ch, all the rest of the substrates gave 1<sup>st</sup> to 3<sup>rd</sup> flushes. In some cases, 4<sup>th</sup> flush was also harvested (data not shown). On the other hand, all bags containing pure coffee bean husks showed very slow mycelial growth which totally ceased after 15 days. As a result, all bags containing coffee bean husk were negative for primordia formation and flushes.

### Incubation periods of substrates

The mean duration of incubation between the first and second flushes was 12 days (Table 2). Variations in the

**Table 1.** Mycelial extension corresponding to substrate treatments.

| Substrate          | Mycelial extension (cm) |          | Mean value<br>(cm/day) | Complete invasion<br>(day) |
|--------------------|-------------------------|----------|------------------------|----------------------------|
|                    | 7th day                 | 14th day |                        |                            |
| sd <sup>1</sup> C  | 2.8                     | 6.2      | 0.44                   | 23                         |
| sd <sup>2</sup> A  | 3.5                     | 8.6      | 0.61                   | 20                         |
| Zc                 | 3.2                     | 8.0      | 0.57                   | 23                         |
| Ch                 | 1.5                     | 2.4      | 0.17                   | **                         |
| sd <sup>1</sup> Ch | 3.6                     | 8.4      | 0.60                   | 22                         |
| sd <sup>2</sup> Ch | 3.9                     | 8.4      | 0.60                   | 23                         |
| ZcCh               | 4.2                     | 9.0      | 0.64                   | 17                         |
| sdZcCh             | 4.4                     | 9.6      | 0.69                   | 17                         |
| %CV                | 27.50                   | 32.05    | 32.70                  | 11.35                      |

CV, Coefficient of variation; \*\*, mycelial growth ceased.

**Table 2.** Incubation periods of treatment substrates

| Substrate          | Mean incubation period (days)       |                                         |                                         |                                         |
|--------------------|-------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|
|                    | Inoculation - 1 <sup>st</sup> Flush | 1 <sup>st</sup> - 2 <sup>nd</sup> Flush | 2 <sup>nd</sup> - 3 <sup>rd</sup> Flush | 3 <sup>rd</sup> - 4 <sup>th</sup> Flush |
| sd <sup>1</sup> C  | 31.0 <sup>a</sup>                   | 12.7 <sup>ab</sup>                      | 10.0 <sup>a</sup>                       | -                                       |
| sd <sup>2</sup> A  | 31.3 <sup>a</sup>                   | 11.0 <sup>ab</sup>                      | 9.0 <sup>a</sup>                        | -                                       |
| Zc                 | 31.3 <sup>a</sup>                   | 18.3 <sup>a</sup>                       | 7.7 <sup>a</sup>                        | -                                       |
| sd <sup>1</sup> Ch | 30.7 <sup>a</sup>                   | 13.7 <sup>ab</sup>                      | 5.3 <sup>a</sup>                        | 6.3 <sup>b</sup>                        |
| sd <sup>2</sup> Ch | 31.0 <sup>a</sup>                   | 5.7 <sup>b</sup>                        | 6.7 <sup>a</sup>                        | 5.0b <sup>c</sup>                       |
| ZcCh               | 25.0 <sup>b</sup>                   | 11.7 <sup>ab</sup>                      | 6.7 <sup>a</sup>                        | 9.7 <sup>a</sup>                        |
| sdZcCh             | 25.0 <sup>b</sup>                   | 10.7 <sup>ab</sup>                      | 7.7 <sup>a</sup>                        | 4.7 <sup>c</sup>                        |

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

incubation periods among substrates during 2<sup>nd</sup> to 3<sup>rd</sup> flushes were not significant ( $p > 0.05$ ).

### Maturation period of oyster fruit bodies

Most treatment bags took 3 to 4 days from pinning to maturation of mushroom fruit body. After 4 days, mushrooms became ready for picking (Figure 2f). Figure 2 shows the progressive development of oyster mushroom.

Duration for the maturation of fruit bodies after pinhead formation showed variations among different substrates and replicates. Generally,  $4 \pm 0.7$  days were recorded among the substrates and different flushes. Except at the 3<sup>rd</sup> flush, significant variations ( $p < 0.05$ ) of period of maturation were recorded among the substrates treated (Table 3).

### Yield per flush of substrates

Yield per flush of substrates showed no significant ( $p > 0.05$ ) variations in the 1<sup>st</sup> and 3<sup>rd</sup> flushes, whereas significant ( $p < 0.05$ ) variations were observed in the fresh weight obtained from 2<sup>nd</sup> and 4<sup>th</sup> flushes (Table 4).

### Biological efficiency of substrates

Mushrooms harvested from each treatment bag varied among the substrates in its yield parameters. The highest mean weight of mature mushrooms was obtained from the sdZcCh substrate that is, 309.5 g (Table 5). Mean pileus diameter of mushrooms ranged from 3.8 to 5.2 cm (Table 6). However, variations of pileus diameter among substrates were not significant ( $p > 0.05$ ). Similarly, mean variations of stipe length of mature mushrooms was not significant ( $p > 0.05$ ) among different substrates which was ranged from 1.4 to 1.9 cm (Table 5). Number of mature mushrooms, mean weights of mature mushrooms harvested and aborted pinheads were significantly ( $p < 0.05$ ) varied among substrate types (Table 5).

About 57.14% of the treatment substrates had more than 50% BE (Figure 3). Sawdust of *C. africana* (sd<sup>1</sup>C) gave the least percentage of BE (29.07%) whereas combination from the four original substrates (sdZcCh) showed the highest BE (77.38%). Others, namely, combination of corncobs and coffee bean husks, combination of sawdust of *P. adolfi-friederici* and coffee bean husks, sawdust of *P. adolfi-friederici*, combination of sawdust of *C. africana* and coffee bean husks, and corncobs, had BE

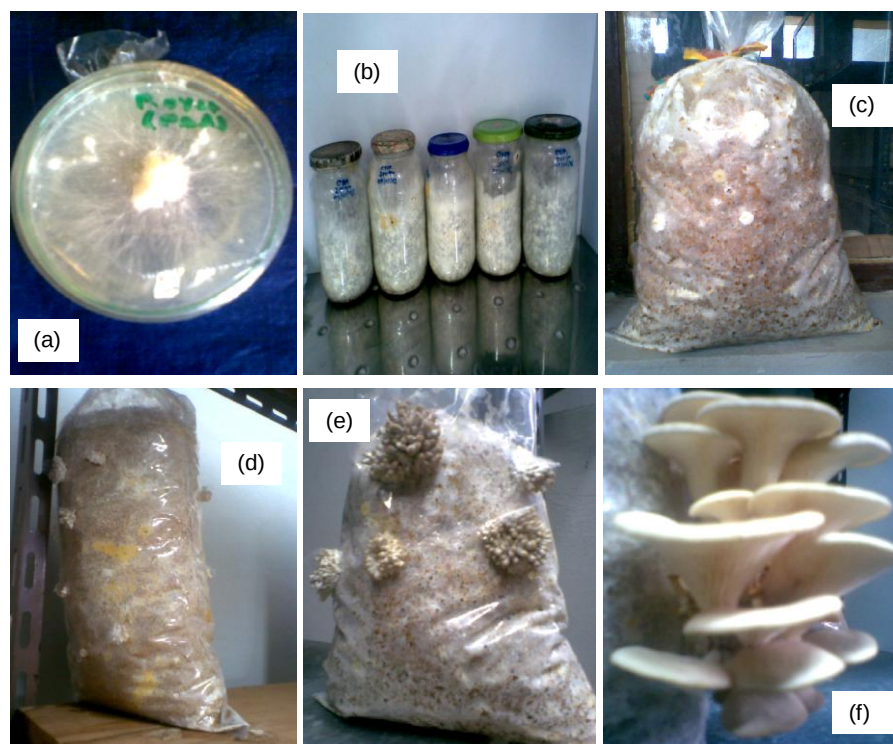


**Figure 2.** Progressive development of mushroom: (a), Pure plate culture of *P. ostreatus*; (b), oyster spawns ready for use; (c), primordia formed inside bags; (d), pinheads emerging out 2<sup>nd</sup> day; (e), elongating pinheads 3<sup>rd</sup> day; (f), mature oysters 5<sup>th</sup> day.

**Table 3.** Period of pinning-to-maturation of mushrooms in substrates

| Substrate          | Mean duration (days) of maturation |                       |                       |                       |
|--------------------|------------------------------------|-----------------------|-----------------------|-----------------------|
|                    | 1 <sup>st</sup> Flush              | 2 <sup>nd</sup> Flush | 3 <sup>rd</sup> Flush | 4 <sup>th</sup> Flush |
| sd <sup>1</sup> C  | 5.0 <sup>ab</sup>                  | 3.7 <sup>b</sup>      | 4.0 <sup>a</sup>      | -                     |
| sd <sup>2</sup> A  | 3.7 <sup>b</sup>                   | 4.0 <sup>ab</sup>     | 3.3 <sup>a</sup>      | -                     |
| Zc                 | 4.3 <sup>ab</sup>                  | 3.7 <sup>b</sup>      | 3.3 <sup>a</sup>      | -                     |
| sd <sup>1</sup> Ch | 4.3 <sup>ab</sup>                  | 4.0 <sup>ab</sup>     | 3.7 <sup>a</sup>      | 3.3 <sup>ab</sup>     |
| sd <sup>2</sup> Ch | 6.0 <sup>a</sup>                   | 4.3 <sup>ab</sup>     | 3.7 <sup>a</sup>      | 4.3 <sup>a</sup>      |
| ZcCh               | 5.3 <sup>ab</sup>                  | 4.0 <sup>ab</sup>     | 4.0 <sup>a</sup>      | 4.0 <sup>ab</sup>     |
| sdZcCh             | 5.3 <sup>ab</sup>                  | 5.3 <sup>a</sup>      | 3.3 <sup>a</sup>      | 3.0 <sup>b</sup>      |

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

in an increasing order of 43.48, 48.55, 52.03, 55.35 and 55.78%, respectively (Figure 3). Generally, BE was significantly different ( $p < 0.05$ ) between sdZcCh and sd<sup>1</sup>C, sd<sup>2</sup>A, and ZcCh; whereas differences in the BE among sd<sup>2</sup>A, Zc, sd<sup>1</sup>Ch, sd<sup>2</sup>Ch, ZcCh, and sdZcCh were not significant ( $p > 0.05$ ).

## DISCUSSION

Despite wide variety and ecological distribution of wild edible mushrooms in Ethiopia, particularly in the south western part, there are no research findings that have

been conducted regarding cultivation of exotic mushrooms or domestication of the indigenous mushrooms in the southwestern part of the country. Regardless of high timber extraction in the country from trees such as *C. africana* and *P. adolfi-frederici*, their usage as main substrates for mushroom cultivation is quite uncommon. Moreover, locally shredded wheat as substrate supplement considered in this study was the first of its kind in our country. Most reports showed that substrates could be supplemented with wheat or rice bran usually 10 to 50% (Peng et al., 2000; Atikpo et al., 2008; Pathmashini et al., 2008) for mushroom cultivation. In this study, however,

**Table 4.** Mean yield per flush per 400 g (dry weight) substrate

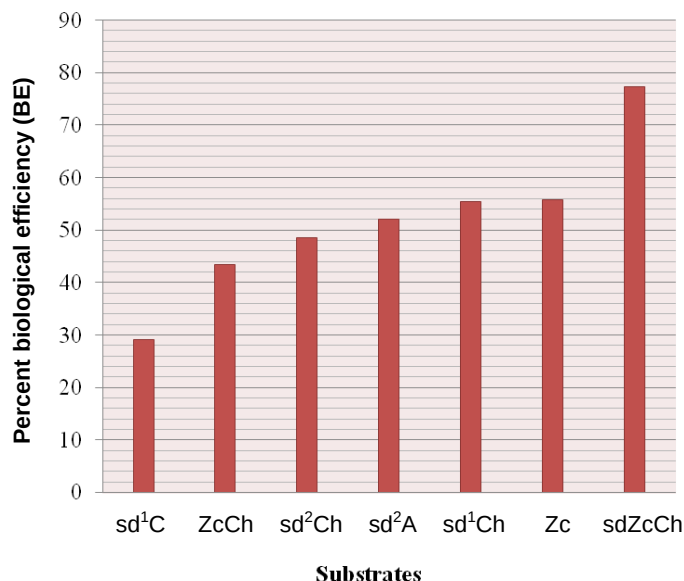
| Substrate          | Mean fresh weight of mushrooms (g) |                       |                       |                       |
|--------------------|------------------------------------|-----------------------|-----------------------|-----------------------|
|                    | 1 <sup>st</sup> Flush              | 2 <sup>nd</sup> Flush | 3 <sup>rd</sup> Flush | 4 <sup>th</sup> Flush |
| sd <sup>1</sup> C  | 35.4 <sup>a</sup>                  | 47.8 <sup>ab</sup>    | 44.9 <sup>a</sup>     | -                     |
| sd <sup>2</sup> A  | 65.0 <sup>a</sup>                  | 72.4 <sup>ab</sup>    | 70.7 <sup>a</sup>     | -                     |
| Zc                 | 68.6 <sup>a</sup>                  | 80.7 <sup>a</sup>     | 73.9 <sup>a</sup>     | -                     |
| sd <sup>1</sup> Ch | 96.0 <sup>a</sup>                  | 52.5 <sup>ab</sup>    | 60.2 <sup>a</sup>     | 12.7 <sup>b</sup>     |
| sd <sup>2</sup> Ch | 70.0 <sup>a</sup>                  | 34.8 <sup>ab</sup>    | 54.5 <sup>a</sup>     | 35.0 <sup>b</sup>     |
| ZcCh               | 64.3 <sup>a</sup>                  | 29.3 <sup>b</sup>     | 47.3 <sup>a</sup>     | 32.9 <sup>b</sup>     |
| sdZcCh             | 93.7 <sup>a</sup>                  | 84.0 <sup>a</sup>     | 67.1 <sup>a</sup>     | 64.7 <sup>a</sup>     |

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

**Table 5.** Effect of substrates on yield parameters of mushrooms

| Substrate          | Mean yield measures/400 g substrate |                   |                    |                     |                     |
|--------------------|-------------------------------------|-------------------|--------------------|---------------------|---------------------|
|                    | Pileus diameter (cm)                | Stipe length (cm) | Number of matures  | Number of aborts    | W/t of mature       |
| sd <sup>1</sup> C  | 5.2 <sup>a</sup>                    | 1.4 <sup>a</sup>  | 6.9 <sup>b</sup>   | 9.3 <sup>bc</sup>   | 116.3 <sup>b</sup>  |
| sd <sup>2</sup> A  | 4.6 <sup>a</sup>                    | 1.6 <sup>a</sup>  | 14.4 <sup>ab</sup> | 14.2 <sup>abc</sup> | 208.1 <sup>ab</sup> |
| Zc                 | 5.1 <sup>a</sup>                    | 1.8 <sup>a</sup>  | 12.1 <sup>b</sup>  | 7.7 <sup>c</sup>    | 223.1 <sup>ab</sup> |
| sd <sup>1</sup> Ch | 4.4 <sup>a</sup>                    | 1.6 <sup>a</sup>  | 14.9 <sup>ab</sup> | 16.2 <sup>abc</sup> | 221.4 <sup>ab</sup> |
| sd <sup>2</sup> Ch | 4.0 <sup>a</sup>                    | 1.8 <sup>a</sup>  | 10.7 <sup>b</sup>  | 18.8 <sup>abc</sup> | 194.2 <sup>b</sup>  |
| ZcCh               | 3.8 <sup>a</sup>                    | 1.8 <sup>a</sup>  | 9.4 <sup>b</sup>   | 22.3 <sup>a</sup>   | 173.9 <sup>b</sup>  |
| sdZcCh             | 4.3 <sup>a</sup>                    | 1.9 <sup>a</sup>  | 21.7 <sup>a</sup>  | 21.6 <sup>ab</sup>  | 309.5 <sup>a</sup>  |

Mean values within a column sharing the same superscript letter(s) are not significantly different by using DUNCAN test at  $p = 0.05$ ; W/t, weight.

**Figure 3.** Biological efficiency per 400 g dry substrates

in this study, findings on the incubation periods of the first pinning were relatively shorter than 35 to 51 and 46 days reported by Pathmashini et al. (2008) and Peng et al.

(2000), respectively. High variability of incubation periods of *Pleurotus* species were also reported by Iqbal et al. (2005) from three different substrates in which sugar bagasse took 16 to 23 days, cotton waste 27 to 32 days and wheat straw took 43.7 to 46.3 days. Relatively shorter mean period of the first pinning in this study might be due to the amount of spawn inoculated into the substrates. Pathmashini et al. (2008) and Iqbal et al. (2005) spawned 1 and 0.25%, respectively whereas 10% sorghum grain spawn was used to inoculate substrates in this study. Relatively higher room temperature could have also resulted in shorter pinning periods as comparable results (27 to 34 days of incubation) obtained from Shah et al. (2004) at 25°C. In general, it takes longer period of incubation for oyster mushrooms at lower temperatures and relative humidity (Zadrazil, 1976; Daba et al., 2008).

One of the important factors for growing mushrooms commercially is cropping period (Islam et al., 2009). With regard to the period of pinning to maturation of mushrooms (this study), the shortest mean duration was 3.3 days and the longest was 6.0 day throughout the treatment substrates and flushes. This agrees with the range of maturation period (3.29 to 4.33) of *Pleurotus* species reported by Islam et al. (2009). Mushrooms took longer mean maturation periods in the first flush than the three other consecutive flushes which was significantly varied.

**Table 6.** Effect of substrates on yield parameters of mushrooms.

| Substrate          | Mean yield measures /400 g substrate |                   |                    |                     |                     |
|--------------------|--------------------------------------|-------------------|--------------------|---------------------|---------------------|
|                    | Pileus diameter (cm)                 | Stipe length (cm) | Number of matures  | Number of aborts    | W/t of mature       |
| sd <sup>1</sup> C  | 5.2 <sup>a</sup>                     | 1.4 <sup>a</sup>  | 6.9 <sup>b</sup>   | 9.3 <sup>bc</sup>   | 116.3 <sup>b</sup>  |
| sd <sup>2</sup> A  | 4.6 <sup>a</sup>                     | 1.6 <sup>a</sup>  | 14.4 <sup>ab</sup> | 14.2 <sup>abc</sup> | 208.1 <sup>ab</sup> |
| Zc                 | 5.1 <sup>a</sup>                     | 1.8 <sup>a</sup>  | 12.1 <sup>b</sup>  | 7.7 <sup>c</sup>    | 223.1 <sup>ab</sup> |
| sd <sup>1</sup> Ch | 4.4 <sup>a</sup>                     | 1.6 <sup>a</sup>  | 14.9 <sup>ab</sup> | 16.2 <sup>abc</sup> | 221.4 <sup>ab</sup> |
| sd <sup>2</sup> Ch | 4.0 <sup>a</sup>                     | 1.8 <sup>a</sup>  | 10.7 <sup>b</sup>  | 18.8 <sup>abc</sup> | 194.2 <sup>b</sup>  |
| ZcCh               | 3.8 <sup>a</sup>                     | 1.8 <sup>a</sup>  | 9.4 <sup>b</sup>   | 22.3 <sup>a</sup>   | 173.9 <sup>b</sup>  |
| sdZcCh             | 4.3 <sup>a</sup>                     | 1.9 <sup>a</sup>  | 21.7 <sup>a</sup>  | 21.6 <sup>ab</sup>  | 309.5 <sup>a</sup>  |

Mean values within a column sharing the same superscript letter(s) are not significantly different by using DUNCAN test at  $p = 0.05$ ; W/t: weight.

This could be the effect of light during the incubation period because it was the only condition differed between the 1<sup>st</sup> and other flushes. The treatment bags were stayed in the dark room before the first flush but not for the rest. With regard to variation in yields of mushrooms per flush, Kimenju et al. (2009) indicated that yields in substrates slightly decline from the first flush. But this is the case only with the substrates of four combinations in this study. In all of the six substrates (except sdZcCh), the phase of flushes did not have any effect on the yield. However, the least result was recorded from the fourth flush in most combination treatments whereas the 4<sup>th</sup> flush was totally absent in pure substrates (Table 6). Results reported from different investigators varied from three to six flushes from the same *Pleurotus* species. For instance, Tan (1981) obtained three flushes and Iqbal et al. (2005) obtained three to four flushes from different substrates. The results of this study indicated that combinations of substrates gave yields for higher number of flushes (four flushes) than pure substrates (three flushes). On the other hand, despite higher number of flushes harvested from substrate combinations, the total yield of pure substrates such as saw dusts of *P. adolfi-friederici*, and corncobs (*Zea mays*) gave relatively higher yields. This could be due to the negative effect coffee bean husks which might lower the yield in substrate combinations which needs further treatment to remove the inhibitory substances.

In general, majority of the substrates showed above 50% BE. Except for the pure coffee bean husks, in which case no yield obtained, all the rest of substrates had yields ranging from 29.07 to 77.38% BE. This is lower than results obtained from Kimenju et al. (2009) which was 68 to 106% but comparable to 44 to 47.1% BE from Shen and Royse (2001), 43.3 to 62.7% BE from Iqbal et al. (2005), and 67.5% average BE reported by Chang (2007). Tan (1981) reported 100% BE from dry weight of the cotton waste substrate. Significant variations in the amount of yield among substrates clearly indicated the quality and quantity of nutritious components contained in the substrates. Higher yields were obtained from pure

substrates than combinations except pure coffee husks.

Generally, those substrates with lower yield gave mature oyster mushrooms with shorter mean stipe length, larger pileus diameter and less number of mature mushrooms. The highest mean pileus diameter was observed in sd<sup>1</sup>C and the lowest in ZcCh. Kimenju et al. (2009) also reported comparable results from sugarcane bagasse (4.8 cm) and sawdust (3.4 cm). Individual observations in this study revealed that big mushroom caps with wider pileus diameter occurred when fewer numbers of fruit bodies accompanied bunches from the same origin in the substrates. This may be because of less localized competition that existed in fewer fruit body containing bunches. Since majority of substrates that gave higher yield also contained higher number of propagating fruit bodies per bunches, they produced smaller pileus with longer stipe lengths. On the contrary, substrates such as sawdust of *C. africana*, from which bunches with fewer numbers of fruit bodies were collected, had oyster fruit bodies with the largest pileus diameter and shortest stipe lengths.

The mean stipe length was longest in mushrooms grown on sdZcCh and ZcCh whereas the shortest observed in sd<sup>1</sup>C. Islam et al. (2009) reported the longest stalk from kadom sawdust (3.59 cm) and the shortest from coconut sawdust (2.20 cm). Although substrates had no significant effect on the stipe length of the mushrooms, it could be deduced that sawdust of *C. africana* and corncobs had some positive effect on the mushroom fruit bodies by producing relatively longer stipe lengths. Regarding acceptable food quality of oyster mushrooms, fruit bodies with larger pileus and shorter stipes are better than that with smaller pileus and longer stipes. However, the stipes contains more insoluble dietary fibers that can be used for the preparation of biologically active polysaccharide complexes utilizable as food supplements than pilei (Synytsya et al., 2008). On the contrary, Peng et al. (2000) described that larger caps break easily during packing resulting in low quality of the product. As to the findings of this study, the criteria of larger pilei and shorter stems best fit to the results from sawdust of *C. africana*, but it was the least with regard to BE. Low yield

from the sawdust of *C. africana* can be associated with accumulation of heavy metal ions such as lead ( $Pb^{++}$ ) and nickel ( $Ni^{++}$ ) from water (Andrabi, 2010); which might had inhibitory effect on the growth of mushroom mycelia. However, the yields obtained from the sawdusts of the tree species in this study were remarkably higher than 3 to 21% BE from different deciduous tree sawdusts reported by Pavlik (2005).

In majority of the substrates, the number of pinhead abortions exceeded number of matures. It was also reported by Kimenju et al. (2009) in which more than 50% of pinheads emerged did not grow into marketable products. However, the number of abortions was less than the number of matures in most of substrates from which higher yields obtained in this study. On the other hand, high number of pinheads aborted from low-yield substrates such as  $sd^1C$  and  $ZcCh$ . This, in general, means that the more productive the substrate is, the less the number of mushroom pinheads to be aborted. Generally, the number of mature mushrooms and aborted pinheads were the highest at the first flush but decreased with the consecutive flushes. This might indicate that vigorous primordia formation occurred in the substrates after the longest period of incubation just before the first flush which might fall with exhaustion of the raw material and period of incubation between flushes.

Coffee bean husks were found resistant to oyster mycelial extension since no complete invasion of oyster mycelia was observed. Coffee bean husks have some chemical components that inhibit mycelial growth. Salmones et al. (2005) reported that caffeine accumulates in fruiting bodies of oyster mushrooms when wheat straw and coffee pulp mixture substrates used. In relation to this, high concentration of caffeine from pure coffee bean husks might have inhibitory effect on invasion of oyster mycelia.

In conclusion, except for pure coffee bean husks, all of the agro-wastes treated in this study gave good yields of *P. ostreatus* which indicates the possibility of oyster mushroom cultivation on the locally available cheap substrates including the sawdusts of *C. africana* and *P. adolfi-friederici*. With regard to the amount of yield obtained, corncobs performed the best, followed by sawdusts of *P. adolfi-friederici* and sawdusts of *C. africana*. Incubation periods of all substrates were relatively shorter than reported by several researchers. Combination of substrates could produce more than three flushes whereas pure substrates gave only three flushes.

It is also imperative to initiate further studies for better utilization of coffee husks by removing the inhibitory component contained in this agro-waste through bioconversion processes. Animal protein is beyond the reach of most people in economically disadvantaged countries. Hence, promotion of mushroom cultivation using agricultural wastes can improve the difficulty of food insecurity, fill the protein gap, and consequently solve malnutrition problems.

## ACKNOWLEDGEMENTS

The financial support of Jimma University is gratefully acknowledged. Authors would also like to thank the anonymous reviewers for their inestimable critics.

## REFERENCES

- Abate D (1998). Mushroom cultivation: A practical approach. Berhanena Selam Printing Enterprise, Addis Ababa, Ethiopia.
- Andrabi SMA (2010). Sawdust of lam tree (*Cordia africana*) as a low-cost, sustainable and easily available adsorbent for the removal of toxic metals like Pb(II) and Ni(II) from aqueous solution. Eur. J. Wood Wood Prod. 69:75-83.
- Atikpo M, Onokpise O, Abazinge M, Louime C, Dzomeku M, Boateng L Awumbilla (2008). Sustainable mushroom production in Africa: A case study in Ghana. Afr. J. Biotechnol. 7:249–253.
- Beetz A, Kustudia M (2004). Mushroom cultivation and marketing: Horticulture production guide. Horticulture Production Guide, ATTRA Publication IP087.
- Chang ST (2007). Mushroom cultivation using the “ZERI” principle: Potential for application in Brazil. Micol. Appl. Int. 19:33-34.
- Daba AS, Kabeil SS, Botros WA, El-Saadani MA (2008). Production of mushroom (*Pleurotus ostreatus*) in Egypt as a source of nutritional and medicinal food. World J. Agric. Sci. 4:630-634.
- Ersado M (2001). Inventory of Woody Species in Bonga Forest. Institute of Biodiversity Conservation and Research. Technical Report No. 1. Addis Ababa, Ethiopia.
- FAO (1968). Coffee Mission to Ethiopia 1964–1965. FAO, Rome.
- FAO (1999). FAOSTAT 1999 <http://apps.fao.org/cgi-bin/nph.db.pl>.
- FAO (2009). Make money by growing mushroom. Rural Infrastructure and Agro-Industries Division Diversification booklet N<sup>o</sup> 7. United Nations, pp. 1-53.
- Gole TW, Denich M, Demel T, Vlek PLG (2002). Human impacts on *Coffea arabica* genetic pool in Ethiopia and the need for its *in situ* conservation. In: Engels J, Ramanatha Rao V, Brown AHD, Jackson M (eds.): Managing plant genetic diversity. CAB International/IPGRI. pp. 237-247.
- Intiaj A, Rahman SA (2008). Economic viability of mushroom cultivation to poverty reduction in Bangladesh. Trop. Subtrop. Agroecosyst. 8:93-99.
- Iqbal SM, Rauf CA, Sheikh MI (2005). Yield performance of oyster mushroom on different substrates. Int. J. Agric. Biol. 7:900-903.
- Islam MZ, Rahman MH, Hafiz F (2009). Cultivation of oyster mushroom (*Pleurotus flabellatus*) on different substrates. Int. J. Sustain. Crop Prod. 4:45-48.
- Jayakumar T, Ramesh E, Geraldine P (2006). Antioxidant activity of the oyster mushroom, *Pleurotus ostreatus*, on CCl<sub>4</sub>-induced liver injury in rats. Food Chem. Toxicol. 44:1989–1996.
- Jayakumar T, Thomas PA, Geraldine P (2007). Protective effect of an extract of the oyster mushroom, *Pleurotus ostreatus*, on antioxidants of major organs of aged rats. Exp. Gerontol. 42:183–191.
- Kimenju JW, Odero OM, Mutitu EW, Wachira PM, Narla RD, Muiru WM (2009). Suitability of locally available substrates for oyster mushroom (*Pleurotus ostreatus*) cultivation in Kenya. Asian J. Plant Sci. 8:510-514.
- Kivaisi AK (2007). Mushroom cultivation in Tanzania. Amelia Kajumulo Kivaisi, Dar es Salam.
- Manzi P, Gambelli L, Marconi S, Vivanti V, Pizzoferrato L (1999). Nutrients in edible mushrooms: An interspecies comparative study. Food Chem. 65:477–482.
- Oei P (2005). Small-scale mushroom cultivation: oyster, shiitake and wood ear mushrooms. Agromisa Foundation and CTA, Netherlands.
- Olumide OJ (2007). Economic analysis of mushroom marketing as a coping strategy for poverty reduction in Ondo State, Nigeria. Afr. Crop Sci. Conf. Proc. pp. 1255-1260.
- Pathmashini L, Arulnandhy V, Wijeratnam SW (2008). Cultivation of oyster mushroom (*Pleurotus ostreatus*) on sawdust. J. Biol. Sci. 37:177-182.

- Pavlik M (2005). Growing of *Pleurotus ostreatus* on woods of various deciduous trees. *Acta Edulis Fungi* 12:306–312.
- Peng JT, Lee CM, Tsai YF (2000). Effect of rice Bran on the production of different king oyster mushroom strains during bottle cultivation. *J. Agric. Res.* 49:60-67.
- Rai RD, Ahlawat OP (2002). Edible fungi: Biotechnological approaches. *Appl. Mycol. Biotechnol.* 2:87–111.
- Rajapakse JC, Rubasingha P, Dissanayake NN (2007). The effect of six substrates on the growth and yield of American oyster mushrooms based on juncao technology. *J. Agric. Sci.* 3:82-85.
- Salmones D, Mata G, Waliszewski KN (2005). Comparative culturing of *Pleurotus* spp. on coffee pulp and wheat straw: Biomass production and substrate biodegradation. *Bioresour. Technol.* 96:537–544.
- Shah ZA, Ashraf M, Ishtiaq M (2004). Comparative study on cultivation and yield performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates (wheat straw, leaves, sawdust). *Pak. J. Nutr.* 3:158–160.
- Shen Q, Royse DJ (2001). Effects of nutrient supplements on biological efficiency, quality and crop cycle time of maitake (*Grifola frondosa*). *J Appl. Microbiol. Biotechnol.* 57:74-78.
- Synytsya A, Mickova K, Jablonsky I, Slukova M, Copikova J (2008). Mushrooms of genus *Pleurotus* as a source of dietary fibers and glucans for food supplements. *Czech. J. Food Sci.* 26:441–446.
- Tan KK (1981). Cotton waste is good substrate for the cultivation of *P. ostreatus* the oyster mushroom. *Mush. Sci.* 11:705–710.
- Tuno N (2001). Mushroom Utilization by the Majangir, an Ethiopian tribe. *Mycologist* 15:78-79.
- Zadrazil F (1976). The ecology and industrial production of *Pleurotus ostreatus*, *P. florida*, *P. cornucopiae* and *P. eryngii*. *Mush. Sci.* 9:621-652.