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Study on suitability of locally available substrates for cultivation of oyster mushroom *(Pleurotus ostreatus)* in Jimma zone, Oromia regional state, southwestern Ethiopia

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A mushroom is the fleshy, spore-bearing fruiting body of a fungus, typically produced above the ground on soil. The nutritional value of mushrooms is greater than one may think. Generally, it is a nature's hidden treasures of nutrition. The aim of this study was to access the suitability of available agro wastes of some lignocelluloses materials containing five different types of main substrates namely, sawdust (Sd), cow dung (Cd), teff straw (Tfs), corn cobs (CbZ) and chat left over (ChC). During this study, rate of mycelia invasion, cap diameters, stipe length, fresh weight per flush and total yield of *Pleurotus ostreatus* were recorded, accordingly. Results indicate that, CbZ alone, CbZ*Tfs, CbZ*Sd and Cd*CbZ showed highest biological efficiency of 83.62, 72.8 to 87.5, 62.6 to 7 and 63.4 to 63.8%, respectively, while the lowest yield was obtained from Cd*ChC and Tfs*ChC (46 to 50.16%). Moreover, CbZ alone as well as in combination with other agro wastes (Tfs*CbZ and CbZ*SdC, Cd*Tfs and Chat (*Catha edulis*)) enhanced the yield of *P. ostreatus*. Thus, the currently used agro wastes, such as corn cobs, teff straw, sawdust and cow dung are promising substrate for domestic as well as industrial production of mushroom.

Key words: Agro wastes, oyster mushroom, spawn, substrate, yields.

INTRODUCTION

People in developing countries like Ethiopia often spend 60 to 80% of their income on food, but Americans spend less than 10%. Moreover, it have been reported that the amount of calories required by world countries keep rising from time to time (Choi et al*.,* 2006). By 2030, global food demand is expected to rise by 35%. On the other hand, only one in seven people are expected to be consuming less than 2,500 kilo calories per day by 2030, but this circumstance seems uncertain in developing countries.

On the other hand, mushrooms production is getting attention globally to resolve the constraints of food

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insecurity, which is also technological and economically affordable. Recently, rapid growth of the industries is increasing the deposit of waste material into the environment (Yildiz et al., 2002). Interestingly, in addition to food source, mushrooms have a great role of decomposing environmental pollutants. In addition to nutritional value, the usefulness of mushrooms for medical purposes has been indicated, such as, antitumor, anticancer, immune modulator, cardiac diseases improve blood circulation, reduce cholesterol, and diabetes (Angeli et al*.,* 2006; Choi et al*.,* 2006; Grind et al*.,* 2006).

Besides mushrooms been endowed with vital nutrients, it has a good aroma and flavoring properties (Pathmashini et al*.,* 2008). It has been understood that mushrooms, such as, oyster and shitake have contributes largely in reducing poverty, by the substitution of plant origin and animal product food, and also a source of income (Masarirambi et al., 2011).

Cultivation of mushrooms is less expensive because it requires little space and inexpensive raw materials including agricultural and industrial waste (Chang, 2007). Thus, it is rational in expanding the mushroom production, particularly in Ethiopia where the mushroom production and consumption is scarce (Yenealem et al., 2013). The mushrooms are considered as delicious and nutraceutical food for the human health but it is still not properly advertised in Ethiopia. To this effect, the present study was designed to evaluate the suitability of locally available substrates for cultivation of Oyster mushroom in Jimma zone, Oromia regional state.

MATERIALS AND METHODS

Spawn production

Pure cultures of *Pleurotus ostreatus* mushroom were obtained from Addis Ababa University. Impurity free sorghum grains had been soaked in tap water for 40 h. After the grain had absorbed water and reached 60% moisture, it was mixed with 1% $CaCO_3$ (Gume et al*.,* 2013). Then, grains supplemented with $CaCO_3$ were filled into glass jar up to ¾ of its volume, and autoclaved for 2 h. This was allowed to cool down, after which 2-pieces of agar block containing *P. ostreatus* culture were inoculated into glass jars containing sterile sorghum. These were then incubated at 25, 30, 35 and 40°C, until the grains were fully colonized by fungal mycelium.

Combinations of substrate

The substrate used for this study were, saw dust (from wood workshops), cow dung, teff straw, corncobs from local farmers around Jimma Zone, and chat gerba from chewing areas. Firstly, the substrates had been chopped into <1 cm pieces, and mixed in different ratios accordingly (1:0.75, 1:0.50, and 1: 0.25). These were then soaked in water for 12 h and the excess water drained off (Bonginkhosi et al*.,* 2012). Next, to the aerated substrate, 3% gypsum, 1% $CaCO_3$, and 5% maize bran was added (Mandeel et al., 2005). The substrates were transferred into rubber bags, and autoclaved at 121ºC for 2 h. Lastly, the polythene bags of the size 35 × 45 cm were filled with sterilized substrates and the top inoculation method was used with mushroom spawn. Both the control (only one substrate, that is, either cow dung or corn cobs or teff straw or chat gereba or sawdust) as well as polythene bags containing various combinations of substrates inoculated with fungal mycelium were arranged in randomized complete block design (RCBD) and incubated in a dark room. The experiment was performed in triplicates. The temperature and humidity of fungal cultivation room was maintained at 25ºC and 80 to 90%, respectively using a thermometer and humidity tester. When the mushroom pinhead emerged via prepared pin holes, sufficient light and air exchange was allowed by opening windows and door in the morning.

Yield measurements

The mushroom biomass, such as, number and weight of flushes per polythene bag, pileus diameter, stipe length, and maturation time were measured for four consecutive flushes (Iqbal et al*.,* 2005). Yield performance and biological efficiency of oyster mushrooms on the five kinds of substrates were calculated based on the following formula (Fan et al*.,* 2006).

$$\text{Biological Efficiency (BE)} = \frac{\text{Weight of fresh mushroom}}{\text{Dry weight of substrate}} \times 100\%$$

Statistical analysis

Results were presented as mean ± SD. Comparison of the yields among substrates was assessed using ANOVA. Statistical significance was set at $P < 0.05$.

RESULTS

Effect of temperature on spawn production

The results indicate that upon inoculation of *P. ostreatus* mycelia, the spawns were fully colonized within 14 days at room temperature as compared to a temperature range of 30 to 40°C (Figure 1). Moreover, the mean of mycelial extension, such as, 0.62, 0.4, 0.31, and 0.12 cm were recorded at 25, 30, 35 and 40°C, respectively. In general as the temperature increases the rate of mycelial ramification was decrease.

Rate of mycelia invasion of substrates

The mycelia of *P. ostreatus* fully colonized the substrates in a range of 17 to 35d (Figure 2). The highest rate of mycelia ramification was recorded in ratio of CbZ*SdC 1:0.75 (0.70 cm/day) and 1:0.50 (0.69 cm/day), while the least in CbZ*Tfs 1:0.25 (0.37 cm/day) (Figure 2). There was no significant difference observed among most of the substrates for both invasion and pinning days of *P. ostreatus* mycelia.

In addition to the combination of CbZ, CbZ*SdC and Ts*CbZ, enhanced *P. ostreatus* mycelia ramification was within a short period of time; it also permitted the development of pin heads to mature mushroom. However, Cd*SdC, and Ts*Ch, were observed as least colonized by *P. ostreatus* mycelia, and also their pin

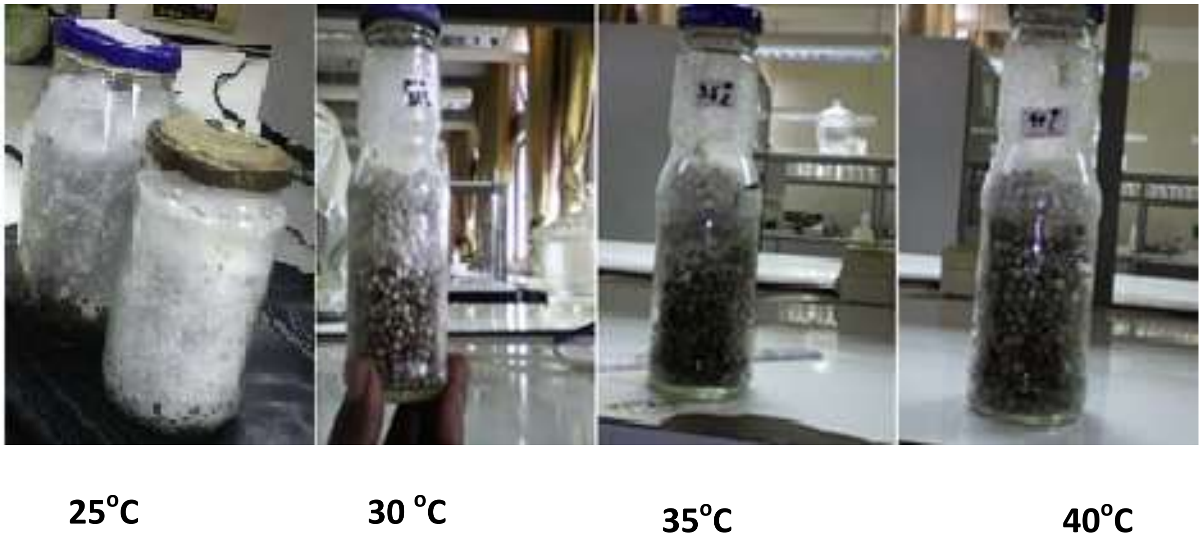

Figure 1. Spawn production at different temperature.

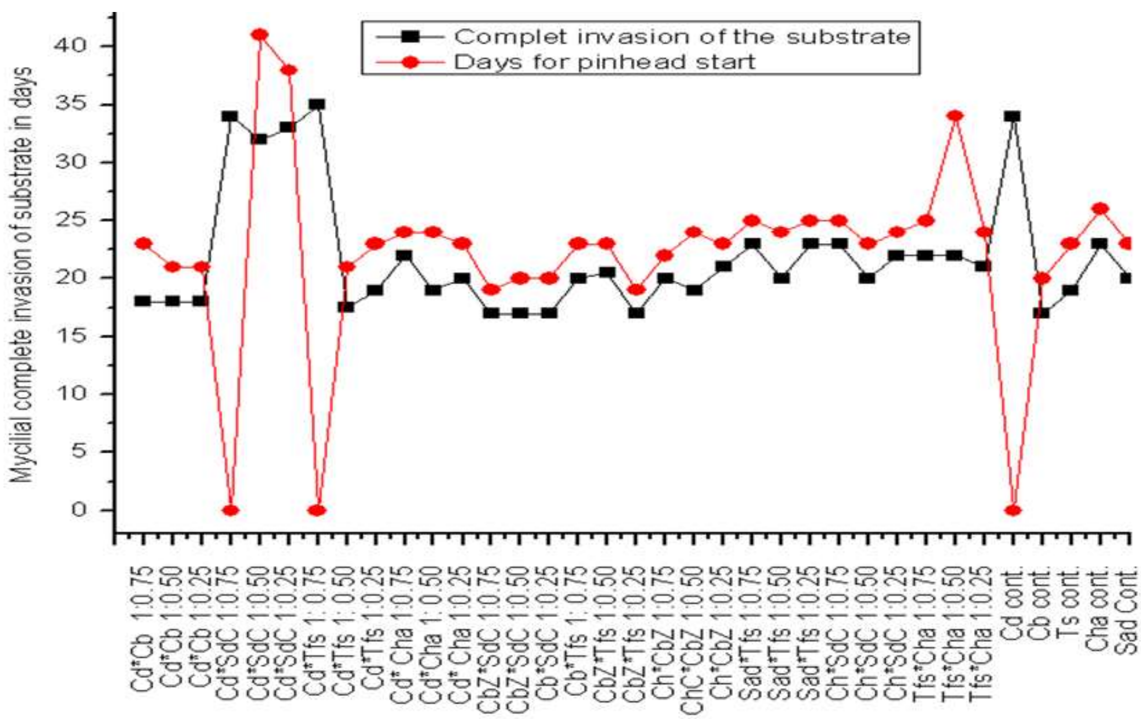

Figure 2. Days of complete invasion and pinning *P. ostreatus* across different substrate ratios. Cd*Cb (cow dung with corn cobs) = 1:0.75,1:0.50,1:0.25; Cd*SdC (cow dung with sawdust of *Cupressus lusitanica*) =1:0.75,1:0.50,1:0.25; Cd*Tfs (cow dung with teff straw) = 1:0.75,1:0.50,1;0.25); Cd*Cha (Cow dung with Chat or khat) = 1:0.75, 1:0.50,1:0.25; CbZ*SdC (corn cobs with sawdust of *C. lusitanica* = (1:0.75, 1:0.50, 1;0.25); CbZ*Tfs (corn cobs with teff straw by 1:0.75, 1:0.50,1;0.25); Cha*CbZ (chat with corn cobs = 1:0.75 1:0.50, 1;0.25); SdC*Tfs (sawdust of *C. lusitanica* with teff straw = 1:0.50, 1:0.25); Cha*SdC (chat with sawdust of *C. lusitanica* 1:0.75, 1:0.50, 1:0.25); Tfs*Cha (teff straw with chat by = (1:0.75, 1:0.50, 1:0.25) ration; cont.= control.

heads were aborted in most of the treatments. There was statistically significant difference among most of the substrates in both mycelia ramification and pinning days of *P. ostreatus* mushroom (Table 1).

Table 1. Ramification of mycelia in different combination of substrate.

Substrate	25%	50%	75%	100%
Cd*CbZ	8.2[a]	7.2[a]	8[a]	5.2[a]
Cd*SdC	7.3[b]	7.4[ab]	9[b]	9.64[b]
Cd*Ts	8.9[c]	8.6[c]	8.2[ac]	9.64[bc]
CbZ*Ts	9[c]	8.7[cd]	8.6[cd]	9[d]
Ch*CbZ	8.2[ad]	8.4[cd]	8.6[cd]	8.6[e]
ChC*Cd	8.4[ad]	8.2[cd]	7.4 [e]	8.4[ef]
SdC*Ch	8.4[ad]	9[cde]	7.8[ef]	8.4[ef]
Cd*Ts	8.2[ad]	8.3[cdf]	8.5[acg]	6.2[g]
Sd*Ts	8.6[acd]	8.4[cdf]	7.9[ef]	8.75[efh]
Ts*Ch	9.6[8e]	6.8[ag]	7.1[ef]	5.8[ai]

Values are least significant difference (LSD). LSD=0.43996.

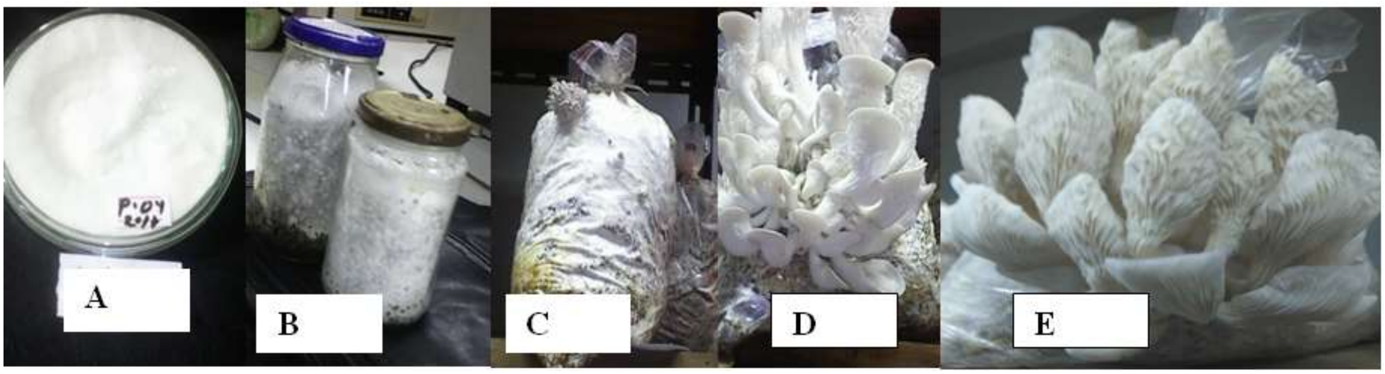

Figure 3. Mushroom cultivation. (A) Pure culture of *P. ostreatus*; (B) Mother oyster spawns; (C) pinheads emerging out at 2-3 day; (D) fruiting body of 3 days old after emerging out. (E) Mature oysters ready for harvest (5-6 days old).

Oyster mushroom maturation

After pinnate had appeared, mushroom maturation is taken 3 to 5 days in most treatment in case of 1st and 2nd flushes (Figure 3). However, Cd*SdC replicates did not provide any yield.

Yield parameters on *P. oysteretus* mushroom

During this study, comparatively, the longest stipe length, largest pileus diameter of mushroom and considerable number of pinning holes was observed in treatment with corn cobs combination as well as cobs alone.

Stipe length

In contrast to cow dung and chat mixtures, the longest stipe and highest bulk density of fruiting bodies of mushrooms were recorded from corn cobs mixtures or alone (Figure 4).

Pileus diameter

Highest pileus diameter were obtained from treatments of corn cob combinations (Cd*CbZ 1:0.50,CbZ*SdC 1:0.25, CbZ alone), and (Cd*Tfs 1:0.5), which were significantly different as compared to the control (Cd) and Cd*SdC (Figure 5).

Products per flush of substrates

The mean yield of mushroom in various substrates showed significant difference among the harvests (df = 10, 24; F = 505.372; $P<0.001$). Moreover, almost more than 75% of the total fruiting bodies were obtained from the first and second harvest, while the third and fourth harvests were relatively lower in quality as well as yield (df = 3, 72; F = 113.830; $P<0.001$) (Figure 6).

Biological efficiency of substrates

Biological efficiency was determined as the ratio of fresh

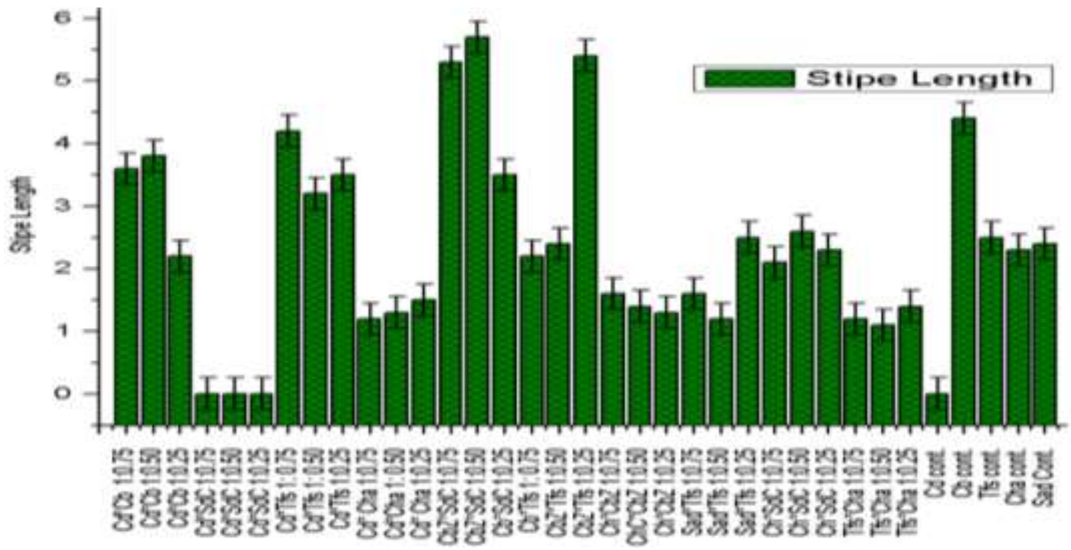

Figure 4. Stipe length (in cm) of *P. ostreatus* mushroom in different combination of substrate.

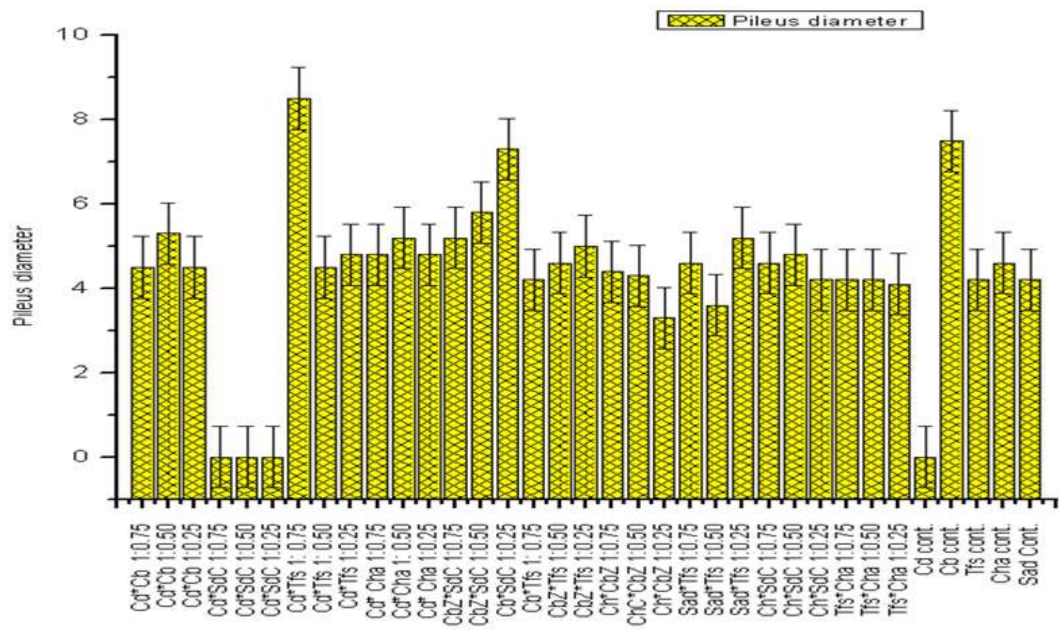

Figure 5. Pileus diameter of *P. ostreatus* mushroom across various combination of substrate.

mushrooms harvested (g) per gram of dry substrates and expressed as a percentage. Highest BE was recorded in combination of Tfs*CbZ, (1:0.75; 1:0.25 and 1:0.50), where BE, was 87.5, 79.5 and 72.8%, respectively. Moreover, the combination of Cd*Tfs (1:0.25) showed 79.9% BE (Figure 7)

DISCUSSION

In recent times, mushroom production has gained attention both globally and nationally because of its nutritional, industrial and medical value as well as its ecosystem sustainability or bioremediation. Generally, it have been understood that mushrooms such as *P. ostreatus* has a potential to turn over various agro-wastes. Thus, the substrates used in this study can be considered practical and economically feasible due to their availability throughout the year at low cost and in huge amounts in southwestern part of Ethiopia.

Utilization of these agro-wastes for the production of *P. ostreatus* mushrooms could be significant to alleviate

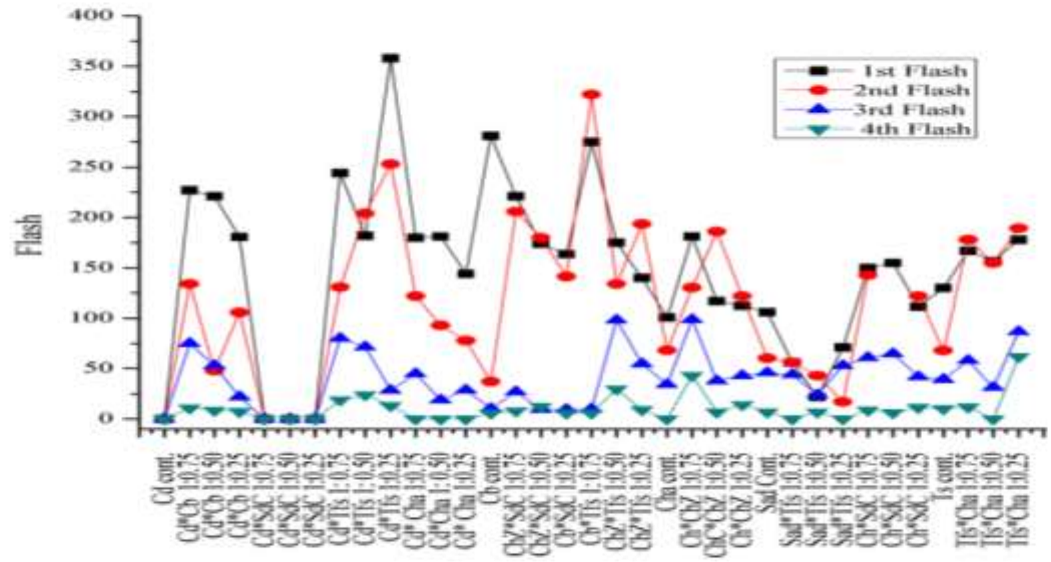

Figure 6. Mean yield per flush of dry weight substrates (400 g).

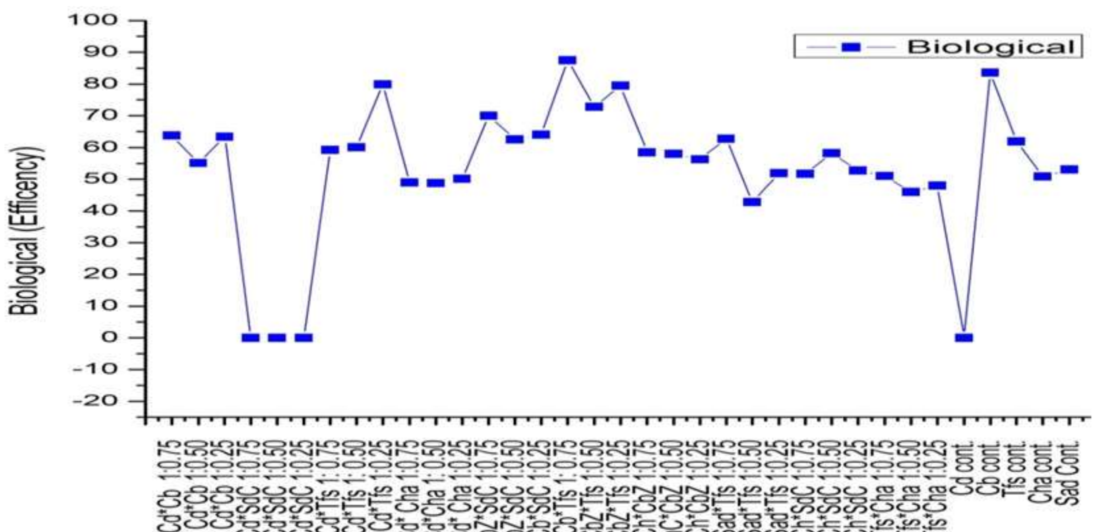

Figure 7. Biological efficiency of different substrates.

food security as well as environmental rehabilitation. This is the first report, in which the utilization of the combination of chat left over (*Catha edulis*) and sawdust (*C. lusitanica*) for *P. ostreatus* cultivation is been reported.

In current study, highest ramification of *P. ostreatus* mycelium was observed at room temperature during spawn production. This could be because room temperature is the optimal condition for *P. ostreatus* growth, as also reported by Siddhant et al*.* (2013).

Although the rate of substrates colonized by mycelium differ within treatments, invasion rate of substrate and pinning days were highly correlated. In contrast to the combination of CbZ*SdC and CbZ, emerging of pinnate in ChC*Cd and CbZ*Ts had taken long days and this could be because those substrates were not permissible for mycelia growth and pinning. This result is in agreement with the finding of Sher et al*.* (2010) as pinheads formation had spent 23 to 27 days. Nevertheless, the pinnate period was lower compared to the work of Pathmashini et al*.* (2008), where it spent 35 to 51 days. This could be because of variation in substrate composition, and environmental factors such as light, temperature, humidity and concentration of CO_2.

Generally, increasing CbZ ratio in the mixtures significantly enhanced mycelia ramification. Narain et al. (2009) also indicated that mushroom mycelia growth and primordial development relay on the composition of lignocellulosic materials, particularly on the C/N ratio.

During this study, paramount stipe length and pileus diameter were harvested from the combination of CbZ*SdC, particularly 1:0.50 ratio (5.7 cm), and CbZ alone. However, the biomass of *P. ostreatus* mushroom was obtained from the combination of ChaC*Cd, and chat alone were disregarded. This could be due to less turnover of chat or offensive chemical released during chat or Cd decomposition that affects mushroom growth. The less productivity of some agro-wastes substrate for mushroom production was also reported by Gume et al. (2013), in which 3.8 cm stipe length was recorded from combination of sawdust and coffee bean husks. Furthermore, considerable amount of mushroom biomass were harvested from 1st and 2nd flushes of the CbZ*Cd, Tfs*CbZ, CbZ*Sd combination as well as CbZ alone, but the least amount obtained from the 3rd and 4th flushes, this might be due to nutrient of substrate depletion, and retard the propagation of mushroom, or immobilization of nutrients as white rote fungus biomass saturated (Gume et al., 2013).

Despite the fact that Dawit (1998) obtained highest mushroom biomass from 2nd and 3rd flushes, others workers obtained significant yield from the 1st (Sher et al., 2010). In this work, the substrates was given up to four phases of flushes, and the duration of time taken for appearing succeeding flushes was statically insignificant among flushes. Interestingly, this study well addressed the suitability of the combination of corn cob, cow dung, saw dust, teff straw, and room temperature, and 80 to 90% of relative humidity for *P. ostreatus* mushroom production. Oei (2003, 2005), also indicated at least 90% of relative humidity is required for primordial formation with room temperature. Furthermore, we determined that the plastic holes size (15 mm^2) is the appropriateness for colonization of substrate by *P. ostreatus* mycelia, which is in accordance with previous work (Tesfaw et al., 2015). Significant amount of BE was recorded from the combination of Tfs*CbZ as well as was corn cob alone. This could be because of corn cob permissible for easily mycelia ramification. Thus, corn cob is a potential agro-waste for *P. ostreatus* cultivation, similarly to straw substrate as indicated by Mateus et al. (2012).

In general, the rate of mycelia invasion in most treatment group was highly related with completed invasion and pinning days. However, stipe length, pilus diameter and other parameters is not always indicator for higher yield obtained as also observed (Gume et al., 2013). Both pinhead maturation and abortion are affected by the type of substrates and environmental factors. During this experiment, in average 6 to 16.5% pinhead were aborted per treatment of bags in all of the substrates. But compared to previous study, we reduced lost of mushroom biomass by 25%, due to optimizing of the approach. It was also reported by Kimenju et al. (2009) in which more than 50% of pinheads emerged did not grow into marketable products. Over all, during this study the various combination of CbZ*SdC and Cd*Tfs were realized as best agro-wastes for *P. ostreatus* mushroom cultivation.

Conclusion

Based on the obtained results, the following conclusions were made. Room temperature, about 80 to 90% of relative humidity, substrates such as corn cob, saw dust, and teff straw are determined as the best promising agro-wastes for *P. ostreatus* mushroom production. Although this is the first work that attempted to use chat left over, and cow dung for mushroom cultivation, which is not suitable for mushroom cultivation. Thus, it needs further investigation for the chemical composition of chat left over. Moreover, high yield of *P*. ostreatus mushroom was obtained from 1st and 2nd flushes. On the other hand, ramification of substrate by mycelia, pinning days/ pinhead maturation, stipe length, and pilus diameter were determined by plastic holes size, substrate composition, temperature and relative humidifies. Finally, this work is a baseline for our institution to establish mushroom production at large scale.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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