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# Suitability of Various Lignocellulosic Substrates for Cultivation of *Pleurotus sajor-caju* (Oyster Mushroom)

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## Abstract

*The objective of this study was to look into the possibility of using the different lignocellulosic biomasses for* **Pleurotus sajor-caju** *(oyster mushroom) cultivation. The mushroom species was cultivated on nine types of substrates; namely three acacia species, three types of straws, coffee husk and saw dust obtained from two types of timber species. Thirty types of treatments (pure and mixed) were used to grow the mushroom and significantly differing results were obtained. Yield data (biological efficiency, spawn running period (SRP) and days to first harvest (DFH) of the mushroom were measured. The shortest SPR and DFH were from the pure chopped barley straw (BS) followed by pure wheat straw (WS) and* **Acacia drepanolobium** *(DC), respectively. The maximum period was from the mixed sawdust of* **Aningeria adolfi-friedericii** *(AS) and* **Cupressus lusitanica** *(CS) in the ratio of 4:6. The least mushroom biological efficiency was also from the ASCS4 (4:6) mixture. No any fruit body appeared on the pure* **C. lustanica** *sawdust (CS) and the mixture of ASCS in a ratio of 3:7. On the other hand, the maximum biological efficiency of the mushroom was obtained from DC followed by BS, WS and SC, respectively. In conclusion, mushroom biological efficiencies of the pure* **A. drepanolobium** *and* **A. seyal** *substrates were found to be better than those of the mixed ones. These biological efficiencies were also found to be better than those of the agri-residues.*

**Key:** *Pleuroteus sajor caju*, *Acacia species,* coffee husk, sawdust, straws, bush control

## Introduction

*Pleurotus species*, oyster mushrooms, are common and primary wood and other lignocellulosic decaying fungi. They grow naturally in the wild in tropical and subtropical rainforests and also commercially cultivated (Bonatti et al., 2004). They can grow in a wide range of temperatures (Khan and Garcha, 1984; Mueller and Gawley, 1983). They have extensive enzymatic systems and are among the most efficient white rot fungi and as a result various forest biomasses and agricultural wastes can be utilized to grow them (Platt et al., 1984). Oyster mushrooms cultivation is becoming popular throughout the world (Chang, 1999; Kues and Liu, 2000).

However, there are only few reports indicating establishment and expansion of mushroom farms in Africa (Justus, 2004; Ukoima et al. 2009, Dawit, 1998).

In Ethiopia, consumption of wild mushroom has been a common practice by people living in the vicinity of natural forests (Shasho, 2004; Dawit, 1998). But owing to severe deforestation, mushrooms collection and eating habit has been in decline. On the other hand, consumption (self and local market) of cultivated mushrooms has been on the rise in Addis Ababa and its suburbs. However, there is no well established and functioning demand and supply channel between cultivators and customers. For this reason, international hotels and some supermarkets in Addis Ababa import canned mushrooms from abroad.

Demand and supply gap in mushrooms cultivation and the supply chain in Ethiopia, should be alleviated through promotion of local cultivation of commercially successful mushrooms species. Amount, abundance and comparative suitability of accessible lingo-cellulosic materials has to be explored. Bush biomass, especially the biomass of aggressive rangeland encroaching acacias, has been one of the viable substrates available in abundance. This biomass is particularly available in excess in the Borana rangelands (Tesfaye *et al*. 2004; Alemayehu 1998; Oba 1998) and the area supporting large population of pastoralists with their cattle and other domestic animals have been shrinking and covered by the native bushes and other plant species. *Acacia* species are among those aggressively encroaching native plant species in the rangeland. The pastoralists have been hopelessly trying to get rid of the bush through clearing and burning. Therefore, utilization of the unwanted and noxious bush biomass for mushroom cultivation has two fold benefits: in one hand it enhances the re-growth of normal grassland vegetation for cattle feed and on the other hand it delivers the biomass for mushrooms farms.

The other potential lingo-cellulosic substrate sources for mushrooms growing are wood industry wastes such as sawdust, trimmings and off-cuts. Sawmills and joineries are one of the major wood industries in Ethiopia. It is common to observe huge hills of softwood and hardwood sawdust in many sawmills of the south and south-west parts of Ethiopia. Coffee husk is another waste but potentially useful substrate found in abundance in the vicinity of coffee processing plants in Sidama, Kaffa, Shaka, Wellega and Illubabour zones. According to Sustainable Tree Crops program (STCP 2003), 1000 Kg of fresh coffee berry generates about 400 Kg of wet waste pulp. Very huge amount of coffee husk is, therefore, discharged and accumulated at the major coffee producing regions of the country every year. Through mushroom cultivation the mentioned problems can be solved yielding nutritious edible mushrooms.

Therefore, the objective of this study was to look into the possibility of using the biomass of tree species that had encroached the rangeland, coffee husk abundantly found in coffee producing areas and saw dusts heaped around sawmills for oyster

mushroom cultivation. It was also to select the best substrates and to explore optimum mixing ratios of the different substrates.

# Materials and Methods

## Culture Source and Maintenance

*Pleurotus sajor-caju* strain was obtained from the United States Department of Agriculture, Forest Service, Forest Products Laboratory, Madison, under the code of FP-140078. The culture was maintained in malt extract agar at 4$^{o}$C.

## Spawn Production

The spawn was prepared following the procedures stated in Pakale (2004). 10Kg of Sorghum grains were boiled for 15 min in 15L of distilled water. The excess water was drained off and the grains were cooled in sieves. The cooled grains were supplemented with 0.35% $CaCO_3$ and 1.3% $CaSO_4$ and filled into 1L bottles and autoclaved for 2 hours. The bottles were inoculated with bits of agar medium colonized with the mycelium, and then incubated at 25$^{0}$C in a dark place for 18 days.

## Substrates Preparation and Cultivation

In the present study, the suitability of different substrates for cultivation of *Pleurotus sajor-caju* was evaluated. The substrates include three encroaching *Acacia* species (*A. drepanolobium*, *A. melifera* and *A. seyal*) from Borana rangeland, coffee husk from Aleta-wondo, sawdust of *Aningeria adolfi-friedericii* and *Cupressus lustanica* from the sawmill of the National Forest Products Utilization Research Program; *Triticum aestivum* (wheat) and *Eragrostis tef* (teff) straws from the research site of Debrezeit Agricultural Research Center and *Hordeum vulgare* (barely) straw from around Holeta town. The branches, thorns and leaves of acacia species (*A. drepanolobium*, *A. melifera* and *A. seyal*) and the straws (wheat, barley, teff) were chopped at 3-5cm length manually. The chopped acacias (mixed), the straws, the sawdust and the coffee husk were soaked in water for two days. Thereafter, they were filtered and thoroughly mixed with 5% wheat bran supplement and 1% chalk (Bonatti et al., 2004). The substrates (pure and mixed) were then filled into polyethylene bags of 30 cm x 60 cm size with 30 μm thick with five replications (table 1). The bags were bottle necked with plastic rings (mouth of bag was pulled through a piece of plastic tube ring, fold over the ring and then tied) and plugged with cotton (Kwon and Thatithatgoon, 2004) and pasteurized. The bags were cooled overnight and spawned and incubated at room temperature and 65% relative humidity (RH). Finally, the induction of the fruit bodies formation was achieved in the perforated plastic bags (to increase air exchange), through exposure to light for a period of 12 hrs per day and increasing the RH to 90% using humidifiers.

Table 1 Acronym for substrate type and mixing ratios.

| Code | Description of the mixing substrates | Mixture ratio |
|---|---|---|
| DC | Chopped *A. drepanolobium* | Pure |
| DCAS1 | Chopped *A. drepanolobium* mixed with *A. adolfi-friedericii* sawdust | 9:1 |
| DCAS2 | Chopped *A. drepanolobium* mixed with *A. adolfi-friedericii* sawdust | 8:2 |
| MC | Chopped *A. melifera* | Pure |
| MCAS1 | Chopped *A. melifera* mixed with *A. adolfi-friedericii* sawdust | 9:1 |
| MCAS2 | Chopped *A. melifera* mixed with *A. adolfi-friedericii* sawdust | 8:2 |
| SC | Chopped *A. seyal* | Pure |
| SCAS1 | Chopped *A. seyal* mixed with *A. adolfi-friedericii* sawdust | 9:1 |
| SCAS2 | Chopped *A. seyal* mixed with *A. adolfi-friedericii* sawdust | 8:2 |
| TS | Chopped teff straw | Pure |
| BS | Chopped barley straw | Pure |
| WS | Chopped wheat straw | Pure |
| CH | Coffee husk | Pure |
| CHAS1 | Coffee husk mixed with *A. adolfi-friedericii* sawdust | 7:3 |
| CHAS2 | Coffee husk mixed with *A. adolfi-friedericii* sawdust | 6:4 |
| CHAS3 | Coffee husk mixed with *A. adolfi-friedericii* sawdust | 5:5 |
| CHAS4 | Coffee husk mixed with *A. adolfi-friedericii* sawdust | 4:6 |
| CHAS5 | Coffee husk mixed with *A. adolfi-friedericii* sawdust | 3:7 |
| AS | *A. adolfi-friedericii* sawdust | Pure |
| CHCS1 | Coffee husk mixed with *C. lustanica* sawdust | 7:3 |
| CHCS2 | Coffee husk mixed with *C. lustanica* sawdust | 6:4 |
| CHCS3 | Coffee husk mixed with *C. lustanica* sawdust | 5:5 |
| CHCS4 | Coffee husk mixed with *C. lustanica* sawdust | 4:6 |
| CHCS5 | Coffee husk mixed with *C. lustanica* sawdust | 3:7 |
| ASCS1 | *A. adolfi-friedericii* sawdust mixed with *C. lustanica* sawdust | 7:3 |
| ASCS2 | *A. adolfi-friedericii* sawdust mixed with *C. lustanica* sawdust | 6:4 |
| ASCS3 | *A. adolfi-friedericii* sawdust mixed with *C. lustanica* sawdust | 5:5 |
| ASCS4 | *A. adolfi-friedericii* sawdust mixed with *C. lustanica* sawdust | 4:6 |
| ASCS | *A. adolfi-friedericii* sawdust mixed with *C. lustanica* sawdust | 3:7 |
| CS | *C. lustanica* sawdust | Pure |

## Data Collection and Analysis

The spawn running period (SPR) and the days to first harvest (DFH) were recorded after complete colonization of substrate by mycelia and appearance of matured fruit body, respectively. Fresh mushrooms were collected on maturity (determined visually) and each fruit body was weighed using analytical balance. The yield was determined at the event of collecting three flushes of fruit-bodies' harvest while the average biological efficiency was quantified as total weight of mushrooms harvested per dry weight of the substrate. The main variables analysed were spawn running period, days to the first harvest and fruit-body yield in terms of biological efficiency (BE). Differences existing between the means were evaluated for significance using the Duncan's test at 5% level and SPSS vs. 12.0.

# Results and Discussions

## Spawn Running Period (SRP) and Days to First Harvest (DFH)

SRP is presented in Fig. 1 for all types of substrates, in Fig. 2 for pure substrates only and in Fig. 3 for mixed substrates. Fig. 1 demonstrates that the shortest SRP was observed on barley straw (BS) and it was significantly lower than all tested substrates. Fig. 2 shows that there was no significant difference in mycelial colonization period between the chopped *Acacia drepanolobium* (DC), teff straw (TS) and wheat straw (WS) substrates. The duration of SRP obtained for wheat straw is much shorter than what was reported by Peken and Kucukomuzlu (2004). On the other hand poor mycelial colonization was observed in some of the substrates and their mixture. When the three acacias were compared, the chopped *A. melifera* (MC) was found to be the most resistant to mycelial colonization followed by *A. Seyal* (SC). However, the difference between the two species was not significant. Fig. 3 demonstrates that different mixing ratios of CH with AS in general resulted in lower SRP than when mixing with CS in different proportions. Figs. 1 and 3 demonstrate mixing of *Aningeria adolfi-friedericii* saw dust (AS) with other substrates in various proportions resulted in significantly differing spawn running periods. The higher the AS proportion in the mix, the longer the spawn running period was. Likewise, the higher the proportion of CS in the respective mixes resulted in significantly longer SRP. When AS and CS, in turn were mixed in different proportions, the larger the CS composition was the longer the SRP. Therefore, it could safely be concluded that CS in pure and in mixes had hindered the mycelia colonization of the mushroom species while AS had elongated it. This finding agrees with the report of Croan (2004) in which white rot fungi did not readily colonize coniferous wood because of their extractives content.

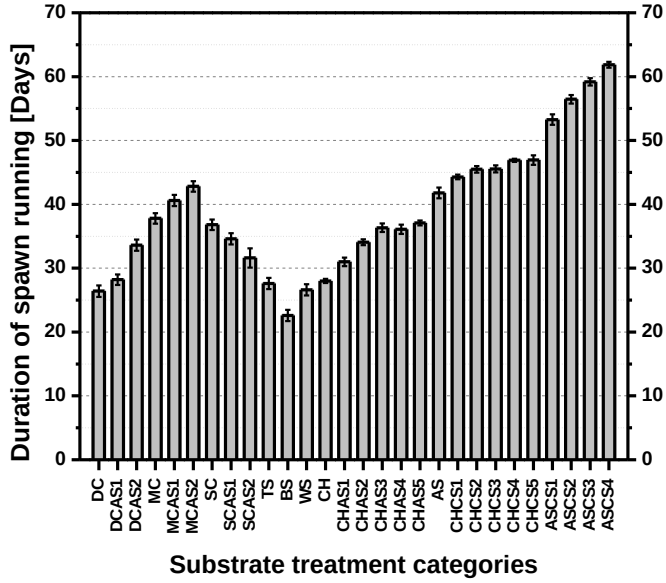


Fig. 1 Comparative plot of the duration needed for full mycelial colonization by pure and mixed substrates

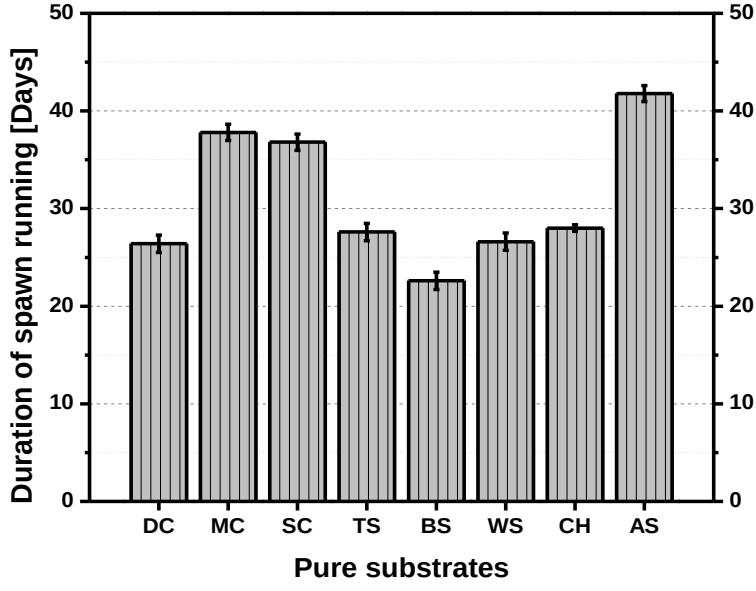


Fig. 2 Comparative plot of the duration needed for full mycelial colonization by pure substrates

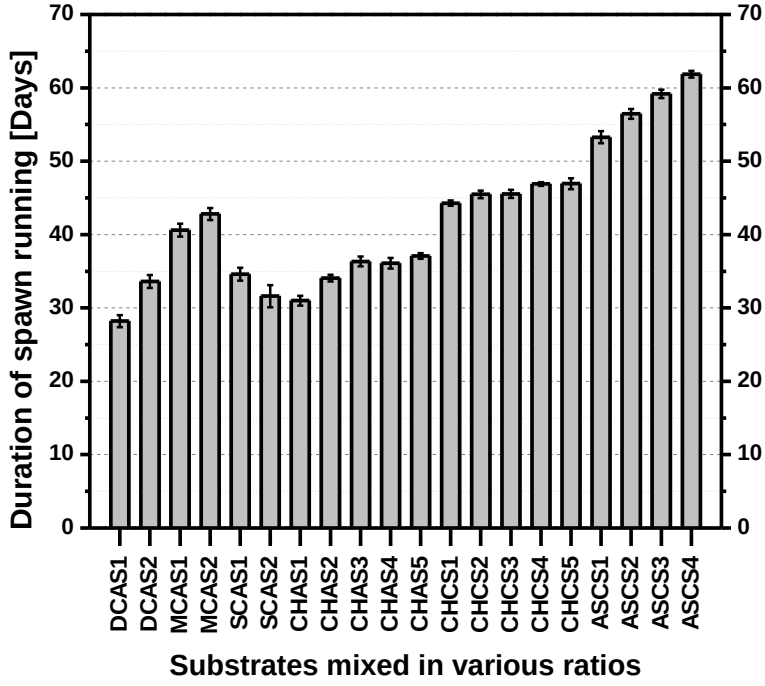


Fig. 3 Comparative plot of the duration needed for full mycelial colonization by mixed substrates

Figs. 4, 5 and 6 show the time duration elapsed up to the first harvest of mushrooms fruit-body for the pure and mixed substrates. The DFH data was also found to be in line with the data for the SRP showing similar pattern. The lowest DFH was observed on BS followed by DC, TS and WS, respectively. Peken and Kucukomuzlu (2004) reported 40.5 SRP and 67.3 DFH on WS for same mushroom species. The DFH result of this study is twofold better than the report made by Peken and Kucukomuzlu (2004) by reducing from 67.3 to 31.8 days on WS substrate. The DFH followed the same pattern as the SRP, even for sawdust of Aningeria.

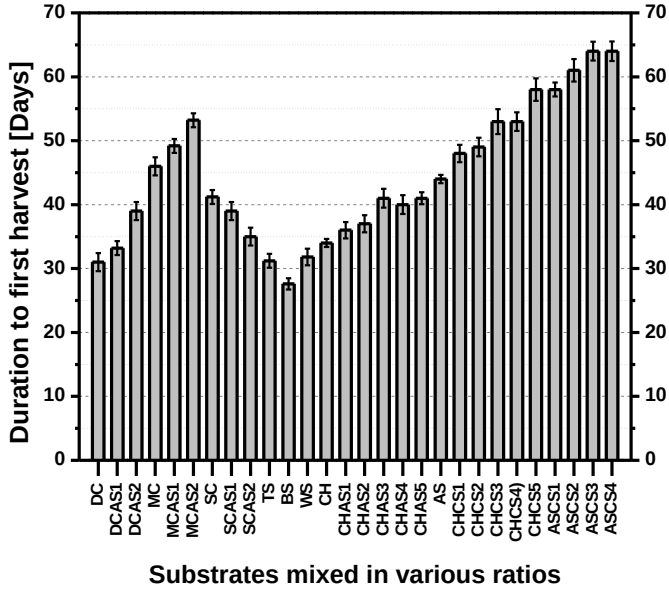


Fig. 4 Comparative plot of the duration elapsed up to the first harvest of fruit body collection including time required for mycelial colonization by pure and mixed substrates

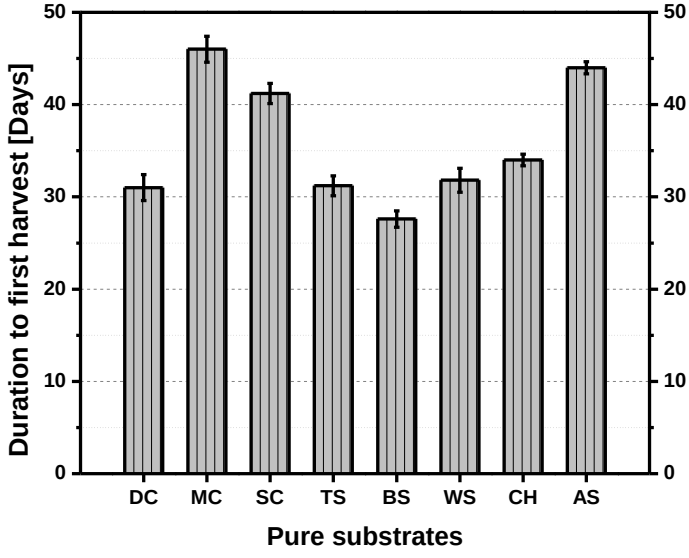

Fig. 5 Comparative plot of the duration elapsed up to the first harvest of fruit body collection including time required for mycelial colonization by pure substrates

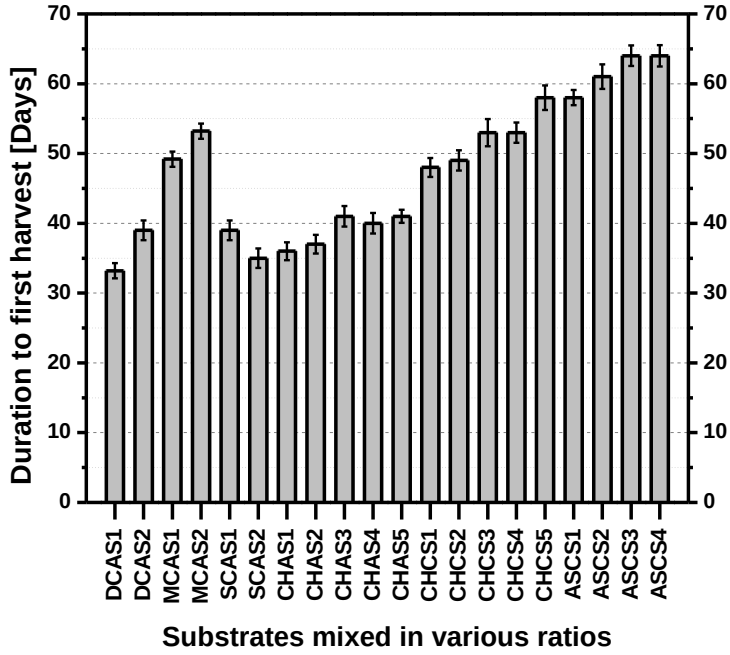


Fig. 6 Comparative plot of the duration elapsed up to the first harvest of fruit body collection including time required for mycelial colonization by mixed substrates

## Biological Efficiency (BE)

BE of the substrates indirectly denotes their suitability to support growth of a given strain of mushroom. The higher the BE is the greater will be the suitability of the substrate for the mushroom cultivation. Table 2 demonstrates that among the types of substrates tested, chopped *A. drepanolobium* showed the best results in biological efficiency (BE). The result was found to be significantly higher than those obtained for other substrates except the barley straw (BS). Similar to the finding obtained for the mycelial colonization in Fig. 1, the lowest BE was obtained for the mixture of ASCS4 as shown in table 2. The high biological efficiency for DS is possibly due to the woods' cell-wall structural components composition. The biological efficiency of CH was one of the lowest and its mixtures with AS and CS were also the smallest in comparison with other mixture-substrates. The AS and CH mixture-substrate showed a decrease in biological efficiency as the amount of AS was increased. The pure CS and ASCS (3:7) did not yield any fruit-body which is in good agreement with the finding of Croan (2004). Table 2 further shows that CS's mixture with CH and AS was also belonged to the lowest in BE. It can be observed that as the proportion of CS was increased, the BE of the mixture happened to be reduced. Here is also the same, as

stated in the above paragraphs that CS elongates the SRP, the yield is also a function of colonization of the substrate with mushroom. Therefore, poor BE is due to the CS extractives which hindered/lowered the mycelia colonization. WS showed equivalent efficiency when compared to BS and DC substrates. The BE of WS and its mixtures reported by various studies range widely from 50.2% to 97% (Zhang et al., 2002; Salmones et al., 2005). In this study the BE of *P. sajor-aju* on WS is less than the one reported by Zhang *et al*. (2002) but it was better than the report of Salmones *et al*. (2005). Banik and Nandi (2004), reported 89.2% BE on rice straw for *P. sajor-caju*.

Table 2: Yield results of *P. sajor-caju* cultivated on different substrates

| Substrate | FB/Bag[1] Mean±SD[2] | Yield (g)/Kg[1] Mean±SD[2] | BE (%)[1] Mean±SD[2] |
|---|---|---|---|
| DC | 563.65±4.28$^{a}$ | 854.11±24.93$^{a}$ | 85.41±2.49$^{a}$ |
| DCAS1 | 430.12±3.97$^{c}$ | 703.99±48.51$^{d}$ | 70.39±4.85$^{d}$ |
| DCAS2 | 368.73±3.87$^{e}$ | 594.78±42.36$^{f}$ | 59.47±4.23$^{f}$ |
| MC | 248.29±2.09$^{k}$ | 342.64±20.11$^{ij}$ | 34.26±2.00$^{ij}$ |
| MCAS1 | 191.3±2.64$^{m}$ | 321.58±13.00$^{ijkl}$ | 32.15±1.30$^{ijkl}$ |
| MCAS2 | 149.45±1.27$^{qr}$ | 248.59±3.54$^{m}$ | 24.86±0.35$^{m}$ |
| SC | 535.49±2.13$^{b}$ | 790.03±19.74$^{c}$ | 79.00±1.97$^{c}$ |
| SCAS1 | 396.24±1.52$^{d}$ | 715.78±37.88$^{d}$ | 71.57±3.78$^{d}$ |
| SCAS2 | 284.79±2.47$^{ij}$ | 645.73±10.47$^{e}$ | 64.57±1.04$^{e}$ |
| TS | 94.11±2.02$^{v}$ | 518.41±13.11$^{g}$ | 51.84±1.30$^{g}$ |
| BS | 161.04±4.17$^{p}$ | 835.66±14.94$^{ab}$ | 83.56±1.49$^{ab}$ |
| WS | 152.05±.89$^{q}$ | 812.50±13.03$^{bc}$ | 81.25±1.30$^{bc}$ |
| CH | 361.23±5.12$^{f}$ | 390.16±7.28$^{h}$ | 39.01±0.73$^{h}$ |
| CHAS1 | 341.68±1.59$^{g}$ | 359.09±3.61$^{hi}$ | 35.91±0.36$^{hi}$ |
| CHAS2 | 334.87±2.10$^{g}$ | 341.01±3.78$^{ijk}$ | 34.10±0.37$^{ijk}$ |
| CHAS3 | 319.36±1.49$^{h}$ | 324.01±3.66$^{ijkl}$ | 32.40±0.36$^{ijkl}$ |
| CHAS4 | 312.87±2.85$^{h}$ | 315.45±2.94$^{jkl}$ | 31.54±0.29$^{jkl}$ |
| CHAS5 | 287.46±5.21$^{i}$ | 301.12±7.22$^{kl}$ | 30.11±0.72$^{kl}$ |
| AS | 278.64±3.23$^{j}$ | 291.26±2.36$^{l}$ | 29.12±0.23$^{l}$ |
| CHCS1 | 206.28±4.84$^{l}$ | 213.64±15.60$^{mn}$ | 21.36±1.55$^{mn}$ |
| CHCS2 | 183.39±3.47$^{o}$ | 191.91±4.19$^{no}$ | 19.19±0.42$^{no}$ |
| CHCS3 | 146.19±4.59$^{qrs}$ | 150.31±3.04$^{p}$ | 15.03±0.30$^{p}$ |
| CHCS4 | 140.27±1.57$^{s}$ | 144.26±2.29$^{p}$ | 14.42±0.22$^{p}$ |
| CHCS5 | 120.97±2.07$^{t}$ | 123.87±3.20$^{p}$ | 12.38±0.32$^{p}$ |
| ASCS1 | 186.60±2.97$^{no}$ | 197.52±4.07$^{n}$ | 19.75±0.40$^{n}$ |
| ASCS2 | 142.65±3.05$^{rs}$ | 153.91±6.83$^{op}$ | 15.39±0.68$^{op}$ |
| ASCS3 | 127.42±1.63$^{t}$ | 133.52±0.83$^{p}$ | 13.35±0.08$^{p}$ |
| ASCS4 | 110.81±1.88$^{u}$ | 116.49±2.87$^{p}$ | 11.64±0.28$^{p}$ |
| ASCS | - | - | - |
| CS | - | - | - |

Mean values within the same column with no common superscript letter differ at the 95% confidence level and the corresponding numbers indicate the mean values; [1]Results reflect observations of five replications; [2]Standard deviation; Values are means of five replicates±SD

BE reports of Salmones *et al*. (2005) for *P. austreatus* and Bermudez *et al* (2001) for *P. florida* on CH were higher than the one obtained in this study on the same substrate. Therefore, coffee husk is more suitable for the cultivation of *P. austeatus* and *P. florida* than it is for *P. sajor-caju*. The biological efficiencies of DC, DCAS1, DCAS2, SC, SCAS1, SCAS2, TS, BS and WS were higher than those reported by Ragunathan and Swaminathan (2003) for cotton stalk, coir fibre and sorghum stoker. But the biological efficiencies of MC, MCAS1, CH, CHAS1, CHAS2, CHAS3, CHAS4 and CHAS5 and AS were equivalent to results reported by Ragunathan and Swaminathan (2003). In general mixing of AS with acacia and coffee husk substrates lower the biological efficiency of the substrate to 30% and the worst substrate mix become the CS which significantly reduced the mushroom yield in all substrates it was mixed with.

## Conclusion

This study showed that chopped *Acacia drepanolobium* has the highest biological efficiency followed by barley straw, wheat straw and *Acacia seyal* in decreasing order. Generally, the acacia species performed well for mushrooms cultivation although it was observed that they require a bit longer spawn running time, especially *A. melifera* and *A. seyal*. It was observed that Cupressus and Aningeria sawdust were not suitable substrates for the growth of *P. sajor-caju* and care should be taken in sawmills not to mix the sawdust with other lingo-cellulosic wastes intended for mushrooms cultivation. Therefore, it can be concluded that encroachment of rangeland by acacia species can be controlled and managed by utilizing the noxious bush biomass resource as a substrate for oyster mushroom cultivation.

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