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*Original Research Article*

# Biodegredation of Different Agro-Endustrial Wastes through the Cultivation of *Pleurotus ostreatus* (Jacq. ex. Fr) Kummer

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## ABSTRACT

**The aim of this study was to investigate the effect of various substrates on spawn running time, yield and biological efficiency (BE) of *Pleurotus ostreatus* (Jacq. ex. Fr) , compositional changes of substrates after growth mushroom and to evaluate its potential use as a feedstock. In the study, chickpea straw (CS) was used as basal substrate (80 %), while some of agricultural wastes such as cotton seed hulls (CSH), olive press cake (OPC), sunflower seed press cake (SPC) and sugar beet pulp (SBP) were added at the rate of 20 % to basal substrate. It was determinated that all of agricultural wastes tested in the study, except SBP, are economically viable alternatives for *P. ostreatus* (Jacq. ex. Fr) growers. On the other hand, the increment in ash, N, P, K, Mg, Fe and Mn content and the decrease in moisture, pH, C, C:N, Zn and Cu, cellulose, hemicellulose and lignin content of substrates were observed after mushroom production. According to this results, we can suggest that CSH, OPC and SPC can be used as a additive material for *P. ostreatus* (Jacq. ex. Fr) cultivation and spent mushroom substrates may be a valuable material as a feedstock, because of higher nutritional content and digestibility.**

**Keywords: *Pleurotus* ostreatus, Mushroom growing substrates, Spent mushroom substrate Lignocellulosic content Macro element content, Micro element content**

## INTRODUCTION

It has been observed that over 70% of agro-industrial products have been discarded as waste (Pala *et al.,* 2014). Agro-industrial residues are rich in case of cellulose, lignin and hemicellulose, but their protein and mineral contents are low. Area of usage of agro-industrial wastes was limited because of these undesirable features.
Mushrooms do not have chlorophyll. So they can not produce carbohydrates by photosynthesis. Mushroom mycelia secrete large amounts of extracellular enzymes that break down compounds such as cellulose and lignin present in the substrate (Kuforiji and Fasidi., 2008). This ability of mushroom mycelia can provide a efficient using for these wastes through mushroom production.

The world mushroom industry has expanded steadily in the last decade in the world. Total mushroom production was reached in 2013 to 9.926.966 tons (Anonymous, 2016). *Pleurotus* spp. are belong to white rot fungi and reported to be efficient colonizers and degraders of lignocelluloses (Rajaratham and Bano, 1989). Commercially most important species is *P. ostreatus* (Jacq. ex. Fr) among *Pleurotus* spp. in the world. It grows wild almost in all regions of Turkey on dead logs or stumps. Local people named it "Kavak mantarı" or "Kayın mantarı"and "İstiridye mantarı". *P. ostreatus* (Jacq. ex. Fr) production has a promising future in Turkey, because its cultivation requires simple and inexpensive cultivation techniques. Although cultivation of this mushroom has significantly increased since five years (Eren and Pekşen, 2016), there are still few producers growing mushrooms on commercial level.

The substrates used in each region depend on the locally available agricultural wastes for *Pleurotus* spp. cultivation. Various types of raw materials such as spent beer grain (Wang *et al.,* 2001), elephant grass (Obodai *et al.,* 2003), sugarcane baggase (Membrillo *et al.,* 2011) coffee husk (Gume *et al.,* 2013) have been examined as alternative substrates for its cultivation. In Turkey, large volumes of unused lignocellulosic by-products can be found. Olive press cake and cotton seed hulls are vast availability in the Aegean and Mediterrian basin, whereas chickpea, sun flower and bettle are widely cultivated throughout middle of Turkey. These fibrous agricultural wastes despite being low in nutritive value are usually used for the feeding of livestocks however, a large quantity of it is burned or incorporated into the soil.

Although mushroom cultivation technology is friendly to the environment, the production of mushrooms generates large volumes of solid waste products that is called spent mushroom substrate (SMS).

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Many countries have no data on SMS discharge, but Medina *et al.* (2012) reported that about 5 kg of waste substrates are generated from the production of 1 kg of mushrooms. Correspondingly, the SMS production in the world was approximately 50 millions tons in 2013. This big waste mass may induce the environmental pollution if they are not eliminated appropriately (Liu, 2009; Rajput *et al.*, 2009). Recycling of SMS by mushroom production may reduced adversely affecting the environment.

The main objective of present investigation was I) to identify the alternative substrates from various agricultural and to assess the growth performance and yield of *P. ostreatus* (Jacq. ex. Fr) II) to determinate correlations between spawn running time, yield and biological efficiency (BE) and chemical composition and lignocellulosic content of substrates III) to investigate changes in the concentrations of lignin, cellulose, hemicellulose, macro and micro elements of substrates after *P. ostreatus* (Jacq. ex. Fr) growth and evaluated its potential as a feedstock.

## MATERIALS AND METHODS

### Materials

*P. ostreatus* HK-35 (from Sylvan)was selected for this study because it is used commercially in Turkey. Cultures were grown and maintained on Potato Dextrose Agar (PDA, Merck; 39 g/l) and the cultures were stored in a refrigerator at 4 °C.

Chickpea straw (CS) was used as main materials and cotton seed hulls (CSH), olive press cake (OPC), sunflower press cake (SPC), sugar beet pulp (SBP) were used as additive materials. Additive materials were added to chickpea straw at ratios of 20% to prepare the growing substrates. Chickpea straw, sun flower press cake, sugar beet baggase were obtained from Kırşehir, cotton seed hulls (CSH) and olive press cake were acquired from villages around İzmir in the Aegean Region.

The mushroom growing process were accomplished in the Mushroom Production Unit of Ahi Evran University's Faculty of Agriculture in Kırşehir, Turkey. Analysis was carried out in the laboratories of Ahi Evran University, Kırşehir, Turkey.

### Spawn production

Spawns were prepared by standard method using wheat grains. Briefly, wheat grains was boiled and then glass bottles filled with boiled wheat grains and 1% w/w $CaCO_3$ were sterilized for 1.5 h at 121 °C, cooled and inoculated with an agar plug (1 cm diam.) cut from the advancing margin of a 5-d-old colony grown on PDA. Bottles were incubated in the dark, at 25 °C until the completion of mycelial growth.

### Preparation of cultivation media

Five different substrates were tested for the cultivation of *P. ostreatus* (Jacq. ex. Fr). CS was used as a base medium and CSH, OPC, SPC and SBP were added to this mixture at ratios of 20% to prepare the substrates. Substrates were soaked in tap water overnight. The excess water was drained out until moisture was reached to 60–70% . Then, 1 kg (wet weight) of each substrate was packed into a polypropylene autoclavable bag of 25 × 45 cm and the bag plugged with a cotton plug. The plastic bags containing substrate were sterilized in an autoclave at 121 ˚ C for 90 min and, after cooling, inoculated in a laminar flow chamber using 3% grain spawn (on a w/w wet weight basis).

### Experimental design and mushroom cultivation

Inoculated bags were incubated at 25±2 $^{o}$C with 80% relative humidity in the presence of light to be colonized by the mycelium. After full colonization, bags were transferred to a cropping room at 15±2 $^{o}$C with a humidity of 80–90% in order to induce fructification. The room was illuminated 8 h/day by fluorescent lamps. Total mushroom yield (g $kg^{-1}$ substrate) was obtained from three flushes in a harvest period. Mushrooms were harvested as soon as the fruiting bodies developed and attained their full size when the in-rolled margins of the basidiomes began to flatten. The following data were recorded; days taken for the completion of substrate colonisation, total mushroom yield (g) and bio- efficiency (BE%).

The biological efficiency percentage (BE) was calculated as follows: ([weight of freshmushrooms harvested/substrate drymatter content] × 100).

The experiment was conducted in a randomized plot design, with ten replications.

**Substrate analysis**

Substrate and spent substrate samples were oven-dried at 60 $^{o}$C for 48 h and ground to pass through a 1 mm sieve. The total moisture, ash, pH and C were determined by standart procedure (Kacar and İnal, 2008).

In order to determine nutrient and phosphorus content in samples, 0.7 g substrate samples were weighed and incinerated in a muffle furnace at 550$^{o}$ C for 16 h; then ash residue was digested in 0.6 mol $L^{-1}$ nitric acid ($HNO_3$). Finally, the solution was used for the determination of nutrients by an atomic absorption spectrometer (AAS) (AAnalyst 800 AAS, PerkinElmer Inc.,Waltham,MA, USA), and phosphorus by a spectrophotometer. The Duchaufour method (Duchaufour, 1970) was used to determine the total nitrogen content of substrates.

Substrates were analysed for cellulose and lignin content using acid detergent fiber methods Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) were determined using the method described by Van Soest *et al.* (1991). Hemicellulose was calculated as the difference between NDF and ADF while cellulose is the difference between ADF and ADL (Zadrazil and Brunnet, 1982).

**Statistical analysis**

The data obtained from the experiment were subjected to variance and means analysis, and the statistical significance was compared employing Duncan's multiple range test, using the SPSS 16.0 for Windows statistical computer program at a significance level of 5%. Correlation analyses were carried out to determinate the relationship between chemical constituents of substrate and spawn running time, yield and BE (%).

## RESULT AND DISCUSSION

**The effect of substrates on spawn running time, yield and BE**

Spawn run time, yield and BEs were all affected by the substrates ($P$ <0.01). The spawning time (day), average yield (g) and biological efficiency (%) of *P. ostreatus* cultivated under controlled condition on various substrates are presented in Table 1.

**Table 1.** Effect of different substrates on spawn running time, yield and BE of *Pleurotus ostreatus* (HK-35).

| Growing media | Spawn running time (day)[a] | Yield (gkg$^{-1}$) [a] | BE (%)[a] |
|---|---|---|---|
| **CS** | 15,3$^{**d}$ | 198.6$^{**d}$ | 68.3$^{**d}$ |
| **CS:CSH** | 17.8$^{c}$ | 312.4 $^{a}$ | 99.8$^{a}$ |
| **CS:OPC** | 21.6$^{b}$ | 232.7 $^{c}$ | 76.5 $^{c}$ |
| **CS:SPC** | 18.4$^{c}$ | 288.6 $^{b}$ | 93.1$^{b}$ |
| **CS:SBP** | 23.2$^{a}$ | 163.2 $^{d}$ | 55.3 $^{d}$ |

[a]Mean of 10 replicates

Asterisks indicate significance at *$P$ <0.05, **$P$ <0.01; Mean values in the same column followed by the same letters are not significantly different by Duncan's multiple range test.

CS Chickpea straw, CSH cottonseed hulls, OPC Olive press cake, SPC sunflower seed press cake, SBP Sugar beet pulp

*P. ostreatus* (Jacq. ex. Fr) colonized the different substrates within a period of 15.3 and 23.2 days of spawn run. Similarly, Sharma *et al.* (2013) reported that the spawn run time of *P. ostreatus* was 22.4- 26.0 days, while Obodai *et al.* (2003) confirmed that this varied in different substrates between 15 and 34 days. Chickpea straw was colonized in a short time as indicated by their incubation time, whereas hyphal growth on CS:SBP was quite slow and less profuse than other substrates. Philippoussis *et al.* (2001) reported that unless substrates are non-thoroughly impregnated with the hyphae, it is sensitive to fungal and bacterial infections resulting in reduced yields. Mycelial ramification was comparatively more condensed and vigorous in substrates colonized by CS: CSH followed by CS:SPC compared to CS:OPC and CS:SBP.

Total yield of *P. ostreatus* (Jacq. ex. Fr) varied between 163.2 and 312.4 g $kg^{-1}$ in the five substrates. Sharma *et al.* (2013) reported that the yield of *P. ostreatus* (Jacq. ex. Fr) were between 247.87 -381.85 gr, while Tisdale *et al.* (2006) confirmed that this varied between 237.7- 334.3 gr in different substrates. Satisfactory productivity (BE 55.3–99.81%) was demonstrated by most of agro-industrial residues, namely cotton seed hulls, olive press cake, sun flower press cake. Philippoussis *et al*. (2001) recorded that BEs between 75% and 100% on cotton wastes and wheat straw. The results in the study are close to these values. 68.3% of BE was attained when the chickpea straw used alone (100%). Chickpea straw gave the fastest mycelial growth rate, however, this did not correspond with yield. Philippoussis *et al.* (2007) reported similar results in *Lentinula edodes* (Berk.) Pegler. This may indicate that mycelial growth and yield of mushrooms have different requirements.

CSH was found to be the best supplement material (99.8% BE), followed by CS: SPC. Whenever 20% (w/w) of CSH and SPC was added to CS, there were 36.3 and 31.2 % increment in mushroom yield, respectively. The best yields of different *Pleurotus* species grown on CSH has been also stated by He *et al.* (1995), Garg (2013) and Yang *et al.* (2013) The advantages of SPC supplementation have already been reported elsewhere for the cultivation of some mushroom species such as *Agaricus blazei* (Matute *et al.*, 2010) and *Hericium erinaceus* (Bull.: Fr.) Pers (Figlas *et al.*, 2007). But when the substrate was supplemented with SBP, relatively lower yields occurred. Data demonstrated an approximate 11.7% reduction of BE when *P. ostreatus* (Jacq. ex. Fr) is cultivated on CS:SBP, as compared to control. Disease density by bacterial and fungal pathogens was also significantly increased in the presence of SBP.

**Chemical composition of the substrates and effect of fungal growth on chemical composition of the substrates**

Significant differences were found among the substrates regarding pH, ash, C and N content and C:N ratio, P, K, Ca,Mg, Fe, Mn, Zn and Cu content ($P$ <0.01), although the moisture and C content was unaffected ($P$ >0.05).

**Table 2.** Chemical composition of substrates (S) and spent mushroom substrate (SMS).

| Growing media | Moisture (%) | | pH | | Ash(%) | | C (%) | | N (%) | | C:N | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GM | SGM | GM | SGM | GM | SGM | GM | SGM | GM | SGM | GM | SGM |
| **CS** | 70.9$^{ns}$ | 62.0$^{ns}$ | 5.5$^{**b}$ | 4.5$^{**a}$ | 5.50$^{**b}$ | 6.3$^{**b}$ | 47.2$^{ns}$ | 46.8$^{**a}$ | 0.75$^{**d}$ | 0.86$^{**c}$ | 63.1$^{**a}$ | 54.7$^{**a}$ |
| **CS:CSH** | 68.7 | 62.9 | 5.9$^{a}$ | 4.5$^{a}$ | 6.2$^{a}$ | 9.3$^{a}$ | 46.9 | 45.3$^{b}$ | 1.0$^{b}$ | 1.4$^{b}$ | 46.9$^{c}$ | 32.8$^{c}$ |
| **CS:OPC** | 69.6 | 61.9 | 5.3$^{c}$ | 4.2$^{b}$ | 5.9$^{ab}$ | 6.9$^{b}$ | 47.3 | 46.5$^{a}$ | 0.74$^{d}$ | 0.98$^{c}$ | 64.5$^{a}$ | 63.4$^{b}$ |
| **CS:SPC** | 69.0 | 61.8 | 5.8$^{a}$ | 4.3$^{b}$ | 5.1$^{c}$ | 10.3$^{a}$ | 47.1 | 44.9$^{b}$ | 1.4$^{a}$ | 1.85$^{a}$ | 33.6$^{d}$ | 24.4$^{d}$ |
| **CS:SBP** | 70.5 | 62.2 | 5.1$^{d}$ | 4.1$^{b}$ | 5.6$^{b}$ | 6.8$^{b}$ | 47.5 | 46.6$^{a}$ | 0.86$^{c}$ | 1.04$^{c}$ | 55.4$^{b}$ | 44.9$^{b}$ |

Asterisks indicate significance at *$P$ <.0.05, **$P$ <0.01, $^{ns}$ Nonsignificant; Mean values in the same column followed by the same letters are not significantly different by Duncan's multiple range test.

CS Chickpea straw, CSH cottonseed hulls, OPC Olive press cake, SPC Sunflower press cake, SBP Sugar beet pulp

The ash content of CS:CSH (6.2%) was the highest. The N content of substrates varied between 0.74% (CS:OPC) and 1.40% (CS:3SPC), while the C:N ratio of substrates varied between 33.6 and 64.5 (Table 2). Our results are corroborated by the findings of Zhang *et al.* (1996) who reported that higher yields of *P. ostreatus* are produced on substrates that have low C:N ratio. The C:N ratios of formulated substrates ranged between 33.64 and 46.94. Levels of P, K, Mg and Zn content of the substrate supplemented with SPC were higher than that of other substrates. The highest Fe content was obtained in control substrate, the highest Ca and Mn contents were in CS:SBP, the highest Cu content was in CS:CSH. Among the substrates, the substrate supplemented with OPC had the lowest macro- and micro-element content.

The most abundant content in substrates was cellulose, the content of which was around 29.9% (CS:SBP) - 36.6% (CS:CSH), while hemicellulose and cellulose contents ranged from 15.9 % (CS:SPC) to 19.4% (CS:SBP) and 8.1% (CS:SBP) to 11.9% (CS:OPC) respectively. Jonathan and Fasidi (2011) obseved that cellulose could serve as an energy source for fungal growth and metabolism.

After the *P. ostreatus* (Jacq. ex. Fr) cultivation, significant alterations were obseved on chemical and lignocellulosic content of spent substrates. Moisture content decreased in all media to a range of 61.8-62.9%. The losing moisture can be occured by means of the harvested fruitbodies and evaporation from substrates. Jahromi *et al.* (2011) suggested that biological treatment reduces the pH of the fermented samples. Similarly, the present study showed that there was a reduction in pH over an cultivation period in all substrates. The decrease in pH of mushroom substrates was reported also by Adenipekun and Okunlade (2012).

C content was decreased by the fungi during the period of degradation of the substrates. The most decreases in C contents were determined in CS:SPC (from 47.1% to 44.9). The N and ash contents of the fungal treated increased significantly throughout the incubation period. Pekşen *et al.* (2011) also observed an increase in the ash content during the growth of the mushroom. After *P. ostreatus* cultivation, total N contents of substrates prepared by the mixtures of CS supplement with SPC and CSH incresed from 1.40 to 1.85% and 1.0 to 1.45%, respectively (Table 2 ). This is also consistent with the findings of Akinfemi *et al. (*2009) who observed an increase in the crude protein contents of maize husk when treated with white-rot fungi. Mushroom mycelia have been observed to be rich in proteins (Jonathan, 2002). The increase in crude protein of spent substrate might be due to an increase in fungal mycelia grown on the substrates during the degradation of the various substrates (Jonathan and Adeoyo, 2011). Belewu and Belawu (2005) also reported increment in protein content of spent substrates due probably to the addition of fungal proteins during solubilization and degradation. Percentage increments in total N contents of spent substrates were higher in CS:SHF medium (45%) than in other substrates. As shown in Table 3 and 4, the concentrations of the macro-micro element components in the postharvest cultivation substrates are largely higher than those in the input cultivation substrates.

**Table 3.** The macro element composition of substrates (S) and spent mushroom substrates (SMS).

| Growing media | P (mg kg$^{-1}$) | | K (mg kg$^{-1}$) | | Ca (mg kg$^{-1}$) | | Mg (mg kg$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | GM | SGM | GM | SGM | GM | SGM | GM | SGM |
| **CS** | 1507$^{**c}$ | 2450$^{**b}$ | 3454$^{**c}$ | 3819$^{**b}$ | 5583$^{**a}$ | 5602$^{**a}$ | 2283$^{**b}$ | 2903$^{**b}$ |
| **CS:CSH** | 1673$^{b}$ | 3143$^{a}$ | 3760$^{b}$ | 3797$^{c}$ | 5341b | 5360$^{b}$ | 2130$^{c}$ | 3030$^{ab}$ |
| **CS:OPC** | 1350$^{d}$ | 2150$^{c}$ | 2751$^{e}$ | 2915$^{e}$ | 5160$^{d}$ | 5150$^{c}$ | 1940$^{d}$ | 2363$^{c}$ |
| **CS:SPC** | 2883$^{a}$ | 3377$^{a}$ | 3862$^{a}$ | 3964$^{a}$ | 5214$^{c}$ | 5143$^{c}$ | 2510$^{a}$ | 3163$^{a}$ |
| **CS:SBP** | 1620$^{b}$ | 1773$^{e}$ | 3340$^{d}$ | 3465$^{d}$ | 4700$^{d}$ | 4814$^{d}$ | 2173$^{bc}$ | 2420$^{c}$ |

Asterisks indicate significance at $*P < 0.05$, $**P < 0.01$, $^{ns}$ Nonsignificant; Mean values in the same column followed by the same letters are not significantly different by Duncan's multiple range test.
CS Chickpea straw, CSH cottonseed hulls, OPC Olive press cake, SPC Sunflower press cake, SBP Sugar beet pulp

There were no significant difference in Ca content of the substrate and spent substrate samples though a general decrease was observed. P contents in spent substrates showed significant increases compared with the CS, CS:CSH and CS:OPC. Similar results have been reported by the percentage increment in P content in CS mixed with CSH was highest (46.77%) while the increments in CS substrates prepared with SPC was lowest (8.65%).

The concentrations of the Mg, Fe and Mn in the spent substrates are higher than those in the substrates. The highest increasment of Mg, Fe and Mn contents were usually determined in the spent substrates prepared with CSH, 29.7%, 6.7% and 39.7%, respectively. Distinctively, the Zn and Cu contents of untreated substrates were higher than the spent substrates. The percentage decrement in Zn content in CS:SPC was 139.2%, while the percentage decrements in Cu content in CS:OPC was 89%. Our results are corroborated by the findings of Lee et al. (2009) who reported that contents of K, Mg, Fe and Mn are higher while contents of Zn and Cu are lower in spent substrates.

**Table 4.** The micro element composition of substrates (S) and spent mushroom substrates (SMS).

| Growing media | Fe (mg kg$^{-1}$) | | Mn (mg kg$^{-1}$) | | Zn (mg kg$^{-1}$) | | Cu (mg kg$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | GM | SGM | GM | SGM | GM | SGM | GM | SGM |
| **CS** | 649$^{**a}$ | 687$^{**a}$ | 15.7$^{**c}$ | 25.7$^{**b}$ | 33.7$^{**b}$ | 15.7$^{ns}$ | 3.7$^{**d}$ | 1.9$^{**e}$ |
| **CS:CSH** | 553$^{e}$ | 593$^{c}$ | 18.5$^{c}$ | 30.7$^{a}$ | 33.2$^{bc}$ | 16.6 | 13.3$^{a}$ | 7.8$^{a}$ |
| **CS:OPC** | 569.4$^{d}$ | 589$^{c}$ | 16.5$^{c}$ | 23.7$^{b}$ | 34.6$^{b}$ | 16.5 | 6.3$^{b}$ | 3.3$^{d}$ |
| **CS:SPC** | 605$^{c}$ | 626$^{b}$ | 22.3$^{b}$ | 35,0$^{a}$ | 40.5$^{a}$ | 16.9 | 6.8$^{b}$ | 4.6$^{b}$ |
| **CS:SBP** | 620$^{b}$ | 635$^{b}$ | 26.4$^{a}$ | 33.7$^{a}$ | 31.6$^{c}$ | 17.8 | 4.7$^{c}$ | 4.0$^{c}$ |

Asterisks indicate significance at **P* <0.05, ***P* <0.01, $^{ns}$ Nonsignificant; Mean values in the same column followed by the same letters are not significantly different by Duncan's multiple range test.
CS Chickpea straw, CSH cottonseed hulls, OPC Olive press cake, SPC Sunflower press cake, SBP Sugar beet pulp

In the study, levels of cellulose, hemicellulose and lignin were found to be significantly lower in the spent substrates. The pronounced changes are shown in Table 5 occurred in cellulose, hemicellulose and lignin.

**Table 5.** The lignocellulosic contents of substrates (S) and spent mushroom substrates (SMS).

| Growing media | Cellulose (%) | | Hemicellulose (%) | | Lignin (%) | |
|---|---|---|---|---|---|---|
| | GM | SGM | GM | SGM | GM | SGM |
| **CS** | 33.2$^{** b}$ | 21.8$^{** b}$ | 17.4$^{**a b}$ | 7.7$^{ns}$ | 9.3$^{** c}$ | 6.3$^{** c}$ |
| **CS:CSH** | 37.7$^{a}$ | 23.5$^{a}$ | 15.2$^{b}$ | 7.8 | 11.9$^{a}$ | 7.2$^{b}$ |
| **CS:OPC** | 32.8$^{b}$ | 22.4$^{ab}$ | 16.8$^{b}$ | 7.9 | 10.9$^{b}$ | 8.4$^{a}$ |
| **CS:SPC** | 36.6$^{a}$ | 20.3$^{c}$ | 15.9$^{b}$ | 7.7 | 11.7$^{a}$ | 6.3$^{c}$ |
| **CS:SBP** | 29.9$^{c}$ | 21.6$^{bc}$ | 19.4$^{a}$ | 7.8 | 8.1$^{d}$ | 5.7$^{d}$ |

Asterisks indicate significance at **P* <0.05, ***P* <0.01, $^{ns}$ Nonsignificant; Mean values in the same column followed by the same letters are not significantly different by Duncan's multiple range test.
CS Chickpea straw, CSH cottonseed hulls, OPC Olive press cake, SPC Sunflower press cake, SBP Sugar beet pulp

The decrease observed in this study has been reported earlier by Kuforiji and Fasidi (2004) and Jonathan *et al.* (2010). The main function of straw is to provide a reservoir of cellulose, hemicellulose and lignin, which is utilized during the growth of 'spawn' and during fructification (Yıldız *et al.,* 2002). Mushroom hyphae secrete large amounts of extracellular enzymes which bring about the degradation of macromolecules such as cellulose, hemicellulose, lignin and protein in the substrates (Kuforji and Fasidi, 2008). The existing trend leads to the conclusion that cell-wall components are being extensively degraded by *P. ostreatus* (Jacq. ex. Fr) enzymes. Chen *et al.* (1995) determinated the decrease in crude fibre and crude fibre fractions of the fungal biodegraded samples and suggested that may be due to the extensive utilization of cellulose and hemicellulose by the test fungi. Morever, Alemawor *et al.* (2009) reported that during the fermentation process, the weekly changes in the fibre fractions indicated the degree of lignocellulose biodegradation as well as the enzyme activities exhibited by *P. ostreatus* (Jacq. ex. Fr) on cocoa pod husk.

It seems that cell-wall components have different rates of degradation when the components was exposed to *P. ostreatus* (Jacq. ex. Fr) enzymes. Percentage decrements in celulose and lignin contents of spent substrates were higher in CS:SHF substrate (44.5 and 49 %, respectively) than in other substrates. Lignin is more difficult to break down than cellulose or hemicellulose due to its complicated structure (Hatakka, 1994). Removal of lignin contents also has a contribution in the digestibility of lignocellulosic substrates (Zadrazil and Brunnet, 1982). The lowest lignin degradation observed in CS:OPC and CS:SBP. It might be ascribed to the compact structure of OPC (Baetta-Hall *et al.,* 2005). and SBP that did not allow an adequate aeration with subsequent negative impact on ligninolysis. Lopez *et al.* (2002) also reported that the degradation activity of white root fungi may be promoted by either intermittent or continuous forced aeration.

Hemicelulose content was also decreased by the fungi during the period of cultivation. The most percentage decrements in hemicellulose contents were determined in CS:SBP (58.2% ). Those results confirm data obtained in other investigation by Thomas *et al.* (1998) and Belewu and Belewu (2005).

The increased proportion of nitrogen and macro-micro elements and decreased proportion of cellulose, hemicellulose and lignin in the spent substrates can facilitate the use of spent substrates as a good animal feed. Although, Philippoussis *et al.* (2002) reported that the nutrient composition of the substrate is one of the factors limiting colonization as well as quantitative and qualitative yield of cultivated mushrooms, there was not a significant correlation between spawn running time yield, BE and N, C:N and macro-micro element content of substrates except Ca in the study (P>0.05) (Table 6). Supplementation with a nitrogenous source is not a key for higher yields in combinations. Our findings are confirmed by Tisdale *et al.* (2006) who reported that there is no correlation between mean N concentration and mean yield was found. *P. ostreatus* (Jacq. ex. Fr) grow on woods which are very poor in nitrogen and macro-micro element contents in the nature. So, the lack of nitrogen may also be factor affecting the overall yield. Yıldız *et al.* (2002) reported also there was no corelation between N, C:N and lignin content with spawn running time. Significant negative relationship was determinated between Ca content of substrates and spawn running time. Calcium ions, therefore, play important roles in the regulation of the growth of hyphal apices and the formation of branches (Gadd, 1995; Royse and Sanchez-Vazguez, 2003).

**Table 6.** Correlations between spawn run time, mushroom yield and BE and constituents of the substrates.

| Constituents of substrates | Spawn run time (days) | Yield (g $kg^{-1}$ substrate) | BE(%) |
|---|---|---|---|
| **Ash** | 0.230 | 0.163 | 0.070 |
| **C** | 0.651 | -0.911* | -0.904* |
| **N** | -0.275 | 0.631 | 0.681 |
| **C:N** | -0,239 | 0,646 | 0.682 |
| **P** | -0.309 | 0.490 | 0.560 |
| **K** | -0.551 | 0.453 | 0.488 |
| **Ca** | -0.924** | 0.389 | -0.413 |
| **Mg** | -0.538 | 0.177 | 0.259 |
| **Fe** | -0.234 | -0.704 | -0.687 |
| **Mn** | 0.583 | -0.254 | -0.248 |
| **Zn** | -0.418 | 0.578 | 0.656 |
| **Cu** | -0.086 | 0.795 | 0.733 |
| **Cellulose** | -0,829 | 0,967** | 0,977** |
| **Hemicellulose** | 0,653 | -0,956* | -0,962** |
| **Lignin** | -0,615 | 0,981** | 0,979** |

**Significant at 0.01, *significant at 0.05

On the other hand yield and BE were significant negatively (P<0.01) related to C ($r^2$= - 0.91 and - 0.90) and hemicellulose ($r^2$ = -0.96 and - 0.96) and significant positive (P<0.01) related to Ca ($r^2$ =0.99 and 0.99) , cellulose ($r^2$ =0.97 and 0.98) and lignin (r2=0.98 and 0.98). Our findings support the conclusion of Ramasamy and Kandaswamy (1976) in that cellulase production was positively correlated with yield of sporophores. Morever substrates contained rich cellulose are suggested as good materials for cultivation of mushrooms by previous studies (Obodai *et al.*, 2003).

## CONCLUSIONS

Based on result, it is appear that chickpea straw, cotton seed hulls, olive press cake and sunflower hulls are economically viable alternatives for *P.ostreatus* (Jacq. ex. Fr) growers. Morever, it was observed that in selecting the right type of material for *P.osteratus* (Jacq. ex. Fr) cultivation, high percentage of cellulose and lignin content of substrate is more important than high nutrient content of substrate.

On the other hand, the increased proportion of nitrogen and macro-micro elements and also decreased proportion of cellulose, hemicellulose and lignin by *P. ostreatus* (Jacq. ex. Fr) growth in the spent substrate can facilitate the use of SMS as a animal feed. So it may increase sustainability and help farm economy and also decrease environmental pollution.

## REFERENCES

Adenipekun, CO and Okunlade, OA, (2012)Biodegradation of Rattan Wood and Maize Stovers by *Pleurotus ostreatus*. Natural Science, 10 (5): 49-57.

Akinfemi A, Adu OA, Adebiyi OA (2009). Use of white-rot-fungi in upgrading maize straw and the resulting impact on chemical composition and *invitro* digestibility. Livestock Research for Rural Development, Vol: 21. http://www.lrrd.org/ lrrd21/10/akin21162.htm

Alemawor F, Victoria PD, Emmanuel OKO, James HO (2009). Effects of *Pleurotus ostreatus* on cocoa pod husk composition: Influence of fermentation period and Mn2+ supplementation on the fermentation process. African Journal of Biotechnology, 8(9): 1950-1958.

Anonymous (2016). Food and Agriculture Organization of the United Nations (FAO), FAOSTAT Database on Agriculture. Rome, Italy: 2016 http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/S

Baeta-Hall L, Saagua MC, Bartolomeu ML, Anselmo AM , Rosa MF (2005). Bio-degradation of olive oil husks in composting aerated piles. Bioresource Technology, 96: 69–78.

Belewu, MA, Belewu KY (2005). Cultivation of Mushrooms (*Volvariella volvacea)* on banana Leaves. African Journal of Biotechnology, 4 (12): 1401-1403.

Chen J, Fales SL, Varga GA, Royse DJ (1995). Biodegradation of Cell wall component of Maize Stover colonized by white rot fungi and resulting Impact on In Vitro Digestibility. Journal of Science of Food and Agriculture, 68: 91-98.

Duchaufour P (1970). *Precis de Pedologie*. Masson & Cia, Paris.

Eren E, Pekşen A (2016). Türkiye'de kültür mantarı sektörünün durumu ve geleceğine bakış. Turkish Journal of Agriculture Food Science and Tecnology, 4(3):189-196.

Figlas D, González Matute R, Curvetto N (2007). Cultivation of culinary-medicinal Lion's Mane mushroom *Hericium erinaceus (Bull:Fr) Pers* on substrate containing sunflowerwer seed hulls. International Journal of Medical Mushrooms, 9, 67- 73.

Gadd GM (1995) Signal transduction in fungi. In: Gow, A.R., Gadd, G.M. (Eds.), The Growing Fungus. Chapman and Hall, London, UK, pp. 183–210.

Garg A (2013). Evaluation Of Different Agricultural Residues For Productivity And Morphological Variations In Oyster Mushrooms *(Pleurotus* spp.). Internet Journal of Microbiology, 12:1.

Gume B, Muleta D, Abate D, (2013). Evaluation of locally available substrates for cultivation of oyster mushroom (*Pleurotus ostreatus*) in Jimma, Ethiopia. African Journal of Microbiology Research , 7(20): 2228-2237.

Hatakka A (1994). Lignin-modifying enzymes from selected white-rot fungi: production and role in lignin degradation. FEMS Microbiol Rev 13: 125–130.

He HQ, Bao DP, Wen MZ, Lu XM (1995). Test on cultivation *Pleurotus ostreatus* with different formula of straw and cotton seed hulls. Journal of Anhui Agrotechnical Teach College, 9**:**36–44.

Jahromi MF, Liang JB, Rosfarizan M, Goh YM, Shokryazdan P, Ho YW (2011). Efficiency of rice straw lignocelluloses degradability by *Aspergillus terreus* ATCC 74135 in solid state fermentation. African Journal of Biotechnology, 10(21): 4428-4435.

Jonathan SG, Fasidi IO (2001). Effect of carbon, nitrogen and mineral sources on growth of *Psathyerella atroumbonata* Pegler), a Nigerian edible mushroom. Food Chemistry, 72: 479-483.

Jonathan SG (2002). Vegetatative growth requirements and antimicrobial activities of some higher fungi in Nigeria. Ph.D Thesis,University of Ibadan, Nigeria.p.268.

Jonathan SG, Akinfemi A, Adenipekun CO (2010). Biodegradation and in-vitro digestibility of maize husk treated with edible fungi (*Pleurotus tuber-regium* and *Lentinus subnudus)* from Nigeria. Electronic Journal of Environmental Agricultural and Food Chemistry, 9(4): 742-750.

Jonathan SG, Adeoyo OR (2011). Effect of environmental and nutritional factors on mycelia biomass yield of ten Nigerian mushrooms during cellulase and amylase production. Electronic Journal of Environmental Agricultural and Food Chemistry, 10 (9): 2891-2899.

Kacar B, İnal A (2008). Bitki Analizleri. Nobel Yayın Dağıtım, Ankara.

Kuforiji OO, Fasidi IO (2004). Effect of Volvariella volvacea cultivation on the components of the substrates. Journal of Applied Science, 7: 4292-4299.

Kuforiji OO, Fasidi, IO (2008). Enzyme activities of Pleurotus tuber-regium (Fries) Singer, cultivated on selected agricultural wastes. Bioresource Technology, 99: 4275-4278.

Lee CY, Park JE, Kim BB, Kim SM, Ro HS (2009). Determination of Mineral Components in the Cultivation Substrates of Edible Mushrooms and Their Uptake into Fruiting Bodies. Mycobiology, 37(2) **:** 109-113.

Liu H (2009) Utilization of spent mushroom substrate in China. http://www.cefa.org.cn/html/2009/10/14/181.html

Lopez, MJ, Elorrieta, MA, Vargas-Garcia, MC, Suarez-Estrella and F, Moreno, J, (2002). The effect of aeration on the biotransformation of lignocellulosic wastes by white-rot fungi. Bioresource Technology, 81 (2): 123–129.

Matute, RG, Figlas D, Curvetto N (2010). Sunflower seed hull based compost for Agaricus blazei Murrill cultivation. International Biodeterioration & Biodegradation, 64: 742- 747.

Medina E, Paredes C, Bustamante MA, Moral R, Moreno-Caselles J (2012). Relationships between soil physic-chemical, chemical and biological properties in a soil amended with spent mushroom substrate. *Geoderma*, 173-174: 152-161.

Membrillo I, Sánchez C, Meneses M, Favela E, Loera O (2011).Particle geometry affects differentially substrate composition and enzyme profiles by Pleurotus ostreatus growing on sugar cane bagasse. Bioresource Technology, 102: 1581–1586.

Obodai M, Cleland-Okine J, Vowotor KA (2003). Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on different lignocellulosic by-products. Journal of Industrial Microbiology and Biotecnology, 30: 146-149.

Pala SA, Wani A, Boda RH, Wani BA (2014). Mushroom refinement endeavor auspicate non green revolution in the offing. Nusantara Bioscience, 6 (2): 173-185.

Peksen, A, Yakupoglu, G, Yakupoglu , T, Gulser, C, Ozturk, E and Ozdemir, N, (2011).Changes in chemical compositions of substrates before and after Ganoderma lucidum cultivation. World Journal of Microbiology and Biotechnology, 27: 637–642.

Philippoussis A, Zervakis G, Diamantopoulou P (2001). Bioconversion of agricultural lignocellulosic wastes through the cultivation of the edible mushrooms *Agrocybe aegenita*, *Volvariella volvacea* and *Pleurotus spp.* World Journal of Microbiology and Biotechnology 17**:** 191-200.

Philippoussis A, Diamantopoulou P, Israilides C (2007). Production of functional food from the sporophores of the medicinal mushroom *Lentinula edodes* through exploitation of lingocellulosic agricultural residues. International Biodeterioration and Biodegradation, 59(3): 216–219.

Philippoussis A, Diamantopoulou P, Zervakis G (2002). Monitoring of mycelium growth and fructification of *Lentinula edodes* on several agricultural residues. In: S´anchez JE, Huerta G, Montiel E (eds), Mushroom Biology and Mushroom Products, UAEM, Cuernavaca.

Rajarathnam S, Bano Z (1989). *Pleurotus* mushrooms Biotransforma-tions of natural lignocellulotic waste. Critical Reviews Food Science Nutrition. 28:31–123.

Rajput R, Prasad G, Chopra AK (2009). Scenario of solid waste management in present Indian context. Caspian Journal of Envirenmental Science, 7: 45–53.

Ramasamy K, Kandaswamy TK (1976). Effect of certain amendments on cellulases and yield of straw mushroom. Indian Journal of Mushrooms, 2: 8-12.

Royse D, Sanchez-Vazquez JE (2003). Influence of precipitated calcium carbonate (CaCO3) on shiitake (Lentinula edodes) yield and mushroom size. Bioresource Technology, 90: 225–228.

Sharma S, Yadav RKP, Pokhrel CP (2013). Growth and Yield of Oyster mushroom (*Pleurotus ostreatus*) on different substrates. Journal on New Biological Reports, 2(1): 03-08.

Thomas M, van Vliet T, van der Poel AFB (1998). Physical Quality of Pelleted Animal Feed: 3. Contribution of Feedstuff Components. Animal Feed Science and Technology, 76: 59-78.

Tisdale TE, Miyasaka SC, Hemmes DE (2006). Cultivation of the oyster mushroom (Pleurotus ostreatus) on wood substrates in Hawaii. World Journal of Microbiology and Biotechnology, 22: 201–206.

Van Soest PJ, Robertson JB, Lewis BA (1991). Methods for dietary fiber, neutral detergent fiber, and nostarch polysaccharides in relation to animal nutrion. Journal of Dairy Science, 74: 3583–3597.

Wang D, Sakoda A, Suzuki M (2001). Biological efficiency and Nutritional Value of *Pleurotus ostreatus* Cultivated on Spent Beer Grain. Bioresource Technology, 78: 293-300.

Yang WJ, Guo FL, Wan Z (2013). Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. Saudi Journal of Biological Science, 20: 333–338.

Yildiz S, Yildiz UC, Gezer ED, Temiz A (2002). Some lignocellulosic wastes used as raw material in cultivation of the Pleurotus ostreatus culture mushroom. Process Biochemistry. 38: 301–106.

Zadrazil F, Brunnert H (1982). Solid state fermentation of lignocellulose containing plant residues with *Sporotrichum pulverlentium.* Nov. and *Dichotimus squalens* (Karsl) Reid. European Journal of Applied Microbiology and Biotechnology, 16: 45- 51.

Zhang CK, Gong F, Sheng XF, Li DS, Feng YJ (1996). Ventilation fermentation of cotton seed hulss population dynmics of microflora and biochemical change furing fermentation. Mushroom Biology and Mushroom Products. Ed. Royse, Penn State Univ. ISBN 1-883956-01-3.