



## Effect of substrates (cotton stalks and cotton seed hulls) on growth, yield and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus florida*)

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**ABSTRACT:** A study was conducted to evaluate the effect of two different substrates (cotton stalks and cottonseed hulls) on growth and yield of two oyster mushrooms, *Pleurotus ostreatus* (PO) and *Pleurotus florida* (PF). The cotton stalks (CS) and cotton seed hulls (CH) were taken in the ratio (100:0, 75:25, 50:50, 25:75 and 0:100) as substrates for growth of oyster mushroom. The growth and yield characteristics (mycelial growth, primordial initiation, cropping period, total yield and biological efficiency), morphological characteristics (cap diameter, stipe length, number of effective fruiting bodies per bunch) and nutritional composition (moisture, protein, fat, fibre, carbohydrates, ash and free gossypol) were investigated. The study showed that the substrate composition had significant effect on growth characteristics, morphological characteristics and nutritional composition of oyster mushroom PO and PF. The increase in cotton seed hulls in the substrate composition increased the mycelial growth and thereby reduced the cropping period. Among the different substrates tested, the substrate composition of 75:25 (CS and CH) was found to have significantly higher yield ( $P<0.05$ ) (926 and 900 g), biological efficiency (23.2 and 22.5 %) and nutritional composition in two oyster mushrooms (PO and PF) respectively. The free gossypol content was absent in all mushroom fruiting body.

**Key words :** Biological efficiency, Cotton stalks, Cotton seed hulls, Mushroom yield, *Pleurotus florida* and *Pleurotus ostreatus*

Mushroom are the fleshy sporophores of fungi known to grow in nature on decaying cellulosic materials, dead wood, soil and manure pits (Josephine, 2014). Majority of these fungi belong to the class Basidiomycotina and a few to the class Ascomycotina. Edible fungi are classified under the order Agaricales and the families Agaricaceae, Polyporaceae and Pluteaceae have been under commercial cultivation. The edible mushrooms are delicacy in food and form one of the choicest table dishes.

They are rich in protein and an excellent source of vitamins and minerals. Most of the mushroom have very low starch content and can form an ideal food for diabetic patients. There are four major edible mushroom cultivated on a commercial scale. They are, *Agaricus bisporus* (white button mushroom), *Volvariella* spp (tropical mushroom or paddy straw mushroom), *Lentinus edodes* (Japanese mushroom) and *Pleurotus* spp (Oyster mushroom). The button mushroom requires low temperature and grows on

fermented substrates, whereas paddy straw mushroom grows on unfermented substrates and at an elevated temperature of 35°C. The Japanese and oyster mushrooms could grow on unfermented substrates at temperature of around 20°C and 30°C, respectively (Satankar *et al.*, 2018).

Agro-residues are commonly used for artificial cultivation of oyster mushroom. The most common agro-residues used are wheat straw, rice straw and pearl millet straw. Soybean straw has also been reported as promising substrate for oyster mushroom cultivation (Deshmukh and Deshmukh, 2016). *Pleurotus sajor-caju* was cultivated in chopped cotton stalks and yield of mushroom was reported as 250 – 400g per kg of dry cotton stalks (Balasubramanya and Kathe, 1996). Mushroom cultivation is highly efficient method of disposing agriculture wastes and simultaneously producing nutritious food. Oyster mushroom cultivation in cotton stalks brings additional income to the farmers of about Rs. 10,000/- per acre besides utilizing stalks generated in the field (Mageshwaran *et al.*, 2017). The degradation of lignin, cellulose, hemicelluloses and tannin in the lignocellulosic residue during mushroom cultivation makes the spent cotton stalks as an ideal animal feed. Among the different substrates tested, cotton stalks has been found to give significantly higher yield in respect of number and weight of sporophores of oyster mushroom (Tupatkar and Jadhao, 2006, Chitamba *et al.*, 2012 and Ashraf *et al.*, 2013) while, Dundar *et al.* (2008) reported that mushroom grown on cotton stalks had higher vitamin content. Cotton seed hulls are the outer covering of cottonseed, made up of lignocellulose and commercially used for

cultivation of oyster mushroom (Yang *et al.*, 2013). In this study, the effect of substrates, cotton stalks and cotton seed hulls was evaluated for the growth yield and nutritional composition of oyster mushroom (PO and PF).

## MATERIALS AND METHODS

**Cotton stalks and cotton seed hulls:** CS and CH were obtained from Ginning Training Center, ICAR-CIRCOT, Nagpur. CS was chipped to 3 to 4 cm in length. The initial moisture content of CS and CH was about 10%.

**Experimental substrate analysis:** The experimental substrate, CS and CH and their combinations were dried in oven at 60° C for 3-4 hours. The dried samples were ground to powder analyzed for pH, Carbon (%), Nitrogen (%) and Free Gossypol. The substrate was mixed completely in distilled water in the ratio of 1:10 and pH of the aliquot was determined using a pH meter. The organic carbon and total nitrogen content was estimated by standard methods. The CN ratio was calculated by dividing the value of carbon to nitrogen. The free gossypol was determined using the method AOCS Ba-7-58 (AOCS, 1989a).

**Oyster mushroom culture:** The oyster mushroom culture PO and PF were obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore, India. The mushroom (PO and PF) was grown in petriplates containing Malt Extract Agar medium at 30 ! at 48 h and stored at 4° C until use.

## Oyster mushrooms spawn preparation:

Two hundred g of sorghum grains were soaked in water for overnight. Next day, the water was drained and the soaked grains were mixed with 1 %  $\text{CaCO}_3$  and 1 %  $\text{CaSO}_4$ . The grains were kept in autoclavable poly propylene (PP) bags ( $11 \times 7$  in.) and sterilized at  $121^\circ\text{C}$ , 15 lbs/in<sup>2</sup> for 15 minutes. After autoclaving, the grains were cooled to room temperature. Two mycelial plugs (dia. 1 cm) of each mushroom strains (PO and PF) were inoculated in separate bags containing sterilized grains. The bags were incubated at  $28 \pm 2^\circ\text{C}$  until the substrate fully colonized with the inoculated fungus.

**Substrate preparation:** Four kg of dry substrate was used for each batch of cultivation. The CS and CH alone (100:0 and 0:100) and its combination (75: 25, 50:50 and 25:75) were soaked in water for overnight. For example, in 50: 50 combinations, 2 kg of CS and 2 kg of CH were taken. After overnight soaking, the water was drained and the substrate was boiled at  $80^\circ\text{C}$  for 2 h. The hot water was drained and the substrate was cooled to room temperature. The approximate moisture content prevailed in pretreated cotton stalks was 50%. The pretreated substrate was further used for oyster mushroom cultivation.

**Oyster mushroom cultivation:** Oyster mushroom cultivation was done at Ginning Training Center, ICAR-CIRCOT, Nagpur, Maharashtra, India during the period June to September 2017. The mushroom bed was prepared by alternative layering of substrate and spawn in PP bags. The dimension of PP bags used was  $24 \times 6$  inches. The mushroom spawn (PO or PF) was layered in the periphery between 2 to 3

inches of substrate. Four layers of substrate were made in each bag. In each bag, 5 % of spawn was layered between the substrate. The random holes were made in the bags to provide aeration. The bags were kept in hanging ropes under dark conditions till complete mycelial growth. The bags were opened after mycelial growth. The temperature and relative humidity maintained during the cultivation period are  $25$  to  $35^\circ\text{C}$  and 60 to 90 % respectively. Two to three harvests were made from each bag. Three batches of mushroom cultivation were done during the reporting period. In each batch, 4 kg of dry substrate was used and filled in 4 to 5 PP bags. In every batch, the yield (g), mycelial growth (days), primordia initiation (days), cropping period (days) and Biological efficiency (%) were determined. The average values of batches of each parameter were recorded.

The biological efficiency was determined using the formula

$$\text{Biological efficiency (\%)} = \frac{\text{Total yield}}{\text{Initial substrate (dry weight)}} /$$

The morphological characteristics of oyster mushrooms (PO and PF) such as cap diameter (mm), stipe length (mm) and no. of effective fruiting bodies / bunch at each batch of cultivation was determined. Ten fruiting bodies of each batch of cultivation were evaluated for the above said characteristics and the average value of batches was recorded.

**Nutritional composition:** The fresh oyster mushrooms (PF and PO) from each batch were dried at  $45^\circ\text{C}$  for 6 to 7 hours in a hot air

oven. The dried mushrooms were powdered and analyzed of its nutritional composition. The percentage of moisture, protein, fat, fibre, carbohydrates, ash and free gossypol were determined. The average values of batches of each parameter were recorded. The moisture content was determined using the formula Initial weight- Final weight (after drying)/ Initial weight × 100. The fat content (%) in the sample was determined by soxhlet method AOCS Ba 3-38 (AOCS, 1989b). The protein content in the sample was determined by kjeldhal method (AOAC, 1999). The fibre content was determined using the method of Tecator *et al.* (1978). The carbohydrate content was determined Nelson-Somoyogi method (Sadasivam and Manickam, 2008). The ash content was determined using muffle furnace. The free gossypol in mushroom sample was determined using the method AOCS Ba-7-58 (AOCS, 1989a).

**Spent substrate analysis:** The substrate obtained after mushroom cultivation was called spent substrate. The spent was dried in oven at 60 °C for 3 to 4 h. The dried spent was powdered and analyzed for pH, carbon (%), Nitrogen (%), CN ratio and free gossypol (%) as detailed in the experimental substrate analysis section.

**Statistical analysis:** The yield data of study were analyzed in a completely randomized design (CRD) using one way analysis of variance (ANOVA) (WASP.1; ICAR research complex, Goa). For all analysis, the differences were considered to be significant at P<0.05.

## RESULTS AND DISCUSSION

**Experimental substrate analysis:** The experimental substrates of different combinations were evaluated for parameters such as pH, Organic carbon, Nitrogen, CN ratio and free gossypol. The results are presented in Table 1. The analysis of experimental substrate is important as these factors determine the mycelial colonization and development of fruiting bodies. The pH of the experimental substrate was in the range of 6.4 to 6.9. In a similar study, the pH of the substrate of different agricultural residues for *P. ostreatus* cultivation was in the range of 6.7 to 6.9 (Hoa *et al.*, 2015). The organic carbon was found higher, 31.8 in 75:25 (CS and CH) and lower, 27.3 in 50: 50 (CS and CH). The nitrogen was found higher, 0.98 in 100 % CH and lower, 0.5 in 100 % CS. The CN ratio was higher, 56.7 in 75:25 (CS and CH) and lower, 31.8 in 100 % CH. Yang *et al.*, 2013 reported the CN

**Table 1.** Chemical properties of experimental substrate

| Substrate        | pH  | Organic Carbon (%) | Total Nitrogen (%) | C/N ratio | Free Gossypol (%) |
|------------------|-----|--------------------|--------------------|-----------|-------------------|
| 100 CS           | 6.9 | 28.1               | 0.5                | 56.2      | 0.03              |
| 75: 25 CS and CH | 6.7 | 31.8               | 0.56               | 56.7      | 0.06              |
| 50 :50 CS and CH | 6.8 | 27.3               | 0.84               | 32.5      | 0.07              |
| 25:75 CS and CH  | 6.5 | 31.5               | 0.91               | 34.6      | 0.15              |
| 100 CH           | 6.4 | 31.2               | 0.98               | 31.8      | 0.52              |

CS: Cotton Stalks; CH: Cottonseed Hulls

ratio of the substrates, rice straw, cottonseed hull and wheat bran for mushroom (*P. ostreatus*) cultivation was in the range of 34.8 to 57.2. The free gossypol content (%) was found higher with increase in addition of CH with CS. The free gossypol was higher, 0.52 in 100 % CH and lower, 0.03 in 100 % CS.

**Growth and yield :** Different combinations of CS and CH were taken for cultivation of oyster mushroom (*P. florida*). The mycelial growth (days) was faster with the increase in addition of CH in CS than only CS. The total yield (g) and biological efficiency (%) was found significantly higher ( $P<0.05$ ) in substrate combination, 75:25 (CS and CH), 926 and 23.2 respectively (Table 2). The mycelial growth period was less, i.e 12 days in 50:50 and 25: 75 (CS and CH) while the mycelial period was high, i.e 23 days in 100 % CS. The primordia initiation was 17 to 19 days from the date of cultivation in all combinations except 100% CS

where the primordia initiation was 25 days. The cropping period (days) was higher, 40 in CS (100 %) and lower, 24 in CH (100 %). In a similar study, the biological efficiency of *P. florida* was higher in corn cob (55 %) followed by oil palm spadix (38%) and corn straw (31.6 %) (Salami *et al.*, 2017). While, Vanathi *et al.* (2016) reported that biological efficiency of *P. florida* was higher in paddy straw (83.4 %) compared to sorghum straw (50.3 %) and sugarcane trash (44.7 %).

Similar to *P. florida* the effect of different combination of substrate (CS and CH) on growth and yield of PO were evaluated. The total yield (g) and biological efficiency (%) of PO was found significantly higher ( $P<0.05$ ) in substrate combination, 75:25 (CS and CH) and the corresponding value was 900 and 22.5 (Table 2). Similar to PF, the mycelial growth was faster with the increase in addition of CH in CS than only CS. The primordia initiation was lower, 17 days in 100 % CH and higher, 25 days in 100% CS. Similarly, the cropping period was higher,

**Table 2.** Effect of substrate on growth and yield of oyster mushroom

| Substrate           | Total yield (g)  | Mycelial growth (days) | Primordia Initiation (days) | Cropping period (days) | Biological efficiency (%) |
|---------------------|------------------|------------------------|-----------------------------|------------------------|---------------------------|
| <i>P. florida</i>   |                  |                        |                             |                        |                           |
| 100 CS              | 736 <sup>b</sup> | 23                     | 25                          | 40                     | 18.4                      |
| 75: 25 CS and CH    | 926 <sup>a</sup> | 13                     | 19                          | 31                     | 23.2                      |
| 50 :50 CS and CH    | 695 <sup>c</sup> | 12                     | 17                          | 29                     | 17.3                      |
| 25:75 CS and CH     | 643 <sup>d</sup> | 12                     | 17                          | 28                     | 16.0                      |
| 100 CH              | 547 <sup>e</sup> | 15                     | 19                          | 24                     | 13.6                      |
| <i>P. ostreatus</i> |                  |                        |                             |                        |                           |
| 100 CS              | 822 <sup>b</sup> | 23                     | 25                          | 42                     | 20.6                      |
| 75: 25 CS and CH    | 900 <sup>a</sup> | 19                     | 20                          | 36                     | 22.5                      |
| 50 :50 CS and CH    | 808 <sup>b</sup> | 18                     | 20                          | 25                     | 20.2                      |
| 25:75 CS and CH     | 532 <sup>c</sup> | 17                     | 18                          | 30                     | 10.6                      |
| 100 CH              | 359 <sup>d</sup> | 16                     | 17                          | 24                     | 8.97                      |

CS: Cotton Stalks; CH: Cottonseed Hulls. The results are the average values of three different batches. Each batch was made with 4 kg of dry substrate. Treatment values followed by same alphabet do not differ significantly at  $P<0.05$ .

42 days in 100 % CS and lower, 24 days in 100 % CH.

In a similar study, Yang *et al.*, (2013) observed that addition of cotton seed hull to rice straw and wheat straw substrate slowed spawn running, primordial development and fruit body formation of *P. ostreatus*. While, our results showed faster mycelial and primordial development and fruiting body formation with the increased addition of cotton seed hulls to cotton stalks. However, Yang *et al.* (2013) reported that increasing the amount of cotton seed hull increased the uniformity of mycelium, yield and biological efficiency and increase mushroom weight, enlarge cap diameter and shorten stipe length. The results were in agreement with our results to certain extent where the addition of 25 % of cottonseed hulls in cotton stalks resulted in increase in yield and biological efficiency (%) of PO. Dundar *et al.* (2009) reported that the yield (g) of fresh mushroom (*P. ostreatus*) in 100 g of dry substrates, wheat straw, cotton stalks, millet stalks and soybean stalks was 17.9, 14.3, 22.7 and 31.5g, respectively. The growth characteristics of three species of *Pleurotus* spp. viz., *P. sarjo-caju*, *P. ostreatus* and *P. djmor*, like spawn running, primordial initiation, harvesting stage, number of fruiting bodies and yield were observed higher in the substrate, cotton stalks than wheat straw and paddy straw (Ashraf *et al.*, 2013).

**Morphological characteristics:** The effect of substrate on morphological characteristics of PF like cap diameter (mm), stipe length (mm) and no. of effective fruiting bodies / bunch were evaluated. The cap diameter (mm) was higher, 77 in substrate combination,

25:75 (CS and CH) and lower, 50 in 100 % CS. The stipe length (mm) was higher, 53 in substrate combination, 50:50 (CS and CH) and lower, 25 in 100 % CS. The number of effective fruiting bodies per bunch was higher, 7.5 in 75:25 (CS and CH) and lower, 5.0 in 25:75 (CS and CH) (Table 3).

Similar to PF, the effect of substrate on morphological characteristics of PO like cap diameter (mm), stipe length (mm) and no. of effective fruiting bodies / bunch were evaluated. The cap diameter (mm) was higher, 69 in substrate combination, 75:25 (CS and CH) and lower, 52 in 100 % CS. The stipe length (mm) was higher, 68 in 50:50 (CS and CH) and lower, 45 in substrate combinations, 100 % CS and CH. The number of effective fruiting bodies/bunch was higher, 16 in 75: 25 and 50:50 (CS and CH) and lower *i.e.* 8.5 in 100 % CS (Table 3). The

**Table 3.** Effect of substrate on morphological parameters of oyster mushroom

| Substrate           | Cap diameter (mm) | Stipe length (mm) | No. of effective fruiting bodies/bunch |
|---------------------|-------------------|-------------------|--|
| <i>P. florida</i>   |                   |                   |  |
| 100 CS              | 50                | 25                | 5.5                                    |
| 75: 25 CS and CH    | 57                | 38                | 7.5                                    |
| 50 :50 CS and CH    | 70                | 53                | 6.5                                    |
| 25:75 CS and CH     | 77                | 38                | 5.0                                    |
| 100 CH              | 64                | 32                | 5.2                                    |
| <i>P. ostreatus</i> |                   |                   |  |
| 100 CS              | 52                | 45                | 8.5                                    |
| 75: 25 CS and CH    | 69                | 56                | 16                                     |
| 50 :50 CS and CH    | 64                | 68                | 16                                     |
| 25:75 CS and CH     | 60                | 53                | 12                                     |
| 100 CH              | 53                | 45                | 9                                      |

CS: Cotton Stalks; CH: Cottonseed Hulls. The results are the average values of three different batches.



**Fig. 1.** Morphology of fruiting body of oyster mushroom (A- *P. florida*; B- *P. ostreatus*)

morphological analysis of fruiting body showed, PO and PF was having serrated and entire margins respectively. The oyster mushroom cultivated in 100 % CS is shown in Fig. 1.

In a similar study, Tupatkar and Jadhao (2006) reported that among the different substrates (wheat and paddy straw, pearl millet, sorghum, cotton and soybean stalks), the substrates containing cotton stalks and leaves was found to have higher weight of sporophore (5.12 g) and stipe length (31.7 mm). The results were in agreement with our results.

**Nutritional composition:** The substrate composition significantly affects the nutritive quality of oyster mushroom (Dunkawal and Jood, 2009; Hoa *et al.*, 2015). In this study, the effect of substrate on nutritional composition (%) of PF like moisture, protein, fat, fibre, carbohydrate, ash and free gossypol were evaluated. The moisture content (%) of PF was in the range of 84.2 and 91.2. Among different treatments, the moisture content of PF was lower, 84.2 in 100 % CS and higher, 91.2 in 100% CH. The protein content of PF (%) was higher, 24.5 in 75:25 and

**Table 4.** Effect of substrate on nutritional composition of oyster mushroom

| Substrate           | Moisture (%) | Protein (%) | Fat (%) | Fibre (%) | Carbohydrate (%) | Ash (%) |
|---------------------|--------------|-------------|---------|-----------|------------------|---------|
| <i>P. florida</i>   |              |             |         |           |                  |         |
| 100 CS              | 84.2         | 18.3        | 2.5     | 12.4      | 59.5             | 7.3     |
| 75: 25 CS and CH    | 87.5         | 24.5        | 3.5     | 11.5      | 53.7             | 6.8     |
| 50 :50 CS and CH    | 89.3         | 24.5        | 3.2     | 13.2      | 52.0             | 7.1     |
| 25:75 CS and CH     | 88.8         | 23.5        | 1.1     | 14.6      | 54.3             | 6.5     |
| 100 CH              | 91.2         | 20.0        | 1.4     | 11.9      | 61.2             | 5.5     |
| <i>P. ostreatus</i> |              |             |         |           |                  |         |
| 100 CS              | 83.4         | 22.8        | 2.1     | 10.5      | 58.4             | 6.2     |
| 75: 25 CS and CH    | 85.0         | 28.0        | 3.7     | 12.5      | 49.9             | 5.9     |
| 50 :50 CS and CH    | 88           | 22.8        | 2.9     | 10.8      | 58.0             | 5.5     |
| 25:75 CS and CH     | 90.5         | 24.0        | 1.5     | 9.6       | 60.0             | 4.9     |
| 100 CH              | 83.4         | 17.5        | 1.2     | 10.2      | 65.9             | 5.2     |

CS: Cotton Stalks; CH: Cottonseed Hulls. The results are the average values of three different batches.

50:50 (CS and CH) and lower, 18.3 in 100 % CS. The fat (%) was higher, 3.5 in 75:25 (CS and CH) and lower, 1.1 in 25:75 (CS and CH). The fibre content (%) was higher, 14.6 in 25:75 (CS and CH) and lower, 11.5 in 75:25 (CS and CH). The carbohydrate content (%) was higher, 61.2 in 100 CH and lower, 52 in 50:50 (CS and CH). The ash content (%) was higher, 7.3 in 100 % CS and lower, 5.5 in 100 % CH (Table 4). The free gossypol in PF sample was found nil in all treatments.

Similar to PF, the effect of substrate on nutritional composition (%) of PO as described earlier was evaluated. The moisture content (%) of PF was in the range of 83.4 and 90.5. The moisture was lower, 83.4 in 100% CS and CH and higher, 90.5 in 25:75 (CS and CH). The protein content (%) of PO was higher, 28.0 in 75:25 (CS and CH) and lower, 17.5 in 100 CH. The fat (%) of PO was higher, 3.7 in 75:25 (CS and CH) and lower, 1.2 in 100 % CH. The fibre content (%) was higher, 12.5 in 75:25 (CS and CH) and lower, 9.6 in 25:75 (CS and CH). The carbohydrate content (%) was higher, 65.9 in 100 % CH and lower, 49.9 in 75:25 (CS and CH). The ash content (%) was higher, 6.2 in 100 % CS and lower, 4.9 in 25:75 (CS and CH) (Table 4). The free gossypol in PF sample was found nil in all treatments.

In a similar study, the maximum protein (27.23 %) was found in oyster mushroom (*P. ostreatus*) grown on cotton wastes as substrate (Ashraf *et al.*, 2013). The range of value of moisture, protein, fat, fibre, carbohydrates and ash contents in oyster mushroom, *P. ostreatus*, *P. sajor-caju*, *P. florida* and *Calocybe indica* grown in Bangladesh were in the range of 86-88%, 20 - 25 %, 4-5 %, 13-24 %, 37-48% and 8-13 %

respectively (Alam *et al.*, 2008). Similarly, the range of value of moisture, protein, fat, fibre, carbohydrates and ash contents in oyster mushroom, *P. ostreatus* and *P. cystidiosus* were in the range of 86-92 %, 15-27 %, 1.3 – 3.3 %, 20-28 %, 37-55 % and 6.3 – 6.7 % respectively (Hoa *et al.*, 2015). In the present study, the moisture, protein, fat, fibre, carbohydrates and ash contents in oyster mushroom, PF and PO were 83-91 %, 18-28 %, 1.1 – 3.7 %, 9.6 – 14.6 %, 52 – 61.2 % and 5.5 – 7.3 % respectively. The present study results are in agreement with the previous reports except the fibre content in mushroom samples where the values in the present results are lesser than the previous reports. As previously stated, the nutritive properties of mushroom depend on the substrate used for its cultivation (Dunkawal and Jood, 2009).

**Characteristics of spent substrate:** The pH of the *P. florida* spent substrate was in the range of 5.3 to 5.8 and found to be slightly acidic. The organic carbon (C) (%) was in the range of 24 to 35.3. The C (%) was found higher, 35.3 in 75:25 (CS and CH) and lower, 24.0 in 100 % CH. The total nitrogen (N) (%) in the spent substrate was in the range of 2.1 to 4.2. The N (%) was found higher, 4.2 in 75:25 (CS and CH) and lower, 2.1 in 25:75 (CS and CH). The C/N ratio was found lower, 8.4 in 75:25 (CS and CH) and higher, 14.6 in 25:75 (CS and CH). The residual free gossypol (%) in the spent substrate was 0.09 (100 % CS and 75:25 CS and CH), 0.04 (50:50 CS and CH), 0.03 (25:75, CS and CH) and nil in 100 % CH (Table 5).

The pH of the *P. ostreatus* spent substrate was between 5.5 and 6.0 which were slight acidic in

**Table 5.** Chemical properties of spent substrate after oyster mushroom cultivation

| Substrate           | pH  | Organic Carbon<br>(%) (C) | Total Nitrogen<br>(N) (%) | C/N ratio | Free Gossypol<br>(%) |
|---------------------|-----|---------------------------|---------------------------|-----------|----------------------|
| <i>P. florida</i>   |     |                           |                           |           |                      |
| 100 CS              | 5.8 | 27.0                      | 3.7                       | 7.8       | 0.09                 |
| 75: 25 CS and CH    | 5.5 | 35.3                      | 4.2                       | 8.4       | 0.09                 |
| 50 :50 CS and CH    | 5.7 | 33.6                      | 2.5                       | 13.4      | 0.04                 |
| 25:75 CS and CH     | 5.7 | 30.6                      | 2.1                       | 14.6      | 0.03                 |
| 100 CH              | 5.3 | 24.0                      | 2.2                       | 10.9      | nil                  |
| <i>P. ostreatus</i> |     |                           |                           |           |                      |
| 100 CS              | 5.6 | 29.4                      | 3.4                       | 8.6       | 0.05                 |
| 75: 25 CS and CH    | 5.5 | 31.7                      | 2.2                       | 14.4      | 0.04                 |
| 50 :50 CS and CH    | 5.6 | 38.1                      | 3.4                       | 11.2      | 0.02                 |
| 25:75 CS and CH     | 5.9 | 32.1                      | 2.8                       | 11.5      | nil                  |
| 100 CH              | 6.0 | 33.9                      | 2.5                       | 13.6      | nil                  |

CS: Cotton Stalks; CH: Cottonseed Hulls. The results are the average values of three different batches.

nature. The C (%) was found higher, 38.1 in 50:50 (CS and CH) and lower, 29.4 in 100 % CS. The N (%) in the spent substrate was in the range of 2.5 to 3.4. The higher N (%), 3.4 was found in 100 % CS and 50:50 (CS and CH) and lower, 2.2 in 75:25 (CS and CH). The C/N ratio varied between 8.6 and 14.4 in *P. ostreatus* spent substrate. The free gossypol (%) in the spent substrate was 0.05 (100 % CS), 0.04 (75:25 CS and CH), 0.02 (50:50 CS and CH) and nil in (25:75 CS and CH) and 100 % CH (Table 5).

The mushroom spent substrate is the left over substrate after the harvesting of mushroom. It consists of mushroom mycelium and residual substrate which are rich in nutrients. The mushroom spent substrate has commercial potential for its use in compost, animal feed, bioremediation, energy feed stock etc. (Phan and Sabaratnam, 2012). As compost, mushroom spent substrate can increase the growth and yield of crop plants (Uzun, 2004; Roy *et al.*, 2015). The potential uses of mushroom spent as discussed here is due its high nutrient content

as evidenced in our study. The results showed that the nitrogen content in the spent mushroom spent substrate was in the range of 2.1 to 4.2 (Table 5).

## CONCLUSION

CS and CH are abundantly available agro-residue in the major cotton growing countries including India. In this study, the effect of CS and CH on growth, yield and nutritional composition of two oyster mushroom, *P. florida* and *P. ostreatus* was evaluated. The results showed that the change in the levels of CS and CH in substrate composition has significantly influenced the growth and yield of mushroom. The increase in the level of CH in the substrate fastens the mycelial growth and reduces the cropping period to 24 days. However, the yield, biological efficiency, fruiting body characteristics and nutritional composition was observed better in the substrate composition (75:25 CS &CH) than the other combinations.

The spent mushroom substrate derived from mushroom cultivation had higher nitrogen content and this could have potential use in compost and other applications.

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