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# Evaluation of Substrate Combinations for Oyster and Milky Mushroom Production

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ABSTRACT

The present investigation was carried out to find the effect of different substrates such as Paddy straw, Sugarcane trash and their combination on growth and yield of different mushroom species. Early bud initiation was found in the paddy straw among all the mushroom species used. In milky mushroom (*Calocybe indica*) early bud initiation was observed in Paddy straw (31.50 days) alone as substrate, which is on par with the combination of paddy straw + sugarcane trash (32.66 days). The fruiting bodies of mushrooms grown on different substrates were harvested at each flush separately up to the third flush. The highest yield was observed in the first flush and it was decreased in the second and third flush on all the substrates. Among different substrate I used, paddy straw substrate was superior and recorded highest yield in all the oyster mushroom species used. In *Hypsizygus ulmarius* the yield on paddy straw (524.33 g $bag^{-1}$) substrate was on par with the Paddy straw + Sugarcane combination (514.17 g $bag^{-1}$). Biological efficiency was significantly varied and found to the highest in Paddy straw substrate in all the oyster mushroom species used. In milky mushroom (*Calocybe indica*) Paddy straw + Sugarcane trash combination recorded higher bio-efficiency of 111.63 per cent and found to be the best substrate for cultivation of milky mushroom. Spent mushroom substrate obtained after mushroom cultivation was analysed for N, P and K content and results showed increase in the N P K content and narrow down of C: N ratio of the spent mushroom substrate over the initial observations. This investigation showed sugarcane trash can be used in combination with the paddy straw for the cultivation of different species of mushroom species.

*Keywords*: Paddy straw, Sugarcane trash, Oyster mushroom, Milky mushroom, Paddy straw, Sugarcane trash

MUSHROOMS are fruiting bodies of fungi belong to Basidiomycetes and Ascomycetes. Edible mushrooms are consumed as good vegetables. They are becoming very popular not only for their taste and flavor but also for their nutritional and medicinal value. Mushrooms are also called as 'White vegetables' or 'Boneless vegetarian meat' containing 20-35 per cent protein (on dry weight basis) which is higher than those of other vegetables and fruits. Oyster mushroom can be used as health beneficial food especially against heart disease and diabetes as it contains low lipid and high fibre (Randive, 2012). Mushrooms can be grown on different agricultural wastes or by-products of agro industry like paddy straw, ragi straw, coir pith, sawdust, cereal straw, corncob, banana leaves, hulled maize cobs and other plant fibres with high cellulose content. Demand for mushroom by consumers has been increasing day by day due to its nutritional and medicinal properties.

For successful mushroom cultivation, three factors must be considered, namely reliable spawn, good substrate and a conducive environment (Rajapakse *et al.,* 2007). Substrates in mushroom cultivation have the same function as soil in plant production Substrate plays an important role in determining yield of mushroom. It is necessary to evaluate different substrates for mushroom growth, yield and also to find the best suitable substrate for its cultivation which in turn helps to utilize agricultural by-products in an economical way. Therefore, the present investigation was carried out to evaluate the effect of different substrates such as paddy straw, sugarcane trash and their combination on the growth and yield of three different species of oyster mushrooms *viz*., *Hypsizygus ulmarius, Pleurotus florida, Pleurotus eous* and a milky mushroom (*Calocybe indica*).

Spent Mushroom Substrate (SMS) is a valuable by-product of mushroom cultivation. It consists of partially degraded paddy or wheat straw, coconut husk, bagasse or other agricultural waste. After a few cultivation cycles, it is bio chemically modified by fungal enzymes into a simpler form and enriched with protein. Fresh and aged mushroom spent substrate has been applied to propagation of fruits, vegetables, flower and foliage crops. Production of mushrooms on these agro-wastes is accompanied by the generation of million tons of residues referred to as spent or used mushroom substrates. Sometimes it is also called mushroom soil, recycled mushroom compost or spent mushroom compost.

## Material and Methods

### Collection of different substrates

Paddy straw was collected from the local farmer's field and Sugarcane trash was collected from VC Farm, Mandya, Karnataka.

### Treatments

*Hypsizygus ulmarius*

$T_1$ – Paddy straw + Hypsizygus ulmarius
$T_2$ – Sugarcane trash + Hypsizygus ulmarius
$T_3$ – (Paddy straw + Sugarcane trash) + Hypsizygus ulmarius

*Pleurotus eous*

$T_1$ – Paddy straw + Pleurotus eous
$T_2$ – Sugarcane trash + Pleurotus eous
$T_3$ – (Paddy straw + Sugarcane trash) + Pleurotus eous

*Pleurotus florida*

$T_1$ – Paddy straw + Pleurotus florida
$T_2$ – Sugarcane trash + Pleurotus florida
$T_3$ – (Paddy straw + Sugarcane trash) + Pleurotus florida

*Calocybe indica*

$T_1$ – Paddy straw + Calocybe indica
$T_2$ – Sugarcane trash + Calocybe indica
$T_3$ – (Paddy straw + Sugarcane trash) + Calocybe indica

*Note:* Combination of paddy straw and sugarcane trash was used in 1:1 ratio on weight basis.

### Spawn Production

Spawn for mushroom cultivation was prepared by following the procedure of Krishnamoorthy (1981). The uninfected, clean sorghum grains were washed thrice in clean water and cooked until the seed coat was just opened. The moisture content of the half boiled grains was adjusted by air drying, to obtain around 50 to 55 per cent moisture. This was followed by mixing with 2 per cent calcium carbonate ($CaCO_3$) and 2 per cent calcium sulphate ($CaSO_4$). This admixture (sorghum + $CaCO_3$ + $CaSO_4$) was filled into Poly propylene bags of 15 × 20 cm of 250 gauge thickness. It was filled to 2/3 capacity to have proper aeration. Mouth of the poly propylene bag was closed with rubber band so as to avoid entry of moisture upon sterilization. The bags were sterilized in an autoclave at 121 °C and 15 psi for 30 minutes. After sterilization the bags were cooled to ambient temperature and inoculated with mother culture of different mushroom species *viz*., *Hypsizygus ulmarius, Pleurotus florida, Pleurotus eous, Calocybe indica* separately. The bags thus inoculated with oyster mushroom species such as *Hypsizygus ulmarius, Pleurotus eous* and *Pleurotus florida* were incubated at 25 °C and those inoculated with milky mushroom *Calocybe indica* were incubated at 30 °C. Mushroom mycelium (cottony growth) covered the entire sorghum in the bag in about 10-12 days. After complete growth of mycelium on substrate, the spawn was used for cultivation.

### Preparation of substrate

Sugarcane trash, paddy straw and combination of both the substrates were used for cultivation of both oyster and milky mushrooms. The substrates were chopped into 3-4 inches and soaked in clean cold water for 10-12 hr in a container. Then excess water was drained off, the substrates were pasteurized using steam for 30 minutes at 80 °C in an autoclave. The pasteurized substrates were spread on a clean cement floor inside the room and cooled to room temperature.

### Spawning and Cropping

The different substrates and their combinations were filled to polythene bag of 30 cm × 45 cm size of 150

gauge thickness. Hundred grams spawn of each mushroom species were used for filling the bags of different treatments separately. Layer spawning was done leaving 5 to 7 cm gap at the top and bag was closed tightly with a rubber band. Three small holes were made at the bottom of the bag and 6 holes all over the bag for drainage and air exchange respectively. These bags were kept on racks in cropping room. Humidity of 70-80 per cent and temperature of 25-28 °C was maintained in the cropping room for oyster mushroom cultivation. After the complete growth of mycelium on substrate, the polythene bags were kept 15 cm apart on racks. Relative humidity was maintained at 80-85 Per cent by using humidifier in the rooms. After the bud initiation, the bags were sprayed with water at regular intervals to avoid drying of the pinhead. Days taken for bud initiation were recorded. The fresh weight of harvested mushroom was recorded at three flushes and total yield per bag was calculated. Watering was continued and crop was maintained up to 45 days. Bio-efficiency was calculated by using the formula as given below (Chang and Miles, 1989).

$$\text{Bio-efficiency (\%)} = \frac{\text{Fresh weight of mushrooms}}{\text{Dry weight of substrate}} \times 100$$

**Cultivation of milky mushroom**

Cultivation of milky mushroom *Calocybe indica* was carried out by following the procedure suggested by Purkayastha and Chandra (1976). The substrate and methods used for milky mushroom is similar to that of oyster mushroom.

**Casing and Cropping**

In milky mushroom cultivation, casing was done after the complete growth of mycelium. Casing mixture include Soil : Compost : Sand in the ratio of 1:1:1 on weight basis with 5 per cent $CaCO_3$. This casing mixture was pasteurized by steaming at 80 °C for 30 minutes and then cooled. Mouth of spawn run bags were opened and folded back and then pasteurized casing mixture was spread over the mycelia grown substrate up to 2.5 cm thickness and moisture was maintained by spraying water as and when required. Days taken for pinhead initiation were recorded. Then fully grown mushrooms were harvested by cutting at the base and recorded the fresh weight for three flushes and total yield per bag was recorded. The crop was maintained up to 60 days. The bio-efficiency of milky mushroom (*Calocybe indica*) was calculated as mentioned for oyster mushroom.

**Chemical analysis of spent mushroom substrates**

Chemical analysis of mushroom spent substrates was carried out for total N, P, K and organic carbon content by adopting following methods.

| Particulars | Methods followed |
|---|---|
| pH | Potentiometric method, pH meter (Piper,1966) |
| Electrical conductivity (ds $m^{-1}$) | Conductometry (Jackson, 1973) |
| Organic carbon (%) | Dry combustion method (Mikhailova *et al.,* 2003) |
| Total nitrogen (%) | Micro Kjeldhal Method (Piper, 1966). |
| Total phosphorus (%) | Vanadomolybdate yellow color method (Jackson, 1973) |
| Total potassium(%) | Flame Photometer (Piper, 1966) |

## Results and Discussion

**Effect of different substrates on pinhead initiation by oyster and milky mushroom species**

Number of days taken for first pinhead initiation by different mushroom species was recorded and results are presented in the Table 1. Early pinhead initiation by *Hypsizygus ulmarius* was observed in paddy straw (21.83 days), which was on par with the combination of Paddy straw and Sugarcane trash and maximum duration for pinhead initiation (28.83 days) was taken in sugarcane trash. Similar trend was observed in *Calocybe indica*. In *Pleurotus eous* and *Pleurotus florida* early pinhead initiation was observed in paddy straw substrate (17.83 and 23.16 days respectively) followed by in the combination of both paddy straw and sugarcane trash and late pinhead initiation (27.16 and 29.33 days respectively) was observed in sugarcane trash substrate.

TABLE 1

Effect of different substrates on pinhead initiation by different mushroom species

| Treatments (Substrates) | Number of days | | | |
|---|---|---|---|---|
| | *Hypsizygus ulmarius* | *Pleurotus eous* | *Pleurotus florida* | *Calocybe indica* |
| $T_1$ - Paddy Straw | 21.83 [b] | 17.83 [c] | 23.16 [c] | 31.50 [b] |
| $T_2$ - Sugarcane trash | 28.83 [a] | 27.16 [a] | 29.33 [a] | 36.33 [a] |
| $T_3$ - Paddy straw + Sugarcane trash | 23.16 [b] | 21.16 [b] | 27.00 [b] | 32.66 [b] |

**Note:** Each value represents the mean of 6 replications.
Means with same superscript are statistically on par at ≤ P 0.05 by DMRT

These results are in agreement with the report of Lalithadevi and Many (2014), where early pinhead initiation of oyster mushroom (*Pleurotus florida*) was observed on paddy straw (16-25 days) when compared to with different fruits and vegetables peel alone and in combination of different proportion with paddy straw as substrates. Sugarcane trash contains high amounts of complex lignin compounds as compared to paddy straw. This might be the probable reason for delayed colonization and pinhead initiation in this substrate. The similar observations were observed by Patel and Trivedi (2016) where in early bud initiation of milky mushroom *Calocybe indica* was observed on paddy straw when compared with other substrates tested *viz.,* wheat straw, mango dry leaves and sugarcane trash. Biswas and Singh (2009) also obtained the similar results.

**Effect of different substrates on oyster and milky mushroom yield at three different flushes**

The fruiting bodies of oyster and milky mushrooms grown on different substrates were harvested at each flush separately up to the third flush and the fresh weight (yield in grams per bag) was recorded.

***Hypsizygus ulmarius***

Effect of substrate combinations on yield of *Hypsizygus ulmarius* at different flushes is presented in the Fig.1a. Perusal of the table indicates that highest yield (g $bag^{-1}$) was obtained in first flush in all the substrates used. However, in second and third flush, the yield of $T_1$ (Paddy straw) was not significantly different from the $T_3$ (Paddy straw + Sugarcane trash) treatment. Significantly lowest yield was recorded on sugarcane trash substrate at all the three flushes. Therefore, total yield of $T_1$ treatment was on par with the $T_3$ treatment.

***Pleurotus eous***

Yield of *Pleurotus eous* at different flushes on different treatment substrates is given in Fig.1b. In the first harvest, highest yield was obtained in paddy straw substrate ($T_1$) in all the three flushes followed by in the combination of Paddy straw + Sugarcane trash ($T_3$). However, lowest yield was recorded in sugarcane trash ($T_2$). In the second and third flushes gradual decrease in yield was observed. Though, it should be noted that except in second flush where $T_1$ and $T_3$ were on par each other and the yield was statistically significant too.

***Pleurotus florida***

Effect of various substrates on *Pleurotus florida* yield at different flushes was recorded and the results are presented in the Fig. 1c. From the results, it is clear that significantly highest yield was observed in $T_1$ (Paddy straw substrate) at all three flushes. In case of second flush, the paddy straw substrate as well as combination paddy straw and sugarcane trash as a substrate were found to be statistically on par with each other and lowest yield was recorded in the $T_2$ treatment (Sugarcane trash) at all the three flushes.

***Calocybe indica***

The results pertaining to the effect of substrate combinations on yield of *Calocybe indica* at three different flushes is presented in the Fig.1d. The yield of *Calocybe indica* was significantly higher in combination of paddy straw and sugarcane trash at all the three flushes (224.50, 166.66 and 61.33 g $bag^{-1}$ respectively), however, it was statistically on par with the substrate paddy straw alone at three flushes (221.16, 147.33 and 57.66 g $bag^{-1}$ respectively). Significantly lowest yield was recorded in $T_2$ treatment (Sugarcane trash) at all three flushes.

Fig. 1a

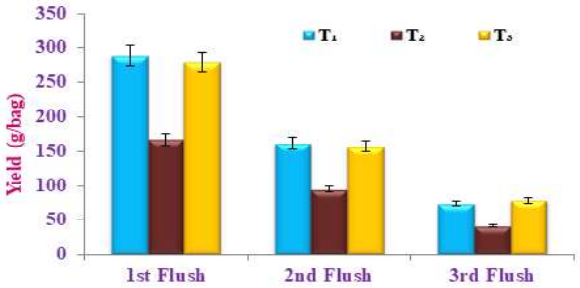


Fig. 1b


Fig. 1c

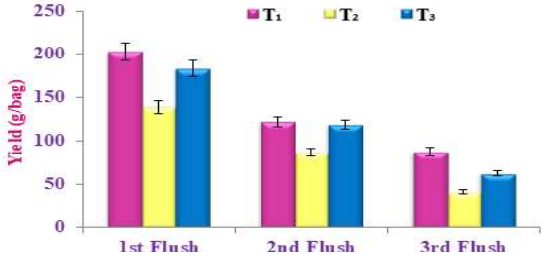


Fig. 1d

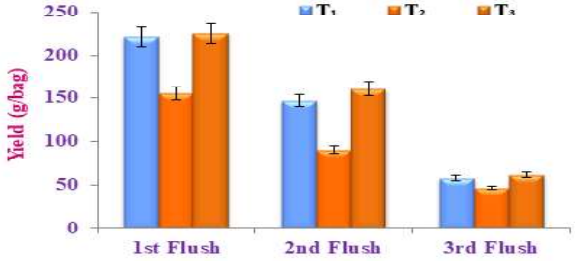


Fig. 1: Effect of substrate combination on oyster and milky mushroom yield at three different flushes

Fig. 1a: *Hypsizygus ulmarius;* Fig. 1b: *Pleurotus eous;* Fig. 1c: *Pleurotus florida;* Fig. 1d: *Calocybe indica*

The highest yield was observed in the first flush and it was decreased in the second and third flush on all the substrates *i.e*., paddy straw, sugarcane trash and paddy straw + sugarcane trash (1:1). Similar results were obtained by Iqbal *et al.,* (2016) in which they have observed that the yield of *Pleurotus florida* was high in the first flush and decreased in the second flush and then decreased further in third flush on all the substrates tested *viz.,* (wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw). Pokhrel *et al.* (2013) also obtained similar results that the *Pleurotus* mushroom grown on all the substrates shown decreasing pattern of yield from first flush to third flush. Vidya *et al*. (2017) confirmed from their work that first flush yield was highest in all treatments followed by second and third flush.

**Effect of different substrates on yield of oyster and milky mushroom species**

Yield of both oyster (*Hypsizy\gus ulmarius, Pleurotus eous* and *Pleurotus florida*) and milky (*Calocybe indica*) mushrooms grown on different substrates is presented in the Table 2 and Fig. 2. Statistically significant difference was observed among the different substrates used for the cultivation of different mushroom species

TABLE 2

Yield of different mushrooms species grown on different substrates

| Treatments (Substrates) | Yield (g bag$^{-1}$) | | | |
|---|---|---|---|---|
| | *Hypsizygus ulmarius* | *Pleurotus eous* | *Pleurotus florida* | *Calocybe indica* |
| $T_1$ - Paddy Straw | 524.33 [a] | 411.83 [a] | 396.00 [a] | 426.16 [b] |
| $T_2$ - Sugarcane trash | 304.00 [b] | 266.83 [c] | 225.00 [c] | 292.00 [c] |
| $T_3$ - Paddy straw + Sugarcane trash | 514.17 [a] | 364.66 [b] | 374.00 [b] | 446.50 [a] |

**Note:** Each value represents the mean of 6 replications.
Means with same superscript are statistically on par at ≤ P 0.05 by DMRT

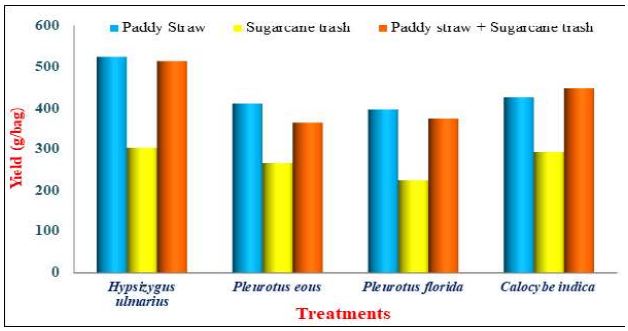


Fig. 2: Yield of different mushroom species grown on different substrates

Among all the oyster mushroom species cultivated, highest yield was observed in *Hypsizygus ulmarius* followed by *Pleurotus eous* and *Pleurotus florida*. In *Hypsizygus ulmarius,* significantly maximum yield of 524.33 gram per bag was obtained on paddy straw ($T_1$) which was on par with the combination of paddy straw and sugarcane trash ($T_3$ with yield of 514.17 g $bag^{-1}$) and minimum yield of 304.00 grams was observed in sugarcane trash substrate.

In case of *Pleurotus eous* and *Pleurotus florida*, highest yield (411.83 g $bag^{-1}$ and 396.00 g $bag^{-1}$ respectively) was observed on paddy straw substrate, followed by combination of paddy straw and sugarcane trash. In both *Pleurotus eous* and *Pleurotus florida* significantly lowest yield of 266.83 gram per bag and 225.00 gram per bag was recorded in sugarcane trash substrate respectively.

Effect of substrates on yield of *Calocybe indica* varied significantly. The maximum yield of 446.50 g $bag^{-1}$ was obtained in treatment $T_3$ (Paddy straw + Sugarcane trash) which is on par with the paddy straw substrate. Significantly lowest yield was observed in sugarcane trash (292.00 g $bag^{-1}$) substrate.

Several studies on the mushroom cultivation confirmed that the use of different strains, different lignocellulosic substrates, different types of spawn, moisture and physicochemical conditions, *etc*. are important for the cultivation productivity of particular mushroom (Ouzoumi *et al*., 2009). Maximum yield by *Pleurotus eous* and *Pleurotus florida* was recorded in $T_1$ (100 % paddy straw alone). It may be due to the nature and nutrient status of the substrates. The result is in agreement with the research work of Karuppuraj *et al*., (2014) who concentrated on yield improvement of *Pleurotus eous* on unexplored locally available lignocellulosic materials such as paddy straw, reeds, banana stem, sugar cane bagasse, coir pith, sorghum husk and sunflower stem. Maximum yield was obtained for paddy straw substrate. Paddy straw substrate was suitable to cultivate *Pleurotus eous* and *Pleurotus florida*, whereas from our study, it is showed that *Hypsizygus ulmarius* and *Calocybe indica* can also be cultivated on the combination of paddy straw and sugarcane trash (1:1 ratio).

## Effect of different substrates on bio-efficiency of different mushroom species

Bio-efficiency of different species of mushroom grown on different substrate materials is given in the Table 3 and Fig.3. A perusal on the table indicates that

TABLE 3

Bio-efficiency of different mushrooms species grown on different substrates

| Treatments (Substrates) | Bio-efficiency (%) | | | |
|---|---|---|---|---|
| | *Hypsizygus ulmarius* | *Pleurotus eous* | *Pleurotus florida* | *Calocybe indica* |
| $T_1$ - Paddy Straw | 131.08 [a] | 102.95 [a] | 99.00 [a] | 106.54 [b] |
| $T_2$ - Sugarcane trash | 76.00 [c] | 66.70 [c] | 56.25 [c] | 73.00 [c] |
| $T_3$ - Paddy straw + Sugarcane trash | 128.54 [b] | 91.16 [b] | 93.50 [b] | 111.63 [a] |

**Note:** Each value represents the mean of 6 replications.
Means with same superscript are statistically on par at ≤ P 0.05 by DMRT

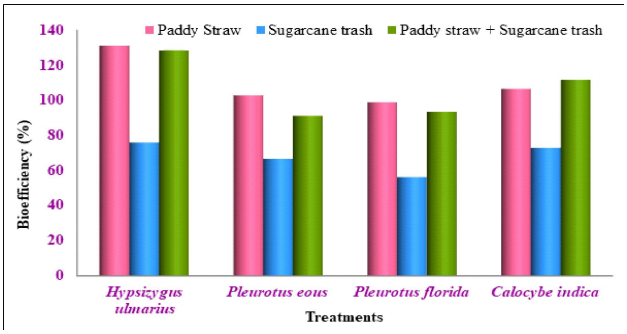


Fig. 3: Bio-efficiency of different mushroom species grown on different substrate

highest bio-efficiency was observed in *Hypsizygus ulmarius,Pleurotus eous* and *Pleurotus florida* when grown on Paddy straw ($T_1$, with bio efficiency of 131.08, 102.95 and 99 per cent, respectively), followed by in treatment $T_3$ (Paddy straw + Sugarcane trash). Significantly the least bio-efficiency was found in $T_2$ (Sugarcane trash).

In case of *Calocybe indi*ca, bio-efficiency of substrate varied significantly and highest bio efficiency of 111.63 per cent was recorded on $T_3$ (Paddy straw + Sugarcane trash) followed by $T_1$ with an bio efficiency of 106.54 per cent and minimum bio-efficiency of 73.00 per cent was observed in sugarcane trash.

Higher biological efficiency was found in those mixed substrates may be caused by sugarcane trash, which is a reservoir of carbon that the *Pleurotus* culture easily utilizes during the growth of spawn and colonization of substrates during the generative stage. In the study of Karuppuraj *et al*., 2014 out of the eight substrates used for the cultivation of *Pleurotus eous*, coir pith compost gave the lowest yield and maximum yield produced when paddy straw was used as the substrate. Raja and Ganesh (2013) have reported that yield potential of milky mushroom (*Calocybe indica*) can be improved when used in combination with paddy straw. Superiority of paddy straw as compared to many other substrates for cultivation of *Calocybe indica* has been reported by many workers (Lakshmipathy *et al*., 2012 and Sarnya *et al*., 2011).

**Initial chemical properties of substrates**

Chemical properties like pH, EC (Electrical conductivity), total per cent organic carbon and total per cent nitrogen, phosphorus and potassium were estimated for paddy straw, sugarcane trash and their combination and the results obtained are presented in the Table 4.

**pH**

The pH of substrates were ranged from 6.85 to 7.23, this indicates the substrates used were nearly neutral. The highest pH of 7.23 was observed in paddy straw followed by the combination of both paddy straw and sugarcane trash (6.93) and the least pH was observed in sugarcane trash.

**Electrical conductivity (dS $m^{-1}$)**

Electrical conductivity of substrates was ranged from 0.58 to 0.65. The lowest EC was observed by sugarcane trash (0.58 dS $m^{-1}$) followed by the combination of paddy straw and sugarcane trash (0.61 dS $m^{-1}$) and Highest EC was found in paddy straw (0.65 dS $m^{-1}$).

**Organic carbon (%)**

Wide variation of organic carbon content was observed among the different substrates. Highest organic carbon content of 68.54 per cent was observed in sugarcane trash followed by the combination of both paddy straw and sugarcane trash (56.45 %) and paddy straw (45.50 %).

**Total per cent nitrogen (N)**

Initial total nitrogen content of substrates was analyzed and results are presented in the Table 4. Nitrogen content varied among the substrates, highest N content of 0.85 per cent was observed in paddy straw followed by combination of substrates (0.74%) and less nitrogen of 0.62 per cent was present in sugarcane trash.

TABLE 4

Initial chemical properties of substrates

| Substrates | pH | EC(dSm$^{-1}$) | OC (%) | N (%) | P (%) | K (%) | C:N Ratio |
|---|---|---|---|---|---|---|---|
| Paddy straw | 7.23 | 0.65 | 45.50 | 0.85 | 0.42 | 1.06 | 53.53 |
| Sugarcane trash | 6.85 | 0.58 | 68.54 | 0.62 | 0.22 | 0.42 | 110.55 |
| Paddy straw + (1:1) Sugarcane trash | 6.93 | 0.61 | 56.45 | 0.74 | 0.37 | 0.93 | 76.28 |

**Total per cent phosphorus (P)**

Phosphorus content of substrates like paddy straw, sugarcane trash and their combination (1:1 ratio) was estimated before using for mushroom cultivation highest P was observed in paddy straw followed by the combination of paddy straw and sugarcane trash and the least P content was found in sugarcane trash.

**Potassium content (K)**

Substrates were analysed for potassium content before using them for mushroom cultivation. The highest potassium content of 1.06 per cent was observed in paddy straw followed by the combination of paddy straw and sugarcane trash and the least P of 0.42 per cent was found in sugarcane trash.

**C:N Ratio**

C:N ratio was calculated by using nitrogen and organic carbon content of substrates and wider range of C:N ratio was observed among the substrates. Maximum C:N ratio was recorded in sugarcane trash (110.55) followed by combination (76.28) and minimum C:N ratio was found in paddy straw (53.53).

**Effect of substrate combination on total per cent organic carbon content of spent mushroom substrate**

Total per cent organic carbon content of the substrates before and after growth of both oyster (*Hypsizygus ulmarius, Pleurotus eous* and *Pleurotus florida*) and milky (*Calocybe indica*) mushrooms are presented in the Table 5. After mushroom cultivation, reduction in organic carbon content was observed in all the substrates used and mushroom species cultivated and the results obtained were statistically significant too. The highest reduction in organic carbon content was observed in sugarcane trash ($T_2$) in all the species of mushroom cultivated followed by the combination of paddy straw and sugarcane and minimum reduction in organic carbon content was observed in treatment $T_1$ (Paddy straw).

TABLE 5

Effect of substrate combination on total per cent organic carbon content of spent mushroom substrate

| Treatments (Substrates) | Organic carbon (%) | | | | |
|---|---|---|---|---|---|
| | *Initial values* | *Hypsizygus ulmarius* | *Pleurotus eous* | *Pleurotus florida* | *Calocybe indica* |
| $T_1$ - Paddy Straw | 45.50 | 37.00 $^{c}$ | 37.93 $^{c}$ | 38.16 $^{c}$ | 36.26 $^{c}$ |
| $T_2$ - Sugarcane trash | 68.54 | 54.20 $^{a}$ | 53.76 $^{a}$ | 57.40 $^{a}$ | 55.63 $^{a}$ |
| $T_3$ - Paddy straw + Sugarcane trash | 56.45 | 46.63 $^{b}$ | 48.66 $^{b}$ | 46.00 $^{b}$ | 47.96 $^{b}$ |

**Note:** Each value represents the mean of 6 replications.
Means with same superscript are statistically on par at ≤ P 0.05 by DMRT

**Hypsizygus ulmarius**

In *Hypsizygus ulmarius*, the highest per cent reduction in organic carbon content of 20.92 per cent was observed in Table 6. sugarcane trash substrate followed by in the paddy straw (18.68%) which is on par with the combination of paddy straw and sugarcane trash combination (17.36%).

TABLE 6

Effect of substrate combination on per cent reduction in organic carbon content of spent mushroom substrate

| Treatments (Substrates) | Percent reduction in organic carbon content (%) | | | |
|---|---|---|---|---|
| | *Hypsizygus ulmarius* | *Pleurotus eous* | *Pleurotus florida* | *Calocybe indica* |
| $T_1$ - Paddy Straw | 18.68 $^{b}$ | 16.26 $^{b}$ | 16.11 $^{b}$ | 20.93 $^{a}$ |
| $T_2$ - Sugarcane trash | 20.92 $^{a}$ | 21.55 $^{a}$ | 16.25 $^{b}$ | 18.83 $^{b}$ |
| $T_3$ - Paddy straw + Sugarcane trash | 17.36 $^{b}$ | 13.78 $^{c}$ | 18.51 $^{a}$ | 15.02 $^{c}$ |

**Note:** Each value represents the mean of 6 replications.
Means with same superscript are statistically on par at ≤ P 0.05 by DMRT

**Pleurotus eous**

Similarly, in *Pleurotus eous* significantly maximum per cent reduction in organic carbon (21.55%) was

observed in sugarcane trash substrate followed by in paddy straw (16.26%) and the least reduction in organic carbon content was observed in the combination of paddy straw and sugarcane trash (13.78%).

**Pleurotus florida**

In case of *Pleurotus florida* significantly highest per cent reduction in organic carbon content of 18.51 per cent was observed in the $T_3$ treatment (Paddy straw + Sugarcane trash) followed by in the treatments which received sugarcane trash (16.25%) and paddy straw (16.11%) alone as substrates.

*Calocybe indica*

Significantly highest per cent reduction in organic carbon content was observed in paddy straw (20.93%) followed by in the sugarcane trash (18.83%) substrate and the lowest per cent reduction in organic carbon content was observed in the combination of paddy straw and sugarcane trash (15.02%). The loss of organic carbon content may be due to the utilization of carbon from lignocellulosic content of the substrate. Shivabasu (2017) observed similar results in *Pleurotus* spp. grown on maize and rice straw showed reduction in organic carbon content.

**Effect of substrate combination on total per cent nitrogen, phosphorus and potassium content of spent mushroom substrate**

Total nitrogen, phosphorus and potassium content of substrates before mushroom cultivation and spent mushroom substrates obtained after mushroom cultivation was determined and the data obtained were presented in the Table 7, 8 and 9, respectively.

**Total nitrogen content (%)**

The total nitrogen content of the substrates before and after the mushroom cultivation is presented in the Table 7. From the table, it is clear that the nitrogen content of the spent mushroom substrates slightly increased compared to unspent substrate. From the data it was observed that the highest total per cent nitrogen content of spent mushroom substrate was found in treatment $T_1$ (Paddy straw) compared to other

TABLE 7

Effect of substrate combination on total per cent nitrogen content of spent mushroom substrates

| Treatments (Substrates) | Nitrogen (%) | | | | |
|---|---|---|---|---|---|
| | *Initial values* | *Hypsizygus ulmarius* | *Pleurotus eous* | *Pleurotus florida* | *Calocybe indica* |
| $T_1$ - Paddy Straw | 0.85 | 2.12 [a] | 1.98 [a] | 1.85 [a] | 2.01 [a] |
| $T_2$ - Sugarcane trash | 0.62 | 1.40 [c] | 1.31 [c] | 1.41 [c] | 1.58 [c] |
| $T_3$ - Paddy straw + Sugarcane trash | 0.74 | 1.82 [b] | 1.69 [b] | 1.61 [b] | 1.80 [b] |

**Note:** Each value represents the mean of 6 replications.
Means with same superscript are statistically on par at ≤ P 0.05 by DMRT

treatments over the initial observations which is less than 1. The second highest was observed in treatment $T_3$ (Paddy straw + Sugarcane trash). The lowest accumulation of total per cent nitrogen was observed in treatment $T_2$ (Sugarcane trash). *Hypsizygus ulmarius* recorded highest accumulation of total per cent nitrogen content among other cultivated mushroom species.

Statistically significant difference was observed among the different substrates and their combination after the cultivation of both oyster (*Hypsizygus ulmarius, Pleurotus eous* and *Pleurotus florida*) and milky mushroom (*Calocybe indica*). In all the mushroom species cultivated, highest nitrogen content was observed in paddy straw followed by the combination of paddy straw and sugarcane trash. Significantly the lowest or minimum increase in nitrogen content was observed in sugarcane trash.

Increase in nitrogen content of mushroom spent substrate may be attributed to the growth of mushroom fungus mycelium. Similar results were obtained by Krishnappa (2012), who has reported that spent mushroom substrate have high nutrient content compared to unspent substrate. It may be due to the degradation of substrates by mushroom which may upgrade the nutrient value of substrate.

**Total Phosphorus content (%)**

Spent substrate after mushroom cultivation was analysed for P content and the data obtained is presented in the Table 8. Significantly the highest total P content of 0.72 per cent, 0.67 per cent, 0.64 per cent and 0.69 per cent was recorded in the treatment $T_1$ which received Paddy straw as substrate by *Hypsizygous ulmarius, Pleurotus eous*, *Pleurotus florida* and milky mushroom *Calocybe indica* spent mushroom substrates respectively. The second highest was observed in $T_3$ treatment (Paddy straw + Sugarcane trash). The lowest accumulation of total per cent nitrogen was observed in treatment $T_2$ (Sugarcane trash).

TABLE 8

Effect of substrate combination on total per cent Phosphorus content of spent mushroom substrate

| Treatments (Substrates) | Phosphorus (%) | | | | |
|---|---|---|---|---|---|
| | *Initial values* | *Hypsizygus ulmarius* | *Pleurotus eous* | *Pleurotus florida* | *Calocybe indica* |
| $T_1$ - Paddy Straw | 0.42 | 0.72 [a] | 0.67 [a] | 0.64 [a] | 0.69 [a] |
| $T_2$ - Sugarcane trash | 0.22 | 0.51 [c] | 0.41 [c] | 0.37 [c] | 0.46 [c] |
| $T_3$ - Paddy straw + Sugarcane trash | 0.37 | 0.61 [b] | 0.57 [b] | 0.52 [b] | 0.57 [b] |

**Note:** Each value represents the mean of 6 replications.
Means with same superscript are statistically on par at ≤ P 0.05 by DMRT

**Potassium content (%)**

Similarly, potassium content of the spent mushroom substrate of different mushroom species cultivated on different substrates was estimated and the data is presented in the Table 9. Slight increase in the K content was observed in all the spent substrate compared to initial value. Significantly the highest K was observed in paddy straw substrate by *Hypsizygous ulmarius, Pleurotus eous*, *Pleurotus florida* and *Calocybe indica* of K content 1.41 per cent, 1.31 per cent, 1.27 per cent and 1.36 per cent respectively. The least accumulation of potassium content was observed in the sugarcane trash substrate in all the mushroom species cultivated. The highest accumulation of potassium content was observed in *Hypsizygus ulmarius* and the least was found in *Pleurotus florida* among other cultivated mushroom species.

TABLE 9

Effect of substrate combination on total per cent Potassium content of spent mushroom substrate

| Treatments (Substrates) | Potassium (%) | | | | |
|---|---|---|---|---|---|
| | *Initial values* | *Hypsizygus ulmarius* | *Pleurotus eous* | *Pleurotus florida* | *Calocybe indica* |
| $T_1$ - Paddy Straw | 1.06 | 1.41 [a] | 1.31 [a] | 1.27 [a] | 1.36 [a] |
| $T_2$ - Sugarcane trash | 0.42 | 0.80 [c] | 0.72 [c] | 0.69 [c] | 0.75 [b] |
| $T_3$ - Paddy straw + Sugarcane trash | 0.93 | 1.31 [b] | 1.23 [b] | 1.16 [b] | 1.29 [c] |

**Note:** Each value represents the mean of 6 replications.
Means with same superscript are statistically on par at ≤ P 0.05 by DMRT

**4.9 Effect of substrate combination on C:N ratio of spent mushroom substrate**

C:N ratio of the substrates before and after growth of both Oyster (*H. ulmarius, P. eous* and *P. florida*) and a milky mushroom (*Calocybe indica*) are depicted in the Table 10. Decrease in C:N ratio was observed after mushroom growth compared to initial C:N ratio (Fig.4). C:N ratio varied significantly among the different substrates used. In *Hypsizygus ulmarius*, the maximum reduction in the C:N ratio was observed

TABLE 10

Effect of substrate combination on C:N ratio of spent mushroom substrate

| Treatments (Substrates) | C: N Ratio | | | | |
|---|---|---|---|---|---|
| | *Initial values* | *Hypsizygus ulmarius* | *Pleurotus eous* | *Pleurotus florida* | *Calocybe indica* |
| $T_1$ - Paddy Straw | 53.53 | 17.47 [c] | 19.45 [c] | 20.73 [c] | 18.09 [c] |
| $T_2$ - Sugarcane trash | 110.55 | 38.94 [a] | 41.08 [a] | 40.90 [a] | 35.15 [a] |
| $T_3$ - Paddy straw + Sugarcane trash | 76.28 | 25.68 [b] | 28.80 [b] | 28.63 [b] | 26.65 [b] |

**Note:** Each value represents the mean of 6 replications.
Means with same superscript are statistically on par at ≤ P 0.05 by DMRT

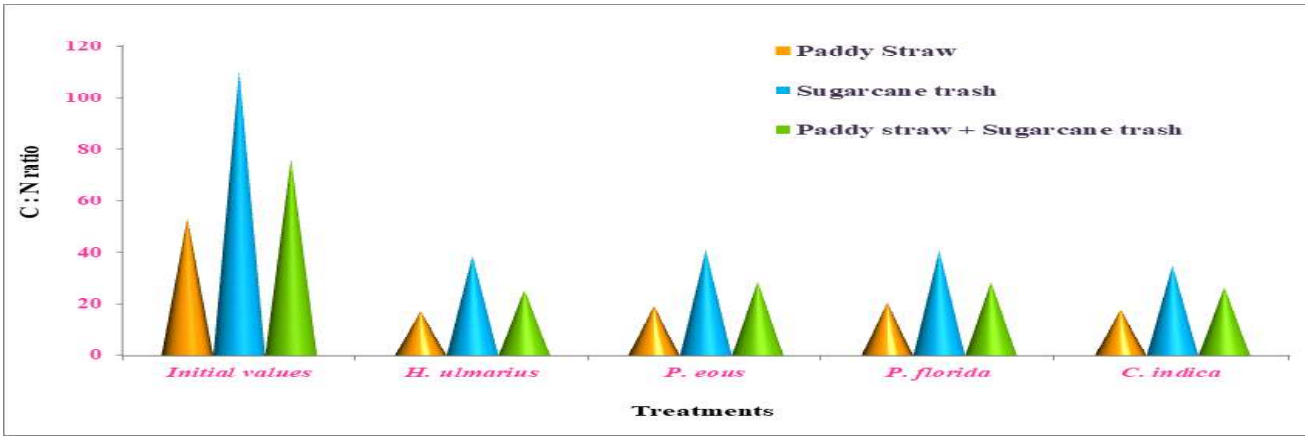


Fig. 6: Effect of substrate combination on C:N ratio of spent mushroom substrate

in Paddy straw followed by treatment $T_3$ and minimum reduction in C:N ratio was obtained from sugarcane trash. Similarly in *P. eous, P. florida* and *C. indica*, significantly narrow C:N ratio (19.45, 20.73, 18.09 respectively) was observed in paddy straw and wide C:N ratio was (41.08, 41.90 and 35.15 respectively) observed in sugarcane trash.

Mushroom spent substrates have narrow C:N ratio compared to unspent substrates. It was mainly due to substrate degradation by mushroom fungus. Mohd *et al*. (2013) also reported C:N ratio of paddy (61.30) straw was more compared to its mushroom spent substrate. Many researchers have reported that the reduction in C:N ratio was mainly due to the declined carbon content lost in form of $CO_2$.

The present study reveals that sugarcane trash can be used as substrate for the cultivation of oyster and milky mushroom species and it was more effective when used with the combination of paddy straw.

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