



Original Research Article

Yield improvement of *Pleurotus florida* fruiting bodies from locally available unexplored lignocellulosic substrates

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A B S T R A C T

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This research concentrated on yield improvement of *P. florida* on unexplored locally available lignocellulosic materials such as paddy straw, reeds, banana stem, sugar cane leaves, sugar cane bagasse milled and crushed coir pith sorghum husk and sunflower stem. The maximum bioefficiency of *P. florida* was obtained from paddy straw (124.50%) followed by reeds (100.30%). Nine spent mushroom substrate were analyzed for reduction of cellulose, hemicelluloses, lignin, and acid detergent lignin. Sugar cane bagasse milled recorded for highest reduction of cellulose (29.40%) and banana stem for hemicelluloses (25.77%). Coir pith recorded for the highest reduction of lignin (24.36%) and acid detergent fiber (15.31%). The reeds can be utilized for successful cultivation of *P. florida* at commercial level than the paddy straw.

Introduction

The diversity and quantity of agricultural cellulosic residues generated by the farming activities and agro industries have raised concerns in many countries. Mushroom cultivation is considered to be one of the economically viable processes for the bioconversion of agriculture and agro - industrial wastes into protein rich food (Bano *et al.*, 1993). Development of production technologies utilizing agricultural wastes have to pace with the food production so as to feed ever increasing population. Use of costly substrate for growing oyster mushroom increases their cost of production. So there was need to search for certain alternative materials

which should be available in sufficient quantity at relatively cheaper price (Arya and Arya, 2003). *Pleurotus* has been reported to grow readily on a number of non-conventional substrates (Mukherjee and Nandi, 2002; Nageswaran *et al.*, 2003). Bioconversion of lignocellulosic residues through cultivation of *Pleurotus* offers the best prospect to utilize renewable resources in the production of protein rich food that will sustain food security for peoples (Naraian *et al.*, 2009).

Pleurotus species are popular and widely cultivated throughout the world mostly in Asia and Europe owing to their simple and

low cost production technology and higher biological efficiency (Mane *et al.*, 2007). *Pleurotus* species are efficient lignin degraders which can grow on wide variety of agricultural wastes with broad adaptability to varied agro-climatic conditions (Jandaik and Goyal, 1995). The practice of mushroom cultivation not only produces a nutritious food but also improves the straw quality. This takes place by reducing lignin, cellulose, hemicellulose, tannin and crude fiber content of straw making it ideal for animal feed (Ortega *et al.*, 1992).

The present study focused on cultivation of *P. florida* by utilizing different locally available agricultural wastes, also exploiting different casing materials and roofing materials to improve the bioefficiency. Reeds as an alternative substrate for cultivation of *P. florida* were explored in this study. The SMS of *P. florida* was analyzed for the lignin, hemicelluloses, cellulose and fiber contents.

Materials and Methods

Mushroom strain

Pure mycelial culture of *P. florida* was obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India and maintained at 25°C. Pure culture was stored at 4°C.

Production of *P. florida* spawn

Sorghum grains were half boiled for 30 min in boiling water until they become soft. Cooled grains were mixed with calcium carbonate (2 %, W/W). Half boiled grains were individually filled (250 g/bag) in a polypropylene bags (13 × 26 cm) and plugged with non absorbent cotton. The grain filled bags were sterilized in autoclave

at 15 lb pressure (121 °C) for 90 min and allowed to cool at room temperature. Seven days old mycelial discs (5 mm) of *P. florida* was aseptically inoculated in the spawn bags and incubated at 28 ± 2°C and dark chamber for 15 - 25 days.

Mushroom bed preparation of *P. florida*

The locally available lignocellulosic substrates such as reeds (RD), paddy straw (PS), banana stem (BS), sugar cane bagasse milled (SBM) and crushed (SBC), sugar cane leaves (SCL), coir pith (CP), sorghum husk (SH) and sun flower stem (SFS) were utilized for mushroom bed production. Mushroom beds were prepared in polypropylene bags of size (30 × 60 cm).

The substrates were sterilized at 121°C for 90 minutes and shadow dried up to 60% moisture. The cylindrical polypropylene bags were filled up to 7 - 8 cm layer height with the processed substrates and 10 g of bed spawn was inoculated on the substrate along the circumference of the bags. The substrate was again layered to 5 cm height and spawn was inoculated along the corners of the mushroom beds with gentle pressing of the substrate in each layer for tight packing. The process was repeated until eight layers of spawn and substrate (90 cm) were packed. The inoculated bag was perforated (12 no's) with sterilized teasing needles.

Construction of *P. florida* mushroom shed and maintenance

P. florida mushroom shed was erected towards east to west direction using locally available materials like coconut thatches, bamboo poles, sticks and wood's pillars. The height of the mushroom shed was 8 feet, length 15 feet and breadth 10 feet. The window of the shed was meshed with net

properly to prevent the entry of mosquitoes and flies. The temperature in the mushroom growing room was maintained at 22 - 28 °C and the humidity was maintained between 75 to 90 %. The floor of the shed was filled with sand up to 1 feet and water was sprinkled thrice in a day. Wet gunnysacks were tied along the corners of the shed to maintain humidity. A hygrometer (Equinox) was kept inside the shed and thrice a day the temperature and humidity were recorded until the final harvest of the fruit body of *P. florida*.

Total weight of all the fruiting bodies harvested from all the four pickings were measured as total yield of mushroom. The bioefficiency (yield of mushroom per kg substrate on dry wt. basis) was calculated by the following formula (Chang *et al.*, 1981).
Fresh weight of mushroom X 100

$$\text{B.E. (\%)} = \frac{\text{Dry weight of substrate}}{\text{Fresh weight of mushroom}} \times 100$$

Bio chemical analysis of substrates before and after harvest

Cellulose, hemicelluloses, lignin and fibre contents in the lignocellulosic substrates were determined by following the methods of Goering and Van Soest, 1975 and AOAC, 1975.

Results and Discussion

Among nine lignocellulosic substrates, paddy straw recorded for the maximum production of fruit bodies (1050.33 ± 45.22 g) harvested from 4 intervals (pickings) (433.33 ± 8.33 g/21 days, 285.0 ± 5 g/28 days, 266.66 ± 13.33 g/36 days and 73.33 ± 18.55 g/45 days) with a bioefficiency of 124.50 ± 0.39 %. However next to the

paddy straw, reeds influenced for the production of fruit bodies (773.33 ± 50.56 g (300 ± 14.43 g/22 days, 248.33 ± 7.26 g/30 days, 150 ± 14.43 g/40 days and 75 ± 14.43 g/50 days) with bioefficiency of 100.30 ± 0.17 % (Table 1). Poppe (2000) reported that there are about 200 kinds of waste in which edible mushrooms can be produced. *P. florida* can be easily cultivated on mushrooms utilizing locally available cellulosic materials were under taken by Sivrikaya and Peker, 1999; Philippoussis *et al.*, 2001; Fan *et al.*, 2003; Shah *et al.*, 2004; Rani *et al.*, 2008; Patill *et al.*, 2008.

Biochemical analysis of substrates before and after harvest

The lignocellulosic substrates and spent mushroom substrate (SMS) of *P. florida* were determined for lignin, hemicellulose, cellulose and acid detergent fibre contents. Among the nine spent mushroom substrate resulted, CP spent substrate recorded for the highest reduction of lignin (24.36 %) and PS spent substrate recorded the lowest reduction of lignin (3.29 %). SBM recorded the highest reduction of cellulose (29.4 %) whereas SH (6.84 %). BS recorded for the highest reduction (25.77 %) of hemicelluloses and CP (2.17 %) recorded lowest reduction. CP (15.31 %) recorded highest reduction of acid detergent fiber and BS (2.93 %) the lowest reduction (Table 2 and 3).

Similar findings were reported by (Kausar, 1988) in which the crude fibre, cellulose and lignin contents dropped significantly in the rice straw biodegraded with *Pleurotus ostreatus* and *Pleurotus sajor-caju*. The results from the present investigation are in consonance with (Moorthy, 1981; Singh *et al.*, 1989) observed that cellulose, hemicellulose and lignin are degraded up to an extent of 75 % during the growth period.

Table.1 Cultivation of *P. florida* using locally available lignocellulosic substrates

| Substrate | SW* | DFSR | DPHF | FH* (g) | SH* | TH* | FH* | TMH* (g) | BE* (%) |
|-----------|-----|------|-------|------------------|------------------|-------------------|------------------|--------------------|------------------|
| PS | 850 | 10 | 17-18 | 433.33 ± 8.33 | 285.0 ± 5 | 266.66 ± 13.33 | 73.33 ± | 1050.33 ± 45.22 | 124.50 ± 0.39 |
| RD | 750 | 10 | 17-19 | 300 ± 14.43 | 248.33 ± 7.26 | 150 ± 14.43 | 75 ± 14.43 | 773.33 ± 50.56 | 100.30 ±0.39 |
| BS | 900 | 11 | 18-19 | 300 ± 14.43 | 255 ± 2.88 | 100 ± 14.43 | 50 ± 0 | 705 ± 31.75 | 78.33 ±6.11 |
| SBM | 750 | 10 | 18-19 | 250 ± 2.88 | 96 ±11.6 | 98 ±1.66 | 50 ±14.43 | 595 ± 30.65 | 79.33 ±5.81 |
| SBC | 700 | 10 | 18-19 | 233.33 ± 8.33 | 248.33 ± | 96.66 ± 11.66 | 50 ± 5 | 628.33 ± 38.01 | 89.76 ±1.09 |
| SCL | 400 | 11 | 18-19 | 103.33 ± 3.33 | 75 ±2.88 | 70 ±7.63 | 56.66 ±3.33 | 305 ±17.19 | 76.25 ±1.25 |
| CP | 600 | 11 | 22-23 | 138.33 ± 7.26 | 100 ± 5.77 | 53.33 ± 3.33 | 528.33 ± 1.66 | 320 ± 18.03 | 53.33 ±0.84 |
| SH | 900 | 10 | 18-19 | 300 ± 5.77 | 206.66 ± 8.81 | 100 ± 5.77 | 35 ± 2.88 | 641.66 ± 23.25 | 71.29 ±0.84 |
| SFS | 550 | 10 | 18-19 | 190 ± 5.77 | 105 ± 7.63 | 76.66 ± 6.00 | 26.66 ± 4.40 | 393 ± 11.66 | 72.41 ±1.39 |

Key: SW - Substrate weight in grams, DFSR - Days for spawn run, DPHF - Days for pinhead formation, Gram, FH - First harvest, SH - Second harvest, TH - Third harvest, FH - Fourth harvest, TMH - Total mushroom harvest, BE – Bioefficiency, PS- Paddy straw, RD- Reeds, BS – Banana Stem, SBM - Sugar cane bagasse milled, SBC-Sugar cane bagasse crushed, SCL – Sugar cane leaves, CP – Coir Pith, SH -Sorghum husk, SFS - Sun flower stem.

Table.2 Biochemical properties of lignocellulosic substrates before and after cultivation (SMS) of PF 01

| Substrates | CELLULOSE (%) | | | HEMI CELLULOSE (%) | | |
|------------|---------------|-------|--------|--------------------|-------|--------|
| | FS | SMS | % of R | FS | SMS | % of R |
| PS | 29.58 | 7.98 | 21.6 | 14.62 | 8.51 | 6.11 |
| RD | 27.55 | 8.50 | 19.05 | 18.93 | 6.72 | 12.21 |
| BS | 26.55 | 13.71 | 12.84 | 31.63 | 5.86 | 25.77 |
| SBM | 36.52 | 6.98 | 29.54 | 28.80 | 12.72 | 16.08 |
| SBC | 32.28 | 8.32 | 23.96 | 30.43 | 16.75 | 13.68 |
| SCL | 19.23 | 7.84 | 11.39 | 15.62 | 11.70 | 3.92 |
| CP | 26.51 | 15.27 | 11.24 | 14.40 | 12.23 | 2.17 |
| SH | 28.34 | 21.50 | 6.84 | 18.16 | 14.91 | 3.25 |
| SFS | 30.55 | 12.23 | 18.32 | 19.34 | 8.87 | 10.47 |

Table.3 Biochemical properties of lignocellulosic substrates before and after cultivation (SMS) of PF 01

| Substrates | LIGNIN (%) | | | ACID DETERGENT FIBRE (%) | | |
|------------|------------|------|--------|--------------------------|-------|--------|
| | FS | SMS | % of R | FS | SMS | % of R |
| PS | 11.23 | 7.94 | 3.29 | 57.23 | 52.56 | 4.67 |
| RD | 13.55 | 9.20 | 4.35 | 59.55 | 53.28 | 6.27 |
| BS | 16.67 | 5.62 | 11.05 | 34.56 | 31.63 | 2.93 |
| SBM | 15.76 | 7.76 | 8.00 | 45.67 | 39.89 | 5.78 |
| SBC | 14.53 | 6.96 | 7.57 | 46.76 | 40.51 | 6.25 |
| SCL | 14.47 | 3.50 | 10.97 | 62.62 | 56.45 | 6.17 |
| CP | 33.56 | 9.20 | 24.36 | 65.76 | 50.45 | 15.31 |
| SH | 12.68 | 3.13 | 9.55 | 56.80 | 43.62 | 13.18 |
| SFS | 17.34 | 9.23 | 8.11 | 45.56 | 40.56 | 5.00 |

Key: FS – Fresh Substrate, SMS – Spent Mushroom Substrate, % of R- Percentage of Reduction, PS- paddy straw, RD-reeds, BS- banana stem, SBM- sugarcane bagasse milled, SBC-sugarcane bagasse crushed, SCL – sugarcane leaves, CP- coir pith, SH- sorghum husk, SFS- sun flower stem.

Karuppuraj *et al.* (2014) similarly reported that *C. indica* spent mushroom substrates, coir pith spent substrate recorded for the highest reduction of lignin (25.29%). Sugar cane bagasse milled recorded highest reduction of cellulose (31.62%) where as banana stem recorded highest reduction of hemicelluloses (22.96 %) and coir pith recorded highest reduction of acid detergent fiber (18.27%)

The present study concluded that *P. florida* can be cultivated by different type of lignocellulosic substrate and among those reeds was a best alternative and replacement of traditional substrates. The spent mushroom substrates as casing materials was enhancing yield of *P. florida*. The present study further leads us to cultivate and commercialize the *P. florida* production using reeds for the benefit of society.

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