

Evaluation Of Different Agricultural Residues For Productivity And Morphological Variations In Oyster Mushrooms (*Pleurotus* spp.)

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Citation

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

Abstract

As agricultural wastes are easily accessible and quite free sources, re-use of them could be considered as a break through in production management, especially if it sustains the production efficiency as standard materials do. In this study, we evaluated combination usage of five substrates viz., Cottonseed hulls, Wheat straw (WS), Sugarcane bagasses (SB), Sunflower stalks (SFS) and Rice straw, collected from agriculture field of Haryana.. The substrates showed better Biological efficiency (BE), when used in combination as compared to alone. The mixture of chopped wheat straw and cotton seed hulls (in ratio of 3:1) with 1% ground limestone was the best combinational substrate among all combination which showed 105.9% biological efficiency for *P. djamor* and 87.76% for *P. florida*.

The fact that basidiomycetes convert waste materials into a highly flavored proteinacious food is clearly relevant to the requirement of both the developing and developed countries. The problem of Inadequate regional food supplies, diminishing quality of health, and increasing environmental deterioration is set to increase as the world's population continues to grow. *Pleurotus* species grows on most lignocellulosic materials such as rotten or rotting wood, wood residues and most of agriculture wastes. This is because of the large capacity of oyster mushrooms that secretes vast number of enzymes which enable them to live on various substrates, decompose lignin, protein, carbohydrate, cellulose and starch containing materials (Madan *et al.*, 1983, Buswell *et al.*, 1993., Rajarathnam *et al.*, 1998., Straatsma *et al.*, 2000). Now-a-days the cultivation of mushroom is disseminated all over the world because of its revenue activity (Ponmurugon *et al.*, 2007) Furthermore, the abundant agricultural wastes found countrywide offers opportunity for production, which in turn provides a more economical and environmentally friendly disposal system (Sanchita, 2008). Keeping in the view of climatic conditions and agricultural residues available in region, there is an ample scope for its commercial exploitation.

The present study was carried out to evaluate the suitable agro wastes for cultivation of *P. djamor*, *P. florida* according

to their climatic condition in this region and to develop a strategy to obtain suitable substrate for large scale cultivation of this mushroom.

MATERIALS AND METHODS

The experiments were conducted in the green house located in the premises of FET, Department of Biotechnology, MRIU, Faridabad. The pure culture of *P. djamor* and *P. florida* were procured from Mushroom Research & Development centre at Murthal (*Sonepat*) in *Haryana*. Isolates were maintained by monthly transfer to Potato Dextrose agar medium (PDA) and stored in refrigerator after growth. Wheat grain spawn of *P. djamor* and *P. florida* was prepared in glass bottles. Six substrates viz., cottonseed hulls, wheat straw (WS), wheat Bran, sugarcane bagasses (SB) and sunflower stalks (SFS) were collected from agriculture field of Haryana.

Comparative study of growth rate on different culture media

Five natural and synthetic media i.e. Potato dextrose agar (PDA), Czapeck's agar (CZ), glucose yeast extract agar (GYE), nutrient agar (NA) and malt extract agar (MEA) were evaluated to determine their effect on linear growth (in mm). The inoculums of both of the strains were initially grown on PDA for 5 days at 25°C. Small discs (5mm in diameter) were transferred to Petri dishes containing the test

medium. A set of five dishes was used for each treatment. Linear growth was measured in mm and recorded when the mycelium reached the edges of the Petri dishes.

Preparation of spawn

Wheat grain spawn of *P. djamor* and *P. florida* was prepared in glass bottles. In spawn preparation grains were submerged in water at 120-130°C for 20 minutes then were taken out and spreaded evenly to drain out excess water. To prevent sticking of grains and to maintain the appropriate pH grains were mixed with 2% CaSo₄ and 0.5% CaCo₃. The grains were filled in bottles and sterilized. The bottles were inoculated with mycelium culture and incubated at 20-25°C for 25 days.

Preparation of different organic substrates

Five substrates viz., cottonseed hulls, wheat straw (WS), sugarcane bagasses (SB), sunflower stalks (SFS) and rice straw were collected from agriculture field of Haryana. Wheat straw and sunflower stalks were chopped (5-8cm long), while chopping is not required for cottonseed hulls, sugarcane bagasses (SB) and rice straw. The substrates were steeped in water containing 75 ppm Bavistin + 500ppm formaldehyde for 18-24 hours (Vijay and Sohi, 1987). Excess water was drained out through strainer and was dried in shade to retain 65-70% moisture content. Spawning was done @ 5% wet weight basis of substrate by thoroughly mixing along with 1% ground limestone was added in each bag to adjust the pH of the medium. Spawned substrate was filled up in perforated polythene bags (45×30 cm), covered with moistened blotting paper on the top and tightly packed. Three replications were maintained for each substrate. These bags were placed on the wooden benches of crop room for spawn run. Sprinklers installed spray mist continuously and helped in maintaining high moisture content (95-98%) of crop room. Only re-circulated air is used for cooling and air distribution. Proper drainage system helped in draining the water. At the end of spawn run period, 4 hours of light was provided daily by cool white fluorescent bulbs. Light intensity measured at various locations in the growing room, may range from 50 to 300 lux. At the time of pinning sufficient fresh air is introduced to lower CO₂ level below 700 ppm. Meterological observations like temperature (maximum and minimum) prevailing in the cropping room was recorded daily. when the bags become full of mycelial growth and or pinhead started appearing on the mycelial surface, the bags mouth were opened or required portion of

the bags were cut-off with blade to facilitate the development of fruiting bodies.

Average value of observations was recorded with respect to their period required for spawn run (days), pin head appearance (days), total yield (g/kg), stipe length (cm) and cap diameter (cm). Biological efficiency indicates conversion of substrate mass to mushroom fruiting bodies. To measure this index, oyster mushroom fresh weight g/100g substrate dry weight was calculated. To measure mean fruiting body weight, the total weight of oyster mushroom from each experimental units was divided to the number of harvested fruiting bodies.

$$BE(\%) = \frac{\text{Fresh weight of harvest}}{\text{Dry weight of substrate}} \times 100$$

Substrates in combination

Various combinations of substrates were tried for getting higher yield. Hence, cottonseed hulls, chopped wheat straw, rice straw showed high productivity among all substrates, their various combination were tried viz: Cottonseed Hulls + Wheat Straw (3:1), Wheat Straw + Rice Straw (3:1), Cottonseed Hulls + Rice Straw (3:1).

RESULTS AND DISCUSSION

Rate and amount of growth of the aforementioned strains of *P. djamor* and *P. florida*, were studied on four different media i.e. PDA, CZ, GYE, MEA. Data (Table 1) showed that *P. djamor* profusely grow on MEA or GYE. Santiago (1983) found that mycelial development was marginally better on a medium containing glucose and sucrose than other carbon sources. *P. florida* showed fair growth on all culture media, slightly showed its better growth on PDA as well as on MEA. Over all growth rate of *P. djamor* was much pronounced than *P. florida* over all culture media.

Data on cultivation of *P. djamor* and *P. florida*, in the month of February to march using four different substrates and their various combinations are given in table 2 and 3 respectively. Values have been calculated by Mean comparison using Duncan method. Results showed that cottonseed hulls had the least growth period of 29 and 31.4 days respectively for both strains of *P. djamor* and *P. florida*. While sunflower stalk led to the longest growth period for oyster mushroom and subsequently was inappropriate for this purpose. The maximum fruiting body number of 25 was pertained to cottonseed hulls for *P. djamor* and minimum was pertained with sunflower stalks. The highest average weight of fruiting

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bodies was achieved for cottonseed hulls (20.12±1.12 gm) and wheat straw (20.05±0.67) for the strain of *P. djamor*. The minimum biological efficiency of 62.89 was recorded on Sugarcane bagasses for *P. florida* and 67.49 on sunflower stalks for *P. djamor*. The highest biological efficiency of 100.6 and 84.12 was recorded on cottonseed hulls for *P. djamor* and *P. florida* respectively. Our result was in accordance with other report as *Periasamy et al.* (2004) showed the great potentiality of the enzymatic activity of the pink colored mushroom *Pleurotus djamor* for lignin enrich substrates (Paddy straw, water hyacinth, groundnut plant, coir pith). *Bermudez et al.* (2000) reported the biological efficiency of *Pleurotus ostreatus* f.sp. florida on coffee pulp (168.5-179.4%), followed by coconut shells(90.0%) and cocoa shells(84.5%). *Pala et al.* (2012) observed biological efficiency of *Pleurotus sajor-caju* was maximum on paddy straw (149.4%) followed by wheat straw (124.7%), apple leaves (95.62 %) and chinar leaves (85.3 %). Addition of wheat straw with cottonseed hulls led to highest biological efficiency of 105.91. It is not only higher productivity but morphological characters (i.e. spawn run, pin head appearance, required lesser time, longer stipe length and greater cap diameter) was recorded. While addition of rice straw with cottonseed hulls could not show such promising result as wheat straw. The higher productivity of *Pleurotus djamor* on cottonseed hulls with wheat straw may be attributed to associated enzymatic activity for solubilization of substrates as compared with rice straw. Studies indicated that adding 6% food supplement to substrate can increase the *Pleurotus cornucopiae* output up to ninety percent (Royse, 2003). Poultry manure, rice bran, wheat bran and peat moss were successfully used as food supplements to improved yield, biological efficiency and growth period via providing sufficient nitrogen and slow nutrient releasing (Baysal et al., 2003; Loss et al., 2009).

SIGNIFICANCE

Haryana's percentage share in India's total area and production of cotton amount to about 6.77 and 11.91 respectively. Jind, Hissar and Sirsa districts are the main producers. In 2010-2011, Haryana's total cotton production area as per Cotton Advisory Board Estimates is 5.33 lakh hectares and production is around 15.00 lakh bales. Cottonseed hulls are comprised of the seed coat with some attached lint that is separated from cottonseed kernel during oil production. Cottonseed hulls are low in crude protein (4-12% of dry matter). The main component of cottonseed hulls is neutral detergent fibre (NDF) which includes a

relatively large proportion of acid detergent lignin. Low protein content and poorly digested high fiber content makes it a poor feedstock. The *Pleurotus* species are able to grow on a wide spectrum of lignocellulosic waste materials due to their ability to secrete a range of degradatory enzymes (cellulose, hemicellulase, xylanases, lignin peroxidase(LIP), manganese peroxidase (MnP)) and laccases). These research findings may be implemented specifically in haryana region where surplus amount of agricultural residues (cottonseed hulls) are available not appropriate as feedstock but can act as an excellent source for mushroom cultivation.

Table 1

Effect of different media on the rate of growth of *P. djamor* and *P. florida*

| strain | Days after inoculation | linear growth measured (cm) | | | |
|-------------------|------------------------|-----------------------------|-----------|-----------|-----------|
| | | P.D.A | C.Z.A | M.E.A | G.Y.A |
| <i>P. djamor</i> | 2 | 1.15±0.02 | 1.23±0.06 | 1.36±0.12 | 1.70±0.08 |
| | 4 | 2.20±0.10 | 2.10±0.12 | 2.73±0.05 | 3.30±0.03 |
| | 6 | 3.26±0.04 | 3.33±0.08 | 4.46±0.09 | 4.40±0.09 |
| | 8 | 4.34±0.08 | 4.43±0.01 | 5.94±0.04 | 5.86±0.04 |
| | 10 | 5.43±0.07 | 5.54±0.02 | 7.43±0.01 | 7.33±0.09 |
| | R | 0.54 | 0.55 | 0.74 | 0.73 |
| <i>P. florida</i> | 2 | 1.00±0.04 | 0.63±0.07 | 0.66±0.08 | 0.60±0.04 |
| | 4 | 1.86±0.12 | 1.43±0.02 | 1.52±0.03 | 1.35±0.11 |
| | 6 | 2.70±0.07 | 2.40±0.10 | 2.60±0.07 | 2.25±0.09 |
| | 8 | 3.70±0.09 | 3.20±0.08 | 3.46±0.04 | 3.00±0.12 |
| | 10 | 4.30±0.11 | 4.00±0.04 | 4.32±0.01 | 3.75±0.02 |
| | R | 0.43 | 0.40 | 0.43 | 0.38 |

(PDA) Potato dextrose agar, (CZ) Czapeck's agar, (GYE) glucose yeast extract agar and (MEA) malt extract agar

Values are the mean ± SE of five replications

R = Average of mycelial growth rate (cm/day)

Table 2

Substrates effect on *P. djamor* and *P. florida* growth characters

| Substrates | Growth Period | Morphological Variations | | | | | Productivity | | |
|-----------------------|---------------|--------------------------|-------------|-------------|-------------------------|-------------------|--------------|--------|--------|
| | | M.R/P.H stage | P.F.B stage | C.F.B stage | Fruiting Body wt (gram) | Fruiting Body no. | Yield (gram) | BE(%) | |
| 1.Cottonseed Hulls | <i>P.d</i> | 29 | 20.8 | 2.5 | 5.7 | 20.12±1.12 | 25 | 503 | 100.6 |
| | <i>P.f</i> | 31.4 | 23.2 | 2.7 | 5.5 | 19.12±0.33 | 22 | 420.64 | 84.128 |
| 2.Wheat Straw | <i>P.d</i> | 32.7 | 25.7 | 2.2 | 4.8 | 20.05±0.67 | 23 | 401 | 80.2 |
| | <i>P.f</i> | 35.4 | 27.3 | 2.6 | 5.5 | 18.07±0.31 | 20 | 361.4 | 72.28 |
| 4. Rice Straw | <i>P.d</i> | 35.3 | 27.5 | 2.7 | 5.1 | 19.46±1.22 | 23 | 369.74 | 73.948 |
| | <i>P.f</i> | 37.3 | 29 | 2.9 | 5.4 | 20.11±0.84 | 20 | 402.2 | 80.44 |
| 5.Sunflower stalks | <i>P.d</i> | 40.1 | 30 | 3.4 | 6.7 | 19.85±0.98 | 19 | 337.45 | 67.49 |
| | <i>P.f</i> | 39.9 | 32 | 2.6 | 5.3 | 17.85±0.63 | 18 | 321.3 | 64.26 |
| 6. Sugarcane Bagasses | <i>P.d</i> | 37.9 | 29.8 | 2.9 | 5.2 | 20.02±0.65 | 20 | 360.36 | 72.072 |
| | <i>P.f</i> | 39.1 | 30.8 | 2.3 | 6 | 16.55±0.91 | 19 | 314.45 | 62.89 |

• M.R mycelium run, P.H. pin head, P.F.B primary fruiting body formation and C.F.B complete fruiting body formation. B.E Biological efficiency.

• Values are the mean ± SE of three randomly harvested fruiting body

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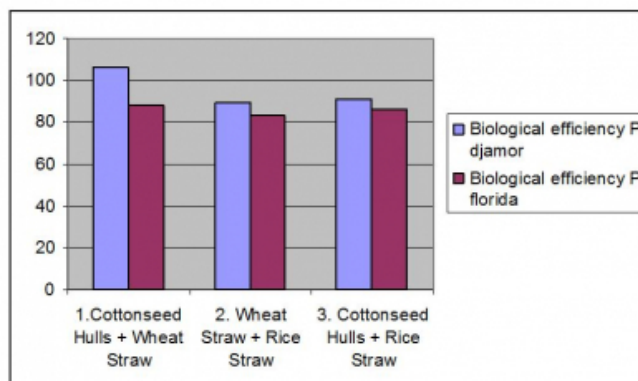
Table 3

Combinational effect of substrates on *P. djamor* and *P. florida* production factors.

| Substrates in combination | strains | Productivity | |
|-----------------------------------|------------|--------------|-----------------------|
| | | Yield | Biological efficiency |
| 1. Cottonseed Hulls + Wheat Straw | <i>P.d</i> | 529.55±0.92 | 105.91 |
| | <i>P.f</i> | 438.8±0.81 | 87.76 |
| 2. Wheat Straw + Rice Straw | <i>P.d</i> | 446.55±0.76 | 89.31 |
| | <i>P.f</i> | 417.45±0.99 | 83.49 |
| 3. Cottonseed Hulls + Rice Straw | <i>P.d</i> | 456.3±0.64 | 91.26 |
| | <i>P.f</i> | 432.55±0.89 | 86.51 |

Figure 1

Combinational effect of substrates on *P. djamor* and *P. florida* production factors.



References

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