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# Enhancement of bioconversion efficiency and chemistry of the mushroom, *Pleurotus sajor-caju* (Berk and Br.) Sacc. produced on spent rice straw substrate, supplemented with oil seed cakes

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**Abstract**

The effect of supplementing the spent rice straw substrate, selected at two different levels of bioconversion efficiency (BCE), 8 and 12%, of *Pleurotus sajor-caju,* with extra organic nitrogen (in the form of oil seed cakes) was studied on further production of mushrooms, their chemistry and the increase in the in vitro dry matter digestibility (IVDMD) of rice straw. The spent rice straw was supplemented with the oil seed cake powders in amounts equivalent to 0.15–0.60% of nitrogen present in the undegraded rice straw, that is used for mushroom culturing. The cotton seed powder proved to be better in enhancing the mushroom yields (up to 12 times those of the unsupplemented spent straw), than the other oil seed cakes such as mustard, niger, soyabean and sunflower. Chemically, the cottonseed powder supplemented mushrooms showed increased protein, fat and decreased carbohydrate contents. Also, there was a significant reduction in the spawnrun period, compared to the unsupplemented rice straw. A considerable increase in the IVDMD of the supplemented spent straw was observed as compared to the IVDMD of the unsupplemented spent straw. Due to cotton seed cake supplementation, there was about a 27% increase in the IVDMD value of the spent straw that resulted in 8% BCE. The mushrooms produced on the spent rice straw substrate, with supplementation of cottonseed powder, did not contain any residues of gossypol, and accordingly, they can be a valuable food or feed. 

*Keywords:* *Pleurotus sajor-caju*; Rice straw; Oil seed cake; Bioconversion efficiency; Biotransformation; Biodegradation; In vitro dry matter digestibility

## 1. Introduction

Annually ~573 million t of rice straw are available in the world (FAO, 1997). The low rate of digestibility of rice straw is attributed to the slow rate of fermentation (Mudgal, Khajuria, & Singhal, 1981). Rice straw is the growth substrate recommended for the economic production of *Pleurotus* species on commercial scales (Rajarathnam & Zakia Bano, 1987). *P. sajor-caju* is known to produce 100% yields (100 kg fresh mushrooms per 100 kg dry straw) on unfermented pasteurized rice straw (Zakia Bano & Rajarathnam, 1982). With every mushroom crop that is harvested, ~40% rice straw is left behind as spent substrate depending on the mushroom yields produced (Rajarathnam & Zakia Bano, 1989).

In view of the large quantities of spent substrate available after commercial mushroom production, the effect of oil seed cake supplementation of the spent rice straw, after the harvest of mushrooms is worth investigating. Supplementing the rice straw after spawnrun (mycelial ramification) with oil seed cakes is already established to increase the mushroom yields considerably without much increase in the period to produce the mushroom yields (Rajarathnam, Zakia Bano, & Patwardhan, 1986; Zakia Bano, Shashirekha, & Rajarathnam 1993).

The present paper deals with the results of supplementing the spent rice straw substrate with oil seed cakes, in enhancing the mushroom yields and quality, and evaluating the spent supplemented rice straw for any increase in IVDMD.

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**Nomenclature**

| | |
|---|---|
| BCE | bioconversion efficiency; |
| IVDMD | in vitro dry matter digestibility. |

## 2. Materials and methods

### 2.1. Culture and spawn

Monocultures of *P. sajor-caju* isolated from tissue cultures of the fruiting primordia were maintained on malt extract (3%) agar (2%) at 25 °C. The culture was subcultured every 3 months. Using this culture, spawn was prepared on wet, chopped rice straw under aseptic conditions (Rajarathnam, Wankhede, & Patwardhan, 1979). A 3-month-old straw spawn was used for all culturing experiments.

### 2.2. Culturing conditions

Chopped rice straw (2–3 cm long) was soaked in hot water at 60 °C for 15 min to ensure that it was free from contamination (Rajarathnam Singh, & Zakia Bano, 1979). Wet straw containing ~ 75% water was spawned (at 10% rate on wet weight basis) along with 1% coarse "horse gram" (*Dolichus biflorus*) powder (mill size ca. 0.5–1.0 mm). The mushroom beds were made in perforated polyethylene (50 μm) bags (25×40 cm). For each treatment, starting with a dry straw substrate of 2.5 kg, eight replicate mushroom beds were prepared, allotted in randomized blocks.

### 2.3. Mushroom yield and collection of spent substrate

The mushroom yields were recorded on the third day of formation of fruiting primordia, and the yields were expressed as grammes (fresh) per kilogram dry straw. The sets of mushroom beds yielding at 8 and 12% BCE levels were identified and, kept separately for supplementation with the oil seed cakes.

### 2.4. Oil seed cake supplementation

The spent substrate (of 8 and 12% BCE levels), starting with a dry straw substrate of 2.5 kg (≡ 8 replicate mushroom beds), was separately supplemented with oil seed cake powders of mustard, niger, sunflower, cotton and soyabean. The oil seed cakes were supplemented at four levels (0.25, 0.50, 0.75 and 1.0 nitrogen units) to give additional nitrogen levels of the dry weight substrate of 0.15, 0.30, 0.45 and 0.60%, respectively. After supplementation, the beds were reformed in polyethylene bags.

### 2.5. Estimation of carbohydrates

Freeze-dried material of the mushroom fruiting bodies, grown on cottonseed supplemented spent rice straw, was subjected to extraction in 70% alcohol and the free and total sugars determined by the phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Reben, & Smith, 1956).

The alcohol-insoluble residue was hydrolyzed using 72% sulphuric acid and the total sugars estimated, as above, in the hydrolysate represented the polysaccharide (alcohol-insoluble carbohydrates) (Rajarathnam, Wankhede, & Patwardhan, 1979).

### 2.6. Estimation of protein content

Total nitrogen in the fruiting bodies (dried at 60 °C) was estimated by the micro-Kjeldahl method (AOAC, 1975). In view of the fact that mushrooms contain a significant amount of non-protein nitrogen in the form of glucosamine in their chitinous cell walls, besides other ninhydrin positive compounds (Altamura, Robbins, Andreotti, Long, & Hasselstrom, 1967), and the findings of Fitzpatrick, Esselen, and Wein (1946), that a purified mushroom protein isolate contained 11.8% nitrogen rather than the expected 16%, a conversion factor of 4.38 is suggested to be more appropriate for estimating mushroom protein, if the nitrogen content of the chitin and free amino acids is to be ignored (Zakia Bano & Rajarathnam, 1988).

### 2.7. Estimation of crude fat and total ash

Dried mushroom powder was subjected to extraction with dry ether, and filtered and the residue left over after evaporating the solvent represented the crude fat (AOAC, 1975).

A known weight of the dried mushroom in a covered porcelain crucible was ignited in a furnace at about 550 °C, to obtain a grey ash. After cooling, the ash was moistened with distilled water followed by drying on a hot plate, and subjected to re-ashing at 550 °C to constant weight (AOAC, 1975).

### 2.8. In vitro dry matter digestibility (IVDMD)

The per cent organic matter lost during a two step digestion was determined by a slightly modified method of Tilley and Terry (1963) as described by Zadrazil (1977; Fig. 1).

### 2.9. Analysis of II stage spent substrate for gossypol residues and mushrooms

Fresh II stage spent substrate (used for IVDMD estimation) was extracted in 70% aqueous acetone for 1 h

Table 1
Effect of oil seed cake supplementation of the spent rice straw substrate (of 12% BCE) on the mushroom yield and IVDMD

| Oil seed cake | Concentration on dry weight. (%N) basis | Spawn run period (days) | Yield (g fresh $kg^{-1}$ dry straw) | IVDMD[a] (%) |
|---|---|---|---|---|
| Cotton seed | 0.15 | 9 (8)[b] | 125 (300)[b] | 56.6 (59.8)[b] |
| | 0.30 | 9 (8) | 110 (280) | 52.4 (54.2) |
| Mustard | 0.15 | 12 (11) | 65 (155) | 44.6 (46.4) |
| | 0.30 | 12 (11) | 50 (125) | 43.0 (43.0) |
| Niger | 0.15 | 14 (13) | 55 (125) | 40.6 (42.6) |
| | 0.30 | 14 (13) | 45 (115) | 41.0 (44.2) |
| Soyabean | 0.15 | 12 (11) | 60 (135) | 40.4 (40.4) |
| | 0.30 | 12 (11) | 45 (115) | 42.6 (42.4) |
| Sunflower | 0.15 | 14 (13) | 50 (120) | 44.8 (46.2) |
| | 0.30 | 14 (13) | 60 (125) | 46.4 (48.4) |
| Control (unsupplemented) | – | 18 (18) | 15 (25) | 37.4 (32.6) |
| | L.S.D. ($P=0.05$) | | 10 (15) | 4.2 (5.8) |

[a] Percent organic matter lost during digestion (Tilley & Terry, 1963; Zadrazil, 1977). Each value of the experimental result is the mean of eight replicates.
[b] Values in parenthesis represent the results obtained at 8% BCE.

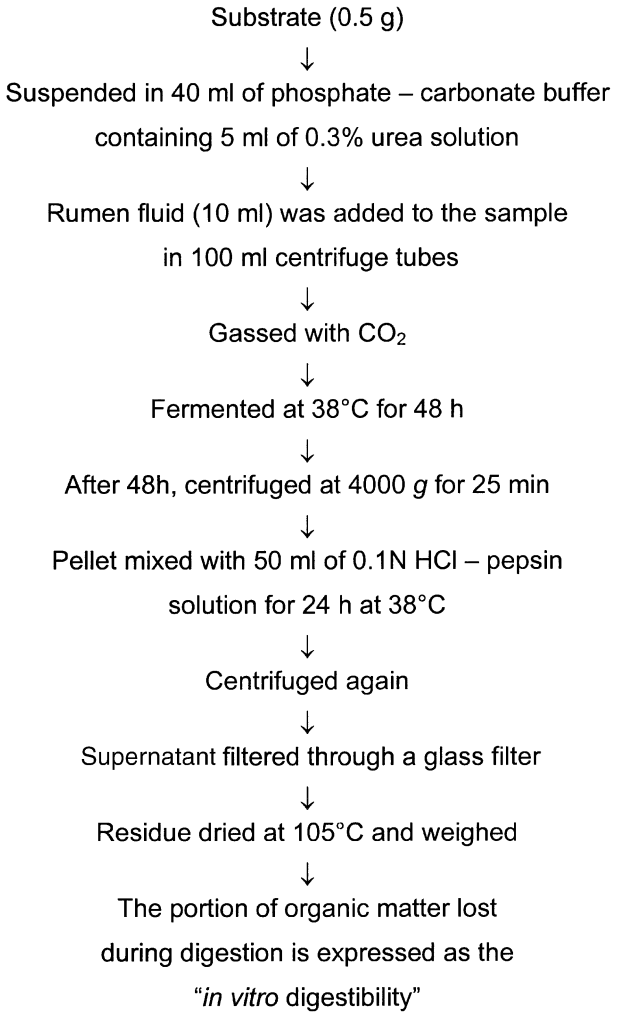


Fig. 1. Scheme adopted for determination of IVDMD.

(three times) and the collections pooled. To an aliquot of the extract, *p*-anisidine reagent was added and heated to 60 °C for 30 min. After cooling to room temperature, the volume was made up to 25 ml with 95% ethyl alcohol and O.D. measured was at 445 nm (Pons & Guthrie, 1949). A standard was prepared using gossypol (Sigma Chem. Co., USA) in the concentration range of 10–100 μg.

A similar procedure was followed for the mushrooms raised on cotton seed supplemented spent rice straw substrate.

## 3. Results and discussion

The present paper deals with the production of higher mushroom yields due to supplementation of spent substrate with oil seed cakes (Table 1). In order to establish the differences in results of yield and substrate IVDMD, the effect of oil seed cake supplementation was studied on spent substrate that had yielded mushrooms at different levels of BCE, i.e. 8 and 12%.

Of the four concentrations (0.15–0.60% N) of each of the oil seed cakes tried as supplements, only 0.15 and 0.30% N levels of oil seed cakes had given significant increase in mushroom yield and hence, only these concentrations were selected for evaluation of IVDMD of the spent substrate (Table 1). Compared to the unsupplemented spent substrate, mushroom yield was significantly increased with oil seed cake supplementation. The increase in mushroom yield was 4- to 12-fold at 8% BCE and 3- to 8-fold at 12% BCE. This indicated availability of more undegraded polymeric carbohydrates in the spent substrate at 8% BCE than at 12% BCE, since the BCE has a relation to the degree of biodegradation of the growth substrate (Rajarathnam, Shashirekha, & Zakia Bano, 1992). The maximum of

Table 2
Chemistry of mushrooms produced on spent rice straw substrate, with and without cottonseed powder (CS) supplementation[a]

| Constituents | Spent rice straw | |
|---|---|---|
| | Unsupplemented (control) | Supplemented (CS) |
| *Carbohydrates* | | |
| Free sugars | 14.2 | 11.0 |
| Alcohol insolubles | 49.6 | 39.5 |
| Protein (N×4.38) | 20.0 | 32.0 |
| Fat | 4.0 | 5.1 |
| Ash | 12.0 | 12.2 |

[a] Each value is the mean of three replicate determinations.

12-fold increase in the mushroom yield of spent substrate (at 8% BCE) was due to supplementation with 0.15% N as cottonseed powder. Further, there was also a significant reduction in the spawnrun period (by $>50\%$ of the unsupplemented lot). Thus, the rice straw left after the harvest of mushrooms in large scale production, can be made to produce a second crop economically. The oil seed cake supplementation enhanced the secretion of cellulase(s), hemicellulase(s) and laccase activities, involved in the degradation of cellulose, hemicellulose and lignin, respectively (Rajarathnam, Shashirokha, & Zakia Bano, 1998). Ultimately, besides a decrease in cellulose and hemicellulose in the rice straw substrate, there was a significant decrease of the lignin content too. Possibly, the added oil seed cake due to the increased secretion of ligninolytic enzymes, aided breakdown of lignin so as to create easier accessibility and degrade other polymeric carbohydrates. The degraded carbohydrates are known to serve as energy sources for the construction of fruiting bodies and also to serve as the structural components of the fruiting bodies, as the fruiting bodies, on dry weight basis are known to contain $\sim$ 60% carbohydrates, which serves to answer the question of enhanced mushroom yields.

Supplementing the spent rice straw substrate with cottonseed powder at as low a concentration as 1.5%, caused significant changes in the composition of mushrooms produced (Table 2). Carbohydrates, both alcohol-soluble (free sugars) and alcohol-insolubles (polysaccharides) showed a total of 13% decrease. Crude protein (Kjeldahl N×4.38) showed an increase of 12% over the unsupplemented mushroom lot. Total fat increased by 1/4th of the control. Ash content tended to remain the same. This sets forth an illustrated example of modifying the chemical properties of mushrooms by minute alteration of growth substrate. Also, this illustrates that cottonseed powder obviously supplements the factor exhausted in the spent rice straw substrate, limiting the production of improved mushroom yields.

There was a clear increase in IVDMD indicating a clear increase in its values, due to oil seed cake supplementation. Probably, this was due to increased breakdown of lignin of the spent substrate, exposing much more of the polymeric carbohydrates in a form more susceptible to carbohydrates. A relative increase in IVDMD values of spent rice straw substrate was noticed at 8% BCE, compared to 12% BCE, effected due to the oil seed cake supplementation. Maximum increase in IVDMD was recorded with the use of cottonseed cake supplementation, probably because of its association with increased BCE.

The major constraint of the rice straw, as feed, is its high lignin and silica contents, low protein and low digestibility (Jackson, 1978). Encrustation of cellulose with lignin is known to impede the digestibility of the former by rumen microorganisms (Pigden & Heaney, 1969). *P. sajor-caju*, after its growth and yield on rice straw, is reported to enhance the dry matter digestibility (in sacco) of the spent substrate (Dhanda, Garcha, Kakkar, & Makkar, 1996). Natarajan, Kaviyarasan and Kadirvel (1993) have observed an increase in the in sacco digestibility of rice straw biodegraded and bioconverted by *P. citrinopileatus*. Negative correlations between the lignin content and in vitro digestibility, for a range of herbages, have been established (Mc Leod & Minson, 1974; Roughan & Holland, 1977). Thus, the prominent ligninolytic property of *P. sajor-caju* (Rajarathnam & Zakia Bano, 1989), appears to have served to raise the IVDMD. Similar increases in the dry matter digestibility due to increased degradation of lignin by several white-rot species have been demonstrated (Asiegbu, Paterson, & Smith, 1996; Suzuki & Miyairi, 1991; Zadrazil, 1985).

Thus, maximum mushroom yields were obtained with supplementation of the spent rice straw substrate with cottonseed powder (Table 1). Cottonseed, in turn, is known to contain the characteristic polyphenolic compound, gossypol, that ranges from 0.4 to 1.5% on a dry weight basis (Pons & Eaves, 1967). Gossypol due to its property of binding with proteins and amino acids, renders them nutritionally unavailable (Damaty & Hudson, 1979). In this context, it was interesting to see whether the spent rice straw substrate, or the mushrooms raised on cottonseed supplemented spent rice straw substrate contained any residues of gossypol. The absence of gossypol residues in both these materials indicate their safety in serving as human food or feed.

Considering the protein contribution by the cotton seed supplementation, absence of gossypol residues and the enhanced IVDMD, it can be safely concluded that the residual II stage spent rice straw substrate could be a valuable animal feed.

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