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Cultivation of two edible mushrooms on pressed leaf fibre, the lignocellulosic by-product of leaf protein production plant

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In recent years mechanical, chemical and biological conversions of various lignocellulosic wastes and by-products from agricultural field to various food processing industries have registered spectacular progress in the emerging field of biotechnology to meet the increasing demand of food, feed, fuel, fertilizer and fibre.

During the bulk extraction of edible protein from any plant source, huge amoumt of lignocellulosic fibrous residue was generated as by-product. Keeping in view of mushroom cultivation for its simple technology and attractive income generating scheme coupled with low initial investment, venture for edible mushroom cultivation using the pressed leaf fibrous residue, the lignocellulosic by-product of leaf protein production was initiated.

Pressed leaf fibre from three tree leaves were collected after complete extraction of protein. Residues after thorough washing with water, sun dried and were analysed for chemical and mineral make up. The pressed fibres were found to be rich sources of nitrogen (3-4 %), lignin (22-29 %), cellulose (25-31 %), pentosan (16-18 %) and minerals (5.5-9 %). After subsequent treatments with formalin and bavistin the three leaf fibres were exploited for fruit body production by two edible mushrooms viz. *Pleurotus sajor-caju* (Fr.) Singer and *P. ostreatus (*Jacq.ex Fr.) Kummer.

Yield performances of three leaf fibre residues from three trees namely *Dalbergia sisso*, *Albizzia procera* and *Delonix regia* were evaluated by using each of them either as whole substrate or in combination with the conventional substrate rice straw in 1:1 ratio. The two mushrooms were grown successfully on the fibrous substrates during the months of November to March. Fruit body productions by all the three substrates were best when used in combination with rice straw. Biological efficiency was maximum (110-120 %) when *Albizzia procera* and *Delonix regia* residue were fortified with rice straw. The chemical, mineral and neutral sugar analysis of the fruit bodies are reported. The efficiency of the pressed leaf fibres as substrates for edible mushrooms was compared with other lignocellulosic substrates.

The fruit bodies were also thick and compact on those substrates thereby indicating their acceptability.

Key words : Edible mushroom, *Pleurotus*, leaf protein technology, fibrous lignocellulosic by-product, biological efficiency

INTRODUCTION

In many developing countries including India, explosive population growth competed with limitations of cultivable land resources are creating serious problem on steady supply of food and nutrition. The problem is accurate in India and more so in eastern states of the country due to very narrow land and man ratio. Agricultural and nutritional researches aim at maximum utilization of existing resources. A lot of lignocellulosic wastes in connection with the harvesting and processing of various agro industrial crops are being generated in various parts of the country. These wastes can be utilized directly through mushroom cultivation converting the nutritionally value less wastes into highly acceptable protein rich nutritious and palatable food. Recently mushroom cultivation has gained a considerable momentum in India. The varied agroclimatic conditions in different parts of the country help in growing mushrooms without artificial temperature and humidity control. Among the various edible mushrooms, *Pleurotus* species are vary potential source to convert lignocellulosic agro as well as industrial wastes and refuses into protein rich food (Basak *et al.*, 1996; Hazarika, 1998). *Pleurotus* species have a unique flavour and aromatic properties and it is considered to be rich in protein, fibre, carbohydrates, vitamins and minerals (Agarwala and Jandaik, 1986). They are poor in substrate specificity and have reported to grow in all kinds of lignocelluloses.

In India *Pleurotus* generally known as oyster mushrooms are cultivated on single substrate commonly wheat or paddy straw. A number of investigators have tried a good number of lignocellulosic agricultural as well as industrial wastes as substrate refuses for *Pleurotus* cultivation like sugracane bagasse, used tea leaves (Awasti, 1991), pressed leaf fiber (Chanda *et al.*, 1993 : Das *et al.*, 1993), bajra and sorghum stalk (Sangwan and Saini, 1995), coir waste (Eyini *et al*, 1995), jute wastes (Basak *et al.*, 1996), common weeds (Justin *et al.*, 1998), sugarcane by product (Chandrasekhar *et al.*, 2001), banana pseudostem (Sharma *et al.*, 2002) ground nut shell, soybean stalk and grasses (Jain and Vyas, 2002), and leaf stalk biomass from coconut palm (Bhawna and Thomas, 2003).

The present study deals with the use of fibrous lignocellulosic residues left after leaf protein production from leaves of *Delonix regia, Albizia procera* and for fruit body production by two edible popular mushrooms, *Plerotus sajar-caju* (Fr.) Singer and *P. ostreatus* Kummar.

MATERIALS AND METHODS

Fresh green leaves were collected from *Albizia procera, Dalbergia sisso* and *Delonix regia* and washed to remove dust. Protein was extracted by IBP pulper (Davys and Pirie, 1969) and press (Davys *et al.*, 1969) and fibrous residue was obtained as a by-product. Fibres were washed with hot water (80°C) and dried in sun for seven days. Rice straw was also dried in sun. Straw was chopped into small pieces. All the test substrates were sterilized by soaking in a solution of bavistin (75 ppm) and formalin (500 ppm) just before use. Rice straw was soaked in the same solution for 18-24 h. Two edible mushrooms *Pleurotus ostreatus* and *P. sajor-caju* were employed for fruit body production. Mushrooms were cultivated according to standardized technique (Fritsche, 1978). Nylon bags were filled with 1 kg of rice straw only, 1 kg of pressed leaf fiber of *Albizia procera, Dalbergia sisso* and *Delonix regia* respectively and 1 kg of mixture of straw and leaf fibre of *Albizia procera, Dalbergia sisso and Delonix regia* mixed in 1 : 1 ratio (w/w). Multilayered spawning at the rate of 3% on wet weight basis was done to inoculate the substrate. The bags were covered by polythene sheets after spraying water. The polythene sheets were removed after complete mycelial colonization of the substrate. The room temperature was maintained between 25-28°C and relative humidity between 80%-90%. The treatments were replicated three times. First, second and third harvests were made after 4, 6 and 8 weeks of spawning, respectively. Rice straw, pressed leaf fibres of *Albizia procera, Dalbergia sesso* and *Delonix rigia* and mixture of those two in 1:1 (w/w) were chemically analysed for total cellulose, total lipid, lignin, total nitrogen and ash content. Nitrogen was calculated as Kjeldahl nitrogen, crude fat was extracted with chloroform : methanol (2:1) in a Soxhlet apparatus. Ash values were obtained by heating the samples at 550°C for 4 h in a muffle furnace. Cellulose and lignin were determined according to standard methods (T.A.P.P.I., 1971). Biological efficiency of substrate was calculated as % yield of fresh mushroom on dry weight of substrate.

RESULTS AND DISCUSSION

The data in Table 1 showed the chemical composition of pressed leaf fibre of *Albizia procera, Dalbergia sisso* and *Delonix regia* and rice straw (as control). Lignin, nitrogen and lipid contents of leaf fibre were higher in comparison to the rice straw. Rice straw was better source of

Table 1 : Yield and Chemical composition of leaf fiber residues from three Tropical tree legumes.

Chemical composition	*Albizia propcera*	*Dalbergia sisso*	*Delonix regia*	*Rice Straw*
Ash	5.52 ± 0.76	5.73 ± 0.95	8.81 ± 0.05	12.61 ± 1.77
Nitrogen	3.87 ± 0.11	3.75 ± .10	3.75 ± 0.11	0.6 ± 0.03
Crude fat	9.54 ± 1.21	9.44 ± 1.40	13.80 ± 2.56	3.53 ± 1.06
Lignin	22.45 ± 4.50	29.30 ± 5.80	28.50 ± 4.65	10.33 ± 2.85
Holocellulose	60.00 ± 3.00	57.88 ± 2.26	51.22 ± 1.66	67.77 ± 7.25
α-celluloses	31.00 ± 1.75	30.74 ± 1.16	25.11 ± 0.88	43.30 ± 6.95
Pentosan	18.46 ± 0.93	16.66 ± 0.76	17.74 ± 0.80	21.15 ± 5.88
MAD	48.05 ± 3.55	55.42 ± 6.26	45.12 ± 3.36	
NDF	60.05 ± 4.45	65.72 ± 5.39	61.25 ± 4.35	
Yield dry wt/kg fresh wt.	156 ± 16	250 ± 36	160 ± 10	

(Values expressed on 100 g oven dry material).
Mean ± SD of five replicates

Table 2 : Comparitive yield of two edible mushrooms in different substrates

Substrate	Harvest	Weeks	Yield(g) per bag (1Kg) [a]	Efficiency	Yield(g) per bag (1Kg) [b]	Efficiency [b]
Rice straw	1	4	240 ± 20	40	550 ± 45	100
	2	6	150 ± 10		300 ± 20	
	3	8	10 ± 2		150 ± 10	
Pressed leaf fibres of *Albizia procera*	1	4	175 ± 20	30	350 ± 25	60
	2	6	100 ± 15		200 ± 15	
	3	8	25 ± 5		50 ± 5	
Mixture of rice straw and pressed leaf fibres of *Albizia procera* in 1:1 (w/w)	1	4	310 ± 25	47	700 ± 50	120
	2	6	140 ± 15		325 ± 25	
	3	8	20 ± 2		175 ± 15	
Pressed leaf fibres of *Dalbergia sisso*	1	4	125 ± 10	21	320 ± 20	53
	2	6	75 ± 5		175 ± 15	
	3	8	10 ± 2		35 ± 5	
Mixture of rice straw and pressed leaf fibres of *Dalbergia sisso* in 1:1 (w/w)	1	4	300 ± 30	42	650 ± 40	110
	2	6	105 ± 15		300 ± 20	
	3	8	15 ± 3		150 ± 10	
Pressed leaf fibres of *Delonix regia*	1	4	165 ± 10	28	350 ± 20	58
	2	6	100 ± 5		180 ± 10	
	3	8	15 ± 3		50 ± 5	
Mixture of rice straw and pressed leaf fibres of *Delonix regia* in 1:1 (w/w)	1	4	300 ± 30	45	675 ± 45	120
	2	6	125 ± 15		350 ± 25	
	3	8	25 ± 5		175 ± 15	

[a] — *Pleurotus ostreatus* [b] — *Pleurotus sajor-caju*

Values are mean of five replicates and ± S.D.

cellulose (Table 1), as well as α-cellulose. The data in Table 2 showed the fruit body production by *Pleurotus ostreatus* and *P. sajor-caju* on various substrates like individual pressed leaf fibres from three leaves, rice straw and the mixture of leaf fibres and rice straw. Guzral *et al.* (1987) used the mixture of *Saccharum manja* and rice straw to cultivate *Pleurotus sajor-caju*, but they obtained highest yield with rice straw alone, where as in the present study the mixture of rice straw and fibres of tree legumes (1:1) showed the best result, 1100-1200 g/kg over 1000 g/1kg rice straw. The data of Bisaria *et al.* (1987) indicated that rice straw mixed with other plant residues provided better substrates for fruit body production by oyster mushroom which has also been proved by the present data (Table 2). Straw being poor in nitrogen, supplementation of nitrogen was required for quick degradation (Kahlon and Kalra, 1983). As leaf fibres contained a greater amount of nitrogen than rice straw (Table 1), it probably helped to increase the yield.

Das and Chanda (1993) and Das *et al.* (1993) worked on the bioregeneration of protein from various leaf fibre residues through cultivation of *Pleurotus sajor-caju.* Combination of leaf fibre residues with rice straw increased the yield of *Pleurotus sajor-caju* and gave the highest yield of 110% biological efficiency. Chanda *et al.* (1993) showed that a tree legume *Sesbania grandiflora* when mixed with rice straw (1:1) yielded *Pleurotus sajor-caju* with biological efficiency of 95% over rice straw alone (B.E. 85%).

Sangwan and Saini (1995) rendered trials on cultivation of *Pleurotus sajor-caju* on various agro industrial wastes. Subtrates trial included wheat straw, paddy straw, sorghum stalk, bajra stalk, sugarcane bagasse, waste paper alone as well as in combinations. The substrates were mixed with each other in equal proportions. Combination of sugarcane bagasse with wheat straw and paddy straw increased the yield of *Pleurotus sajor-caju* and gave the highest yield of 117.5 per cent and 118.5 per cent. Biological efficiency was very close to the present data. On the contrary when sorghum stalk, bajra stalk, and waste paper were mixed with straw the yield decreased as compared with control where straw alone was used.

Abundantly available real wastes like weeds and aquatic weeds which grow without any inputs can be used profitably for oyster mushroom cultivation (Justin *et al.,* 1996). Authors showed that performance of *Pennisetum polystachyon* was better (Biological efficiency 87.2%) over rice straw (B.E. 60.3%) as control. Performance of *Saccharum arundinaceum* (B.E. 20%) and *Themeda cymbaria* (B.E. 18.3%) were much inferior as substrate for mushroom.

Basak *et al.* (1996) used various jute wastes like jute leaves, jute stick, jute caddies etc as substrate for *Pleurotus sajor-caju.* Better results were obtained when such wastes are mixed with rice straw in (1:1) ratio.

Mathew *et al.* (1996) worked extensively on performance of five species of *Pleurotus* in Kerala. They empolyed *Pleurotus sajor-caju, P. citrinopileatus, P. florida, P. Platypus, P. ostreatus* for their yield performance on various substrates like paddy straw, *Eliocharis plantogena* and rubber wood saw dust. Performance of *Pleurotus sajor-caju* was much better than that of *P. ostreatus.* Our study harmonises this finding (Table 2).

Upadhyay and Verma (2000) induced newer substrates like malt industry waste, tea leaf industry waste and dry popular (*Populus* sp.) leaves for growing *Pleurotus* sp. Tea leaves alone (steam pasturized) proved to be poor substrate for mycelial growth of *Pleurotus* sp. but in combination with wheat straw (3:1 and 1:1), it gave better yield than the control (wheat straw). Similarly, malt industry waste which is rich in nitrogen could substitute wheat straw up to 20% for growing *Pleurotus* sp. Dried leaves of *Populus* sp. (*Popular*), a common agro-forestry tree, could be used alone or in various combinations with wheat straw for growing *Pleurotus* sp. Dried *Populus* leaves alone could give 70% biological efficiency. Use of such wastes/ cheap substrate may prove helpful for poorest farmers and reduce the cost of cultivation.

In Table 2, a comparative yield of two mushroom were focussed along with biological efficiencies. The fruit body yield of *P. ostreatus* remained inferior always when compared with *P. sajor-caju.* Jain and Vays (2002) considering the versatility of *Pleurotus* conducted a study where a common substrate wheat straw was tried in various combinations with new substrates to enhance the yield as well as decreasing the dependence on wheat straw which is getting costlier due to its demand for cattle feed. Various substrates used were wheat straw in combination with soyabean

Table 3 : Chemical composition of *Pleurotus sajor-caju* harvested on different substrate.

Substrate	Moisture	Ash	Lipid	Crude protein (Nx 6.25)	Carbohydrate
Rice Straw	89	6.8	12	18.2	70.00
Rice Straw + Leaf fiber of *Albizia procera*	87	10.2	8	31.5	50.2
Rice Straw + *Dalbergia sisoo*	85	9.0	10	25.6	55.4
Rice Straw + *Delonix regia*	87	11.2	7.9	33.0	47.9

(Values expressed on per cent dry weight).
All values shown are mean of three replicates.

Table 4 : Mineral compostion (g/100 g dry weight) of *Pleurotus sajor-caju* grown on different substrate.

Calcium	Sodium	Potassium	Iron	Copper	Zinc
0.022	0.190	3.15	0.016	0.006	0.011
0.031	0.510	2.68	0.004	0.001	0.006
0.029	0.422	2.18	0.015	0.001	0.017
0.035	0.466	2.55	0.027	0.008	0.018

All values shown are mean of three replicates.

straw, groundnut shell, paddy straw, bamboo leaves, grasses, pine needles and used tea leaves. Only two combinations i.e. wheat straw and soyabean straw and wheat straw fortified with used tea leaves found to show biological efficiency of *Pleurotus* sp. Either slightly superior or equal to 93 and 89% respectively, in comparison to wheat straw alone (B.E. 87%).

The results of chemical and mineral analysis of the fruit bodies of *Pleurotus sajor-caju* harvested from promising substrates were projected in Tables 3 and 4. The data were comparable with those shown by Basak *et al.* (1996). The fruit bodies were also thick and compact on those substrates indicating their good acceptibility.

Mushroom cultivation is one of the most profitable and environment friendly enterprises among various horticultural corps in India. It is mainly cultivated indoors in cropping rooms where fluctuations in climatic conditions like draught and rains have little impact on its production. Oyster mushroom (*Pleurotus* sp.) is the third largest cultivated mushroom in the world and contributes 16.3% to the total world mushroom production. In India mushroom varieties like oyster mushroom (*Pleurotus* sp.) are gaining popularity in the tropical and subtropical parts due to their easy cultivation technology at a nominal capital investment. It is cultivated mainly by small and marginal farmers to cater to the demand of local market with an estimated production around 5 tonnes per year. However, there is a vast potential for cultivation of oyster mushroom due to its ability to grow on any kind of agricultural as well as industrial by-products which are rich in cellulose, hemicellulose and lignin.

Vast quantities of renewable lignocellulosic agroforestry as well as agroindustrial residues are generated every year as a result of various extensive practices. Bioconversion of these unmodified ligno-cellulosic residues through mushroom cultivation offers the potential of converting them into protein rich, palatable food to meet the global gap of protein-calorie malnutrition.

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