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Bioconversion of low quality lignocellulosic agricultural waste into edible protein by *Pleurotus sajor-caju* (Fr.) Singer

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Abstract: *Pleurotus sajor-caju* (Fr.) Singer was cultivated on selected agro wastes viz. cotton stalks, groundnut haulms, soybean straw, pigeon pea stalks and leaves and wheat straw, alone or in combinations. Cotton stalks, pigeon pea stalks and wheat straw alone or in combination were found to be more suitable than groundnut haulms and soybean straw for the cultivation. Organic supplements such as groundnut oilseed cake, gram powder and rice bran not only affected growth parameters but also increased yields. Thus bioconversion of lignocellulosic biomass by *P. sajor-caju* offers a promising way to convert low quality biomass into an improved human food.

Key words: *Pleurotus sajor-caju*, Agricultural wastes, Yield
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INTRODUCTION

The use of fungi for the conversion of lignocellulose into food and feed rich in protein offers an alternative for developing unconventional source of proteins as food/feed. Yeast and algal proteins require sophisticated techniques and heavy inputs whereas the beauty of mushroom cultivation lies in its ability to grow on cheap lignocellulosic materials with minimum inputs and a high yield of valued food protein for direct human consumption.

A wide range of diverse cellulosic substrates are used for cultivation of *Pleurotus sajor-caju*. Amongst various cereal straws, paddy straw was reported to be the best substrate for the cultivation of oyster mushroom (Bano and Srivastava, 1962; Jandaik and Kapoor, 1974; Khanna and Garcha, 1982), whereas, next to the paddy straw, wheat straw proved to be the best substrate for the cultivation of *Pleurotus* spp. (Bano and Rajarathnam, 1982; Bhatti *et al*., 1987; Thampi *et al*., 1996; Bonatti *et al*., 2004). Sorghum straw was also effectively used to cultivate *P. sajor-caju* (Bahukhandi and Munjal, 1989; Patil *et al*., 1989). Similarly, Garcha *et al*.(1984) and Diwakar *et al*.(1989) reported the utility of pearl millet stalks in the cultivation of *P. sajor-caju*. Rye straw waste (Pal and Thapa, 1979), lawn grass (Yamashita *et al*., 1983), maize cobs (Bhatti *et al*., 1987), Banana waste (Bonatti *et al*., 2004) and maize straw (Bahukhandi and Munjal, 1989) were reported as suitable substrates for cultivations of different *Pleurotus* spp. Bhandari *et al*.(1991) successfully cultivated *P. sajor-caju* on straws of millets viz. *Echinochloa frumentacea* (Poaceae) and *Eleusine coracana* (Poaceae), and grasses viz. *Heteropogon contortus* (Poaceae) and *Andropogon purtuses* (Poaceae). Many other types of substrates were also reported to be useful for the cultivation of various species of *Pleurotus* spp. (Table 1).

Supplementation of main substrates with nutrient or combination of two or more substrates were reported to increase the yields of *P. sajor-caju* (Jadhav

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Table 1 Substrates reported to be useful for the cultivation of various species of *Pleurotus* spp.

Substrate	References
Chopped branches of *Euphorbia royleana* (Euphorbiaceae)	Kaul and Janardhan, 1970
Saw dust	Gurjar and Doshi, 1995
Waste tea leaves	Bisht and Harsh, 1983
Pulp mill sludge	Mueller and Gawley, 1983
Coffee pulp	Guzman and Martinez, 1986
Oil palm bunch refuse	Ramesh and Ansari, 1987
Water weeds	Jain *et al*., 1988
Sugarcane baggasse	Bahukhandi and Munjal, 1989
Coconut and anjali logs	Suharban and Nair, 1991
Sericulture/agriculture industry wastes	Sharma and Madan, 1992
Wood logs and trunks	Chang and Miles, 1993
Lantana camara (Verbenaceae)	Vats *et al*., 1994
Mustard haulm	Mukherjee and Nandi, 2001
Water hyacinth	Murugesan *et al*., 1995
Straws of *Saccharum munja* (Poaceae), *Abelmoschus esculentus* (Malvaceae) and *Cyamopsis tetragonoloba* (Fabaceae)	Patrabansh and Madan, 1995
Salvinia molesta (Salviniaceae) and *Eichhorinia crassipes* (Pontederiaceae)	Mani and Philip, 1995
Leonotis sp. (Lamiaceae), *Sida acuta* (Malvaceae), *Parthenium argentatum* (Asteraceae), *Ageratum conyzoides* (Asteraceae), *Cassia sophera* (Caesalpiniaceae), *Tephrosia purpurea* (Fabaceae) and *Lantana camara* (Verbenaceae)	Das and Mukherjee, 2007

et al., 1998). Amendments or mixtures of various straws were tried to assess possibilities for main substrates to increase the yield of *P. sajor-caju*. The second objective was to determine an effective substrate combination for the cultivation of *Pleurotus* spp. in the paucity of wheat or paddy straws.

Lignocellulosic materials are generally low in protein content, insufficient for the cultivation of mushrooms, which requires nitrogen, phosphate and potassium. Since the C:N ratio plays an important role in spawn running and the growth of fruiting body, nitrogen supplementation is an important factor for the growth and yield of mushrooms. So far, however, addition of substrates with organic nitrogen supplements has not been much studied yet.

Based on earlier studies and local availabilities of the agricultural wastes, in the present study we utilize cotton stalks, groundnut haulms, soybean straw, pigeon pea stalks and leaves and wheat straw for the cultivation and production of *P. sajor-caju*. We also study the effects of organic nitrogen supplementation on the growth and yield of mushrooms in substrate combinations in which the nitrogen content is relatively low by addition of cheap and locally available nitrogenous materials.

MATERIALS AND METHODS

Culture of *Pleurotus sajor-caju*

Stock culture of *P. sajor-caju* was obtained from the Mushroom Research Centre, College of Agriculture, Pune, India. The cultures were maintained on 2% malt extract agar slants at 4 °C. Subculturing was performed every 15 d.

Spawn preparation

Spawn was prepared in polystyrene packets or 1 L bottles. Wheat grains were boiled in waterbath for 10~15 min and mixed with 4% (w/w) $CaCO_3$ and 2% (w/w) $CaSO_4$. The strains were inoculated with actively growing mycelium of the *Pleurotus* from malt extract slants and incubated at (27±2) °C for mycelial growth for 12 to 15 d until the mycelium fully covered the grains.

Cultivation of *Pleurotus sajor-caju*

Cotton stalks, groundnut haulms, soybean straw, pigeon pea stalks and leaves and wheat straw were used for the cultivation of *P. sajor-caju*, following the method proposed by Bano and Srivastava (1962) with slight modifications. Dried agro wastes were chopped into 1 to 2 cm long bits and soaked in water overnight. Excess water was drained off and these substrates were pasteurised by dipping in hot water at 80 °C. Spawn (20 g/kg) was added and mixed with the wet straw. Polyethylene bags of 30 cm×20 cm with holes were filled with the mixture. One kg of dry substrate was used in each bag. Spawning was done in five layers at the rate of 2% of net substrate. These bags were incubated at room temperature [(27±2) °C] in a room with sufficient light and ventilation for 15 d. Next, the polyethylene bags were cut open on the sides without disturbing the bed and were sprayed with water twice a day using a hand sprayer. The mass of substrate was kept undisturbed for the appearance of fruiting bodies. The spray of water was discontinued a day before the harvest of the fruiting bodies. The mushroom was collected in three flushes, and after each flush a small layer of substrate was scrapped off from all the side of the substrate.

The substrates were supplemented with 1% organic nitrogenous material i.e. gram flour or groundnut oil seed cake or rice bran alone in dry powder form during filling of bags.

RESULTS

The effect of different substrates on yield contributing characters such as pileus size and stipe length varied significantly (Table 2). The pileus size on different substrates ranged from 20.83 to 28.88 cm^2, the largest being on cotton stalks+wheat straw

Table 2 Yield of *Pleurotus sajor-caju* cultivated on various agro waste substrates

No.	Substrate	Spawn running	Stipe length (cm)	Pileus size (cm^2)	Yield (g/kg substrate)
1	Cotton stalks	++	2.95	25.61	510.63
2	Groundnut haulms	+	2.45	22.35	365.05
3	Soybean straw	+	2.24	20.83	257.75
4	Pigeon pea stalks and leaves	++	2.83	24.68	463.36
5	Wheat straw	++	2.65	23.00	447.12
6	Cotton stalks+groundnut haulms	++	3.00	26.35	596.50
7	Cotton stalks+soybean straw	++	3.15	27.83	661.00
8	Cotton stalks+pigeon pea stalks and leaves	++	3.27	28.21	713.27
9	Cotton stalks+wheat straw	++	3.25	28.88	796.12
10	Groundnut haulms+soybean straw	++	2.64	21.32	333.12
11	Groundnut haulms+pigeon pea stalks and leaves	++	2.85	25.00	556.61
12	Groundnut haulms+wheat straw	++	2.78	26.11	635.83
13	Soybean straw+pigeon pea stalks and leaves	++	2.43	24.35	457.05
14	Soybean straw+wheat straw	++	3.10	27.80	668.36
15	Pigeon pea stalks and leaves+wheat straw	++	3.17	28.21	735.89
16	Cotton stalks+groundnut haulms+soybean straw	+++	3.10	24.65	487.25
17	Cotton stalks+groundnut haulms+pigeon pea stalks and leaves	+++	3.37	26.11	533.12
18	Cotton stalks+groundnut haulms+wheat straw	+++	3.45	27.83	657.33
19	Cotton stalks+soybean straw+pigeon pea stalks and leaves	+++	3.27	26.18	633.49
20	Cotton stalks+soybean straw+wheat straw	+++	3.35	27.25	641.36
21	Cotton stalks+pigeon pea stalks and leaves+wheat straw	+++	3.26	28.62	703.22
22	Groundnut haulms+soybean straw+pigeon pea stalks and leaves	+++	2.95	23.55	395.26
23	Groundnut haulms+soybean straw+wheat straw	+++	3.20	25.66	512.96
24	Soybean straw+pigeon pea stalks and leaves+wheat straw	+++	3.00	24.33	450.63
25	Cotton stalks+groundnut haulms+soybean straw+pigeon pea stalks and leaves	+++	3.35	26.65	582.56
26	Cotton stalks+groundnut haulms+soybean straw+wheat straw	+++	3.32	26.83	591.13
27	Groundnut haulms+soybean straw+pigeon pea stalks and leaves +wheat straw	+++	3.45	27.23	615.23
28	Cotton stalks+groundnut haulms+soybean straw+pigeon pea stalks and leaves+wheat straw	+++	3.63	28.50	750.30
	(±)*SE*	−	0.05	0.62	16.62
	CD at 5%	−	0.17	1.92	48.82

+=Sparse growth; ++=Moderate growth; +++=Abundance growth. SE: Standard error; CD: Critical difference

(28.88 cm^2) and the smallest obtained on the soybean straw (20.83 cm^2). The stipe length of mushrooms ranged from 2.24 to 3.63 cm. The highest stipe length was observed in fruit bodies grown on the combination of cotton stalks+groundnut haulms+soybean straw+pigeon pea stalks and leaves+wheat straw (3.63 cm) and the smallest on soybean straw (2.24 cm).

The yield of mushrooms was affected by different substrates. The highest yield of mushrooms was recorded on cotton stalks+wheat straw (796.12 g/kg substrate), which was found to be markedly higher than all other treatments except cotton stalks+groundnut haulms+soybean straw+pigeon pea stalks and leaves treatment.

The supplementation of nitrogenous material was performed to the substrates containing neither groundnut nor soybean wastes. The combination of cotton stalks+pigeon pea stalks and leaves+wheat straw with the supplementation of gram flour yielded 716.50 g/kg substrate, whereas the substrate without this supplementation produced 703.22 g/kg substrate. In cotton stalks alone, the yield was 510.63 g/kg substrate, whereas with the supplementation of gram flour the yield increased to 617.53 g/kg substrate (Table 3).

The addition of groundnut seed cake to cotton stalks+pigeon pea stalks and leaves+wheat straw yielded 914.03 g/kg substrate, whereas without supplementation it was 703.22 g/kg substrate. The yield was 510.63 g/kg substrate in cotton stalks alone vs 845.27 g/kg substrate with the addition of groundnut seed cake. Similar results of other combinations were recorded in Table 4. The supplementation of rice bran to cotton stalks+pigeon pea stalks and leaves+wheat straw yielded 756.88 g/kg substrate, whereas without supplementation it was 703.22 g/kg substrate. In cotton stalks alone the yield was 510.63 g/kg substrate, whereas with supplementation it gave 661.25 g/kg substrate. Listed in Table 5 were similar results from other combinations.

Table 3 Effect of gram flour supplementation on yield of *Pleurotus sajor-caju*

No.	Substrate	Spawn running	Stipe length (cm)	Pileus size (cm^2)	Yield (g/kg substrate)
1	Cotton stalks	+++	3.32	26.42	617.53
2	Pigeon pea stalks and leaves	+++	3.15	25.68	556.89
3	Wheat straw	+++	2.93	24.50	481.19
4	Cotton stalks+pigeon pea stalks and leaves	+++	3.22	25.83	563.56
5	Cotton stalks+wheat straw	+++	3.18	24.00	453.42
6	Pigeon pea stalks and leaves+wheat straw	+++	3.29	26.35	605.24
7	Cotton stalks+pigeon pea stalks and leaves+wheat straw	+++	3.56	29.12	716.50
	(±)*SE*	−	0.05	0.70	15.10
	CD at 5%	−	0.15	2.15	45.50

+=Sparse growth; ++=Moderate growth; +++=Abundance growth. SE: Standard error; CD: Critical difference

Table 4 Effect of groundnut oil seed cake supplementation on yield of *Pleurotus sajor-caju*

No.	Substrate	Spawn running	Stipe length (cm)	Pileus size (cm^2)	Yield (g/kg substrate)
1	Cotton stalks	+++	3.51	57.17	845.27
2	Pigeon pea stalks and leaves	+++	3.32	25.43	754.30
3	Wheat straw	+++	3.26	25.00	693.84
4	Cotton stalks+pigeon pea stalks and leaves	+++	3.38	26.25	712.25
5	Cotton stalks+wheat straw	+++	3.41	26.66	796.27
6	Pigeon pea stalks and leaves+wheat straw	+++	3.48	27.47	883.65
7	Cotton stalks+pigeon pea stalks and leaves+wheat straw	+++	3.63	29.38	914.03
	(±)*SE*	−	0.06	0.65	20.43
	CD at 5%	−	0.19	1.90	60.65

+=Sparse growth; ++=Moderate growth; +++=Abundance growth. SE: Standard error; CD: Critical difference

Table 5 Effect of rice bran supplementation on yield of *Pleurotus sajor-caju*

No.	Substrate	Spawn running	Stipe length (cm)	Pileus size (cm^2)	Yield (g/kg substrate)
1	Cotton stalks	+++	3.45	26.85	661.25
2	Pigeon pea stalks and leaves	+++	3.27	25.43	582.56
3	Wheat straw	+++	3.17	25.10	517.63
4	Cotton stalks+pigeon pea stalks and leaves	+++	3.31	26.00	617.68
5	Cotton stalks+wheat straw	+++	3.27	25.50	542.33
6	Pigeon pea stalks and leaves+wheat straw	+++	3.36	26.43	712.50
7	Cotton stalks+pigeon pea stalks and leaves+wheat straw	+++	3.53	27.20	756.88
	(±)*SE*	−	0.06	0.62	17.23
	CD at 5%	−	0.18	1.88	50.45

+=Sparse growth; ++=Moderate growth; +++=Abundance growth. SE: Standard error; CD: Critical difference

DISCUSSION

Most of the mushroom species possess the ability to degrade lignin, cellulose and hemicellulose and to produce fruiting bodies containing most of the essential amino acids, valuable vitamins, minerals and low energy carbohydrates. *Pleurotus* spp. have the potential to convert cheap cellulosics into valuable protein at a low cost. Following the cultivation on paddy straw for the first time and subsequent standardization by Bano and Srivastava (1962), a great number of investigations have confirmed its wide adaptability and its suitability for developing countries such as India that have vast areas under tropical and sub-tropical zones and produce crop residues in abundance.

Mushrooms get nutrition from cellulose, hemicellulose and lignin, which are abundantly available in cereal straws. If only one-fourth of the world's annual yield of cereal straw (2325 million tonnes) was used to grow mushrooms, about 377.8 million tonnes of fresh mushrooms could be produced and such an amount would provide 4.103 million people with 250 g of fresh mushroom daily (Madan *et al*., 1994; Jandaik and Goyal, 1995; Royse, 2002).

The variation in stipe length was observed in the combination of all five agro wastes used in this study. Cotton stalks gave the longest stipe, followed by pigeon pea stalks and leaves, and wheat straw, with the least stipes in groundnut haulms and soybean straw. There appears a definite co-relation between the substrate composition and stipe length; whenever cotton stalks were present in various combinations, the stipe length was greater than 3 cm, whereas in the absence of cotton stalks the stipe ranged from 2.43 to 3.45 cm. Similar observations on the cultivation of cotton stalks alone were also reported by others (Lavie, 1988; Sharma, 1983) and lower stipe lengths were recorded in groundnut haulms alone (Jadhav *et al*., 1996).

The pileus size of mushrooms varied markedly in different substrates and combinations of different substrates. While the trend of the variation of the pileus size was more or less similar to that of stipe length, the bigger pileus sizes were associated with cotton, pigeon pea and wheat agro waste, whereas the size relationship in groundnut and soybean combination was inconsistent. The superiority of cotton stalks over paddy straw in relation to pileus size for *P. ostreus* and *P. florida* was also reported by others (Lavie, 1988; Leong, 1980). It is clear from the yield on various combinations of substrates that cotton stalks or pigeon pea stalks and leaves or wheat straw in combination with groundnut haulms or soybean straw was considerably higher than a single substrate cultivation. Similar results were obtained by Patil *et al*.(1989). They reported a superiority of cotton stalks over paddy straw. Leong (1980), Lavie (1988), Silanikove *et al*.(1988), Patil *et al*.(1989) and Jadhav *et al*.(1996) also observed relatively higher yields on cotton wastes.

Veena and Savalgi (1991) reported a low yield of mushrooms on groundnut haulms. They attributed the low yield to a high moisture holding capacity and a high susceptibility to fungi and improper aeration. Anastazia *et al*.(1982) observed that legumes rich in nitrogen gave a higher yield in combination with paddy or wheat straw or corncobs. Jadhav *et al*.(1996)

reported lower yields on soybean straw and groundnut haulms. It can be stated that groundnut haulms and soybean straw give good results when used in combination with cotton straw and/or pigeon pea stalks and leaves and/or wheat straw.

Whenever cotton straw and/or pigeon pea stalks and leaves and/or wheat straw were used, the yields decreased; but when supplemented with groundnut haulms and soybean straw, relatively higher yields occurred. This is due probably to the high nitrogen content of groundnut and soybean waste. Supplementation with a nitrogenous source is a key for higher yields in combinations containing neither groundnut haulms nor soybean straw.

Organic supplements viz. soybean meal, alfalfa meal, and cotton seed powder increase not only yields but also proteins of mushrooms (Zadražil, 1980). Several nitrogen sources tried as supplements to the rice straw, yeast mud, cotton seed powder and rice bran proved ideal in increasing the mushroom yields (Jandaik, 1989). Although the supplementations have been reported to enhance the yield, they have not been adopted by growers yet.

Various legume crop wastes viz. soybean, mash clover, kulthi and mixture of soybean, mash and kulthi (1:1:1) were found effective in cultivating *P. sajor-caju* (Pal and Paul, 1985). Rana and Subag (1990) recorded that gram pod waste in combination with wheat straw (1:1) improved mushroom yields by 5.26% over the wheat straw alone. Various legume straws, including groundnut straw and seed husk (Diwakar *et al*., 1989), cowpea and guar straw (Patrabansh and Madan, 1995), and green gram and gram straw (Kadlag, 1997) were found to be suitable substrates for cultivating *Pleurotus* spp.

In the present study, the substrates, such as cotton stalks, with more cellulose and lignin were found to induce more growth of *Pleurotus* spp., consistent with established data (Kandaswamy and Ramaswamy, 1978; Poo-Chow, 1980; Bhatti *et al*., 1987; Muhamad and Khan, 1993). In conclusion, the treatment of lignocellulosic biomass with *P. sajor-caju* offers a promising means to convert low quality biomass into a high protein food. All five agro wastes applied in this study are viable substrates for mushroom cultivation alone or in combinations, and the low cost organic supplementation of substrate increases mushroom yield.

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