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IMPROVEMENT IN THE NUTRITIVE VALUE OF RICE STRAW BY BIODEGRADATION WITH *PLEUROTUS* SPP

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Biodegradation of rice straw with *Pleurotus spp.* enhanced its nutritive value. The protein contents increased from 1.7 to 9.40% and crude fibre, cellulose and lignin decreased from 32.6 to 17.4%, 35.0 to 18.2% and 10.9 to 8.5%, respectively. Reticulo-rumen digestibility of dry matter, cellulose, minerals and protein contents of rice straw, biodegraded by different *Pleurotus* spp., was enhanced from 37.0 to 46.8%, 49.9 to 61.0%, 20.1 to 29.3% and 31.4 to 43.9%, respectively over the non-degraded rice straw.

Key words: Rice straw, Biodegradation, *Pleurotus* spp.

Introduction

Lignocelluloses are the major constituents of agro-industrial wastes. In recent years, biodegradation of lignocelluloses by microbes and its transformation into food and feed has been an area of great interest and represents a tremendous challenge to microbial ecologists (Zadrazil 1977; Bone and Levonen-Munoz 1984).

White-rot *Basidiomycetes* are characterized with the ability to degrade all the components of lignocelluloses i.e., cellulose, hemicellulose and lignin simultaneously. Therefore, white-rot fungi are the true lignin biodegrader, most of the recent investigations on fungal biodegradation of lignin has shown that *Pleurotus* spp have become model organism for biodegradation (Hiroi and Erikson 1976; Rajarathnam *et al* 1979).

Rice is the second most important cereal crop of pakistan, yielding almost 3 million tonnes of crop residues annually (Shah 1980). A major portion of this agricultural waste is disposed off by indiscriminate burning which, in addition to being wasteful, creates serious environmental pollution problems. Therefore, studies were initiated for the biodegradation of rice straw with different strains of *Pleurotus* spp for the development of a system which would providean alternative use of the residue and minimization of the pollution problem.

Materials and Methods

Culture maintenance. The stock cultures of *Pleurotus* spp were maintained on standard malt extract agar medium in test tube slants at 3-5°C. The inoculum was grown on standard malt extract agar medium in 100 mm sterilized petri-plates. The sterilized malt extract medium (at 6.81 kg for 15 min) was poured aseptically into petriplates, cooked aseptically, inoculated with mycelial plugs (1-2 mm) of *Pleurotus* spp and incubated to grow at 25 °C for 7-10 days.

Spawn preparation. The grains of gram, rye, millet, sorghum or wheat were softened by soaking in boiled water. Softened grains (50g) were taken in sterilized wide mouthed, cotton plugged bottles and subcultured aseptically by transferring small mycelial plugs (6mm diameter) of *Pleurotus* spp The bottles containing spawn were placed in an incubator at 25 + 0.5 °C for 7-10 days.

Substrate. Rice straw of IRRI-Pak-6 variety was chopped into 30-50mm length and was soaked in boiling water for 15 min and washed twice with boiling water. The excess water was allowed to drain off, so that moisture contents of the substrate was nearly 80 percent. This was referred to as pasteurized substrate. Two kg of pasteurized substrate was transferred while hot into wooden trays (52 x 38 x 10 cm) having two verticle growing surfaces. A polyethylene sheet was wrapped around the tray and allowed to cool overnight.

Spawning and ramification. After unwrapping the tray after 24 h, 5% spawn of *Pleurotus* spp (w w^{-1} basis) was punched at different spots at a distance of 15 cm. The tray was wrapped with polyethylene sheet again. The mycelium was allowed to ramify the substrate in the spawn running room under controlled temperature of 25-30°C. The relative humidity (r.h.) of the room was maintained between 65-85 percent with the help of a humidifier (Defensor AG 505, Switzerland).

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Fruitifications and harvesting. The bed was uncovedred after 21 days when it had turned white due to the growth of mycelium. After completion of spawn running, the temperature of the spawning room was brought down to 20 ± 1 °C in winter and 25 ± 1 °C in summer, for fruitification. In a week, pinheads appeared which later turned Into full oyster shaped mushrooms. The full sized mushrooms were cut by sharp knife. Two more crops were obtained at the intervals of 8-10 days. After harvesting the three flushes the substrate (ricestraw) infested with different *Pleurotus* species was dried, ground to 100 mesh and used for subsequent analysis. Uninoculated rice straw sample, which served as control, was also subjected to similar analysis.

Analytical procedures. Moisture, ash, fat, crude protein and lignin contents were determined according to standard methods of Association of Official and Analytical Chemists (AOAC 1984) whereas cellulose was determined using the Kurschner and Hanak method (1930), briefly outlined below:

i)*Moisture.*The samples were kept at 100 - 105°C to constant weight

$$\% \text{ Moisture} = \frac{\text{loss in weight of sample}}{\text{weight of sample}} \times 100$$

ii)*Ash.* 2-3g of dried samples was incinerated first on a low flame and then placed into muffled furnace.

iii) *Crude protein.* Nitrogen present in the sample was estimated by micro-Kjeldhal method. A factor of 6.25 was applied for conversion of nitrogen into crude protei contents.

iv)*Fat.* The samples were extracted in Soxhlet extractor using *n*-hexane to extract fat.

v) *Crude fibre.* Fat free sample was refluxed with 1.25% H_2SO_4 and 1.25% NaOH to dissolve all material except fibrous portion. Crude fibre was calculated as reported elsewhere (AOAC 1984).

vi) *Lignin.* About one gram of sample was mixed, stirred with 15ml cold H_2SO_4 and frequently stirred at 18-20°C, washed with water and refluxed with water for 4 h. Residue was washed with hot water, alcohol and either, dried and incinerated at 450-500°C.

$$\text{Lignin} = \frac{\text{dry wt. of digested material-wt. of ash}}{\text{weight of sample taken}} \times 100$$

vii) *Cellulose.* Oven dried sample was mixed with 15 ml of 80% CH_3COOH and 1.5 ml of conc. HNO_3, refluxed for 20 min and filtered. Residue was washed with ethanol, oven dried at 100-105 °C and incinerated at 450-500 °C.

$$\% \text{ Cellulose} = \frac{\text{dry wt. of digested material-wt. of ash}}{\text{wt. of samples take}} \times 100$$

In vivo digestibility trials. A dried Sahiwal cow weighing 350 kg was rumen fistulated and fitted with an aluminium cannula measuring 113x80 mm, weighing 0.51 kg. The cow was maintained on ration according to National Research Council (USA) recommendations i.e. it contained feed ingredients having metabolizable energy, sufficient to maintain the weight of cow (Anon 1971). The ground ricestraw biodegraded by *P sapidus, P ostreatus and P florida* was infused into the rumen in accordance with the rumen technique (Anon 1971) in 6 replicates and the material left after digestion was removed after 2 days. The material was dried at 100-105°C to constant mass. The coefficient of digestability was calculated according to McDonald *et al* (1982) and Ranjhan (1981). The results obtained for various observations were analysed statistically according to Steel and Torrie (1960). Control samples of rice straw were also subjected to similar digestibi lity trials.

Results and Discussion

Rice straw ramified with *P florida, P sapidus, P ostreatus* and the control straw was dried at 100 ± 2°C and analyzed for ash, fat, protein, crude fibre, cellulose and lignin contents (Table 1). The results revealed that the protein contents increased from 1.7% (control) to 9.4% in *P ostreatus*. The rise in protein contents i.e. 7.3, 9.4 and 9.4% when infested with *P sapidus, P florida and P ostreatus* may be viewed as an important development in the enhancement of the nutritive value of the rice straw. It is, therefore, apparent that above mentioned fungi are capable of upgrading the low protein straw to medium protein straw feed.

The crude fibre and cellulose contents significantly decreased in the infested straw. The decrease in crude fibre contents from 32.6% to 17.4% and cellulose contents 35.0% to 18.2%, clearly showed that *P ostreatus, P sapidus* and *P florida* are efficient biodegraders of crude fibre, cellulose and lignin. The decrease in fat contents from 1.6% to 0.9% indicated that fungi metabolized fat. Similarly fungal infestation reduced the lignin contents from 10.9 to 8.5% making the straw more susceptible for the enzyme system. Similar biochemical changes were also reported by other researchers (Gerrit *et al* 1965 Gujral *et al* 1987).

The unbiodegraded and biodegraded rice straw were subjected to reticulo-rumen digestibility i.e. absorption of different ingredients in reticulum and rumen part of the stomach of cow. The improvement in the digestibility of dry matter, cellulose, minerals and protein is presented in Table 2. The dry matter, cellulose, minerals and protein digestibilities of rice straw biodegraded by *P florida,P sapidus and P ostreatus* varied from 38.8 to 41.6%, 31.9 to 34.2%, 57.9 to 62.2% and 39.7 to 43.6%

Table 1

Proximate composition* of rice straw before and after propagation of *Pleurotus* Spp.

Biodegraded with	Protein (%)	Fat (%)	Ash (%)	Crude fibre (%)	NFE** (%)	Cellulose (%)	Lignin (%)
Control (as such)	1.7 ± 0.2	1.6 ± 0.2	17.8 ± 0.5	32.6 ± 1.5	46.3 ± 1.8	35.0 ± 1.3	10.9 ± 0.5
P florida	9.4 ± 0.4	1.1 ± 0.1	32.2 ± 1.4	19.6 ± 1.1	37.6 ± 1.4	20.2 ± 0.8	8.8 ± 0.4
P sapidus	7.3 ± 0.3	1.0 ± 0.1	33.3 ± 1.8	18.2 ± 0.8	40.1 ± 2.0	18. 5 ± 0.5	8.7 ± 0.4
P ostreatus	9.4 ± 0.3	0.9 ± 0.2	31.4 ± 1.5	17.4 ± 1.2	40.8 ± 1.5	18.2 ± 0.8	8.5 ± 0.7

*, On dry matter basis; **, Nitrogen free extract = 100-(protein + fat + ash + crude fibre); NB, All the figures are average of six replicates with standard deviation

Table 2

Digestibility* of r ice straw before and after biodegradation by *Pleurotus* spp.

	Dry matter		Cellulose		Minerals		Protein	
Biodegradation of rice straw with	Digestibility (%) (Weight/Weight	Increase (%) basis)	Digestibility (%) (Cellulose/Cellulose	Increase (%) basis)	Digestibility (%) (Mineral/Mineral	Increase (%) basis)	Digestibility (%) (Protein/Protein	Increase (%) basis)
Control (as such)	28.3 ± 1.2	-	21.3 ± 1.1	-	48.2 ± 2.1	-	30.3 ± 0.5	-
P florida	41.6 ± 1.8	46.8 ± 2.0	33.6 ± 0.8	58.3 ± 1.9	62.2 ± 1.9	29.3 ± 1.0	42.8 ± 0.9	41.5 ± 1.5
P sapidus	38.8 ± 1.4	37.0 ± 1.5	31.9 ± 1.4	49.9 ± 1.6	57.9 ± 2.3	20.1 ± 1.1	39.7 ± 0.7	31.4 ± 0.9
P ostreatus	40.4 ± 1.9	42.6 ± 1.3	34.2 ± 1.2	61.0 ± 2.2	61.4 ± 2.5	27.6 ± 0.8	43.6 ± 0.6	43.9 ± 1.2
Significant difference	HS	HS	HS	HS	HS	HS	HS	HS

*NB, All the figures are average of six replicates with standard deviation; Increase in digestibility over control; H S Highlysignificant ($P < 0.01$)

respectively which showed highly significant improvement ($P < 0.01$) in the nutritive value as compared to the control i.e. unbiodegraded rice straw. The results are in line with the findings of Kurtzman (1976), Zafar *et al* (1981) and Beg *et al* (1986) who observed that biodegradation of cereal straws and rice husk increased their feed values to different extent.

Maximum biodegradation was shown by *P ostreatus* and minimum by *P sapidus*. The dry matter digestibility was enhanced from 37 to 46.8% over the unbiodegraded straw. The conversion into digestible form is further borne out by an analysis of undigested portion of the biodegraded rice straw obtained after termination of cow recticul-orumen digestibility studies.

The values indicated augmentation in the digestibility of cellulose, minerals and proteins to the tune of 49.9 to 61.0%, 20.1 to 29.3%, 31.4 to 43.9%, respectively over the unbiodegraded straw. It is evident that a sufficient portion of the biodegraded materials was ingested and only those components were left which could not be utilized by the microflora present in the rumen. The data showed that of all the three *Pleurotus* spp., *P ostreatus* was comparatively the most efficient biodegrader.

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