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Production of *Flammulina velutipes* on Coffee Husk and Coffee Spent-ground

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ABSTRACT

Solid state cultivation (SSC) was carried out to evaluate the feasibility of using coffee husk and spent-ground as substrates for the production of edible mushroom Flammulina under different conditions of moisture and spawn rate. The strain of F. velutipes LPB 01 was adapted for a coffee husk extract medium. Best results were obtained with 25% spawn rate, though there was not much difference when lower spawn rates (10-20%) were used. Ideal moisture content for mycelial growth was 60% and 55% for coffee husk and spent-ground, respectively. With coffee husk as substrate, first fructification occurred after 25 days of inoculation and the biological efficiency reached about 56% with two flushes after 40 days. With spent-ground as substrate, first fructification occurred 21 days after inoculation and the biological efficiency reached about 78% in 40 days. There was decrease in the caffeine and tannins contents (10.2 and 20.4%, respectively) in coffee husk after 40 days. In coffee spent-ground, the tannin contents decreased by 28% after 40 days. These decrease was attributed to the degradation of caffeine or tannins by the culture because these were not adsorbed in the fungal mycelia. Results showed the feasibility of using coffee husk and coffee spent-ground as substrate without any nutritional supplementation for cultivation of edible fungus in SSC. Spent ground appeared better than coffee husk.

Key words: *Flammulina velutipes*, coffee husk, coffee spent ground, solid state cultivation, fructification, biological efficiency

INTRODUCTION

Flammulina ranks at fourth place in the category of edible mushrooms for production and consumption. During 1990, its production was estimated to be approximately 143,000 tons, which increased to 230,000 tons in 1994, showing a remarkable jump of 61% (Chang 1996). According to Yang (1986) and Wang (1995), it's been first cultivated in China during the 8th century. In 1928, Moriki cultivated it with sawdust and rice bran in Japan (Nakamura, 1981). During the 1960s, its cultivation revolutionized in Japan, which became its largest producer in the world and enjoyed this position till the 1980s. Since the early 90´s, China has occupied the first place in its production. It was estimated that in the Mainland China it was produced about 200,000 tons during 1995 (Meiging, 1997). Production data from different other countries too indicated a faster growth rate in terms of its total production. In the United States, for example, the production of *Flammulina* increased at an estimated rate of 25% or more per year for the last four years (Royse, 1995).

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Production of *Flammulina* is based on synthetic substrate contained in polypropylene bottles or bags. The substrates most utilised are agricultural residues, such as corncobs, cottonseed husk, sugarcane bagasse, etc., besides sawdust (Chang, 1989; Yang, 1986; Fan et al, 1990; Wang, 1995; Royse, 1995).

Coffee husk and spent-ground are the two important agro-industrial residues in the coffee producing countries. According to International Coffee Organisation, there are more than 50 countries producing coffee (ICO, 1998). At different stages from harvesting to the processing and consumption, coffee husk and spent-ground are generated in more than two millions tons quantity yearly (Tango, 1971; Soccol, 1995, Pandey and Soccol, 2000). Brazil is the largest producer of coffee in the world and thus coffee residues too. In Brazil, the coffee cherries are generally processed by the dry method, resulting coffee husk, which is rich in organic nature and nutrients. It contains compounds such as caffeine, tannins, and polyphenols (Fan et al 1999a, 1999b). Coffee spent-ground, the residue, which is obtained during the processing of raw coffee powder to prepare 'instant coffee', is another residue obtained from coffee industry. This also contains caffeine, tannins and polyphenols, although in lesser quantity. Due to the presence of these compounds (caffeine, tannins and polyphenols), these organic solid residues show toxic nature and thus have not been utilised potentially. This has also led the problem of environmental pollution.

With the advent of biotechnology, attempts have increasingly been made globally to make potential use of agro-industrial residues for value addition by production of enzymes, organic acids, bioactive secondary metabolites, single-cell protein, etc. (Pandey et al. 1988, 1999a,b). Solid state fermentation (cultivation) has been often found promising in this regard (Pandey 1992a, 1994, Pandey et al. 2000, Pandey and Soccol 1998, Soccol 1996, Soccol and Krieger 1998). Several attempts have been made to use residues of coffee industries in Brazil for its biological detoxification and production of mushrooms, aroma compounds, etc. (Brand et al. 2000, Fan et al. 2000a,b, Soares et al. 2000). An attempt was made by Thielke (1989) to cultivate *F. velutipes* on coffee spent-ground. Song et al (1993) also reported the cultivation of *F. velutipes* on coffee spent-ground. However, there is no report on application of coffee husk as substrate for the cultivation of F. *velutipes*.

The objective of this work was to use coffee husk and spent-ground for the cultivation of *F. velutipes* in solid culturing, which would primarily provide edible mushroom and simultaneously help in resolving their disposal problem which otherwise poses a serious environmental concern. The work involved adaptation of the strain of *F. velutipes* in coffee husk extract medium and to evaluate mycelial growth at different spawn rates and moisture contents and ability of fructification in the coffee husk and spent-ground as the substrates. The final substrates and fruit body were analysed to determine the contents of caffeine, tannins, protein and fibre in view of finding their possible utilisation after fermentation.

MATERIALS AND METHODS

Micro-organisms and growth medium: A strain of *F. velutipes* LPB 01 was used in the experiment. The strain was routinely maintained on Potato-Dextrose-Agar (PDA) at 4°C. The culture was adapted for a coffee husk extract medium as described earlier for other mushrooms (Fan et al. 2000a,b).

Spawn preparation: The sawdust of *Eucalyptus* sp. (80%) and rice bran (20%) was used for the spawn preparation. The mixture was adjusted at the moisture of 60% (Yang, 1986) and then filled in the glass jar of 500 ml capacity. After autoclaving (121°C, one h), the spawn medium was inoculated with bits (one disc of one cm in diameter) of mycelia of strain growing vigorously in PDA slants and then incubated at 24°C in dark. The spawn in the jars was ready for inoculation to the substrate after 20 days growth when the mixture turned totally white.

Solid state cultivation (SSC): The raw coffee husk and spent-ground (sun dried) were obtained from the local factories. SSC was carried out using substrates filled in plastic bags of 20x35 cm size, by taking100 g substrate in each bag on dry wt basis. These substrates were moistened with water (60%) generally 4-5 h before autoclaving and were autoclaved at 121°C for 1.5 h. When cooled, these were inoculated with the spawn (10%) and mixed thoroughly to facilitate rapid and uniform mycelial

growth. The mouth of bags was sealed using a cotton plug and thread. Then they were incubated in the dark at 24°C. Mycelial development in the bag was observed and noted each day. Three bags were marked for collecting samples (20g) each five days during 25 days for analysis of protein and fibre contents.

Effect of moisture and spawn rate: Substrates were prepared with different moisture such as 45, 50, 55, 60, 65, and 70% for SSC. Similarly, different spawn rates were tested, which included 2, 5, 10, 15, 20, and 25%. After the 20 days fermentation, the protein and fibre contents in the substrate were measured.

Production of fruit body: The substrates were prepared as described above. Moisture and spawn rate were adopted according to the SSC. After 20 days, the jars were transferred to a lighted environmental chamber (90% relative humidity, 20 °C) to allow stimulation of air, humidity and light to facilitate fruiting body development. After the fructification of two flushes, the protein and fibre contents in the residues were measured.

Biological efficiency: Biological efficiency was determined as described previously (Fan et al. 2000a,b).

Analytical methods: The protein contents were determined by Kjeldahl method. The fibre contents were determined by taking 2 g substrate in 200 ml HCl (1.25%) and boiling for 30 minutes. The whole contents were filtered and the solids were again boiled in 200 ml NaOH (1.25%) for 30 minutes. After filtering, the solids were thoroughly washed first with distilled water, then with alcohol and ethyl acetate (20 ml each), respectively and dried at 60°C (AOAC, 1975). The results reported are the average values of triplicate assays. Caffeine was determined using the modified method as described by the IAL (1985), using chloroform as solvent. For this, samples (2-g) were mixed with 15-ml conc. H_2SO_4 in 100-ml glass beaker and heated in a boiling water-bath for 15 min. The mixture was added to 50-ml distilled hot water (boiling) and again heated for 15 min as above. The mixture was filtered using Whatman filter paper and the filtrate was neutralised using NaOH (1N). Caffeine was extracted from the neutral filtrate by treating with chloroform. All the organic fractions were pooled and the concentration of caffeine was determined in the pooled fraction by spectrophotometer (276.5 nm). Tannins were measured according to the method described in the manual by Ministerio de Agricultura (1986). For this, samples (5-g) were mixed with distilled water (200-ml) and heated for 2-h. After filtering, 5-ml sample was mixed with equal amount of Folin-Denis reagent and saturated Na_2CO_3 (10-ml). The volume was made 100 ml by adding distilled water. The concentration of tannins was determined in this by reading the absorbance at 760 nm in a spectrophotometer.

RESULTS AND DISCUSSION

Adaptation of the strain

The strain of *F. velutipes* LPB 01 grew well in coffee husk extract medium, showing 7.87 $mm.day^{-1}$ mycelial growth and 45.8 mg $biomass.plate^{-1}$ in 10 days (data not shown). It indicated that coffee husk could be used as substrate by this fungus.

SSC using coffee husk

Figure 1a shows the content of protein and fibres in the fermenting coffee husk at different periods of time. As is evident, the protein content showed an increasing trend with the increase in cultivation period. The trend with fibre contents was same, though in reverse order, which decreased with the time of cultivation.

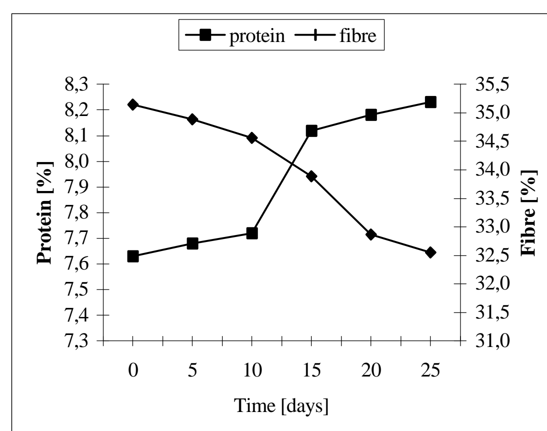

Figure 1a - Changes in protein and fibre contents in coffee husk during 25 days of SSC.

Figure 1b shows the SSC of coffee husk at different moisture levels in the substrate during 25 days of growth. As is apparent, the substrate with

60% moisture resulted in maximum protein and minimum contents of fibres. The mycelial growth in this case was very vigorous (visual observation). At 45% substrate moisture, the growth as evidenced by protein content and visual observation was lowest. When the substrate moisture was 75%, the fermentation was very poor and was almost comparable to that with 45%. Moisture has been termed as a very crucial factor in solid culturing. It is reported that in SSC an optimum level of moisture is crucial a factor as high moisture level results in decreased substrate porosity, which in turn prevents oxygen transfer. At the same time low moisture level leads to poor accessibility of nutrients, resulting poor growth (Pandey 1992a,b).

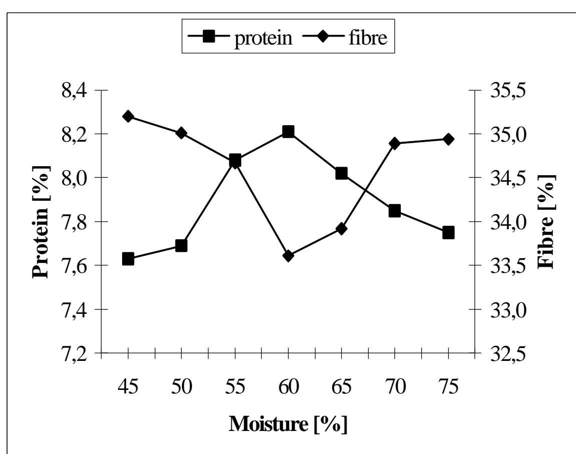

Figure 1b - Effect of moisture on SSC of coffee husk after 20 days of growth.

Figure 1c shows the effect of different spawn rate on protein and fibre contents of coffee husk after 20 days of SSC. With the increase of spawn rate, the mycelial growth was more rapid and active and was maximum with 25% spawn rate. However, there was not much difference in protein contents between 10-25% spawn rate, and the mycelial growth (visual observation) was also not augmenting correspondingly. Hence, a spawn rate of 10% was considered suitable. The spawn rate has also been considered one of the principal factors for edible fungus cultivation in SSC. There has been much variation in spawn rate with different substrate. Rajarathnam and Bano (1987a,b) reported that a spawn rate less than 10% facilitated the contamination and decreased the biological efficiency, and therefore, they recommended higher (20% or more) spawn rate. However, a 2% spawn rate has been recommended by most other authors for mushroom production on different substrates (Yang, 1986; Fan and Ding, 1990 and Wang, 1995).

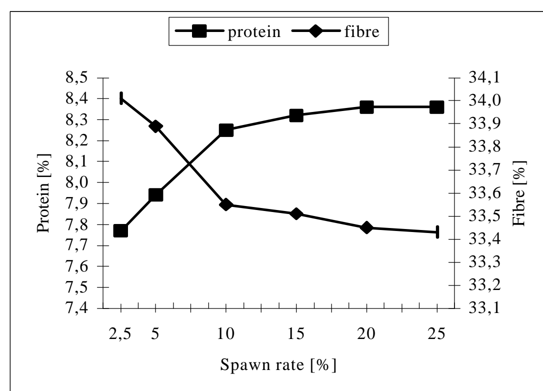

Figure 1c - Effect of spawn rate on SSC of coffee husk after 20 days of growth.

SSC using coffee spent ground

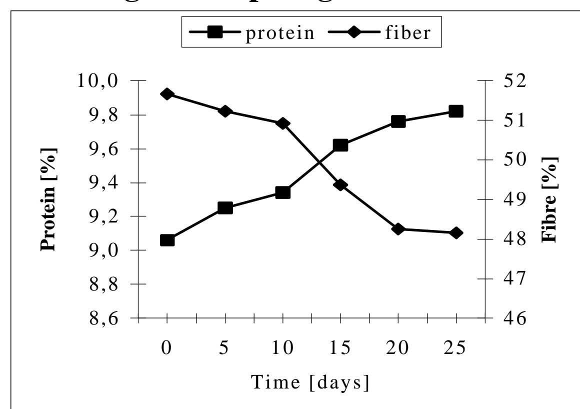

Figure 2a - Changes of protein and fibre contents during SSC of coffee spent ground in 25 days.

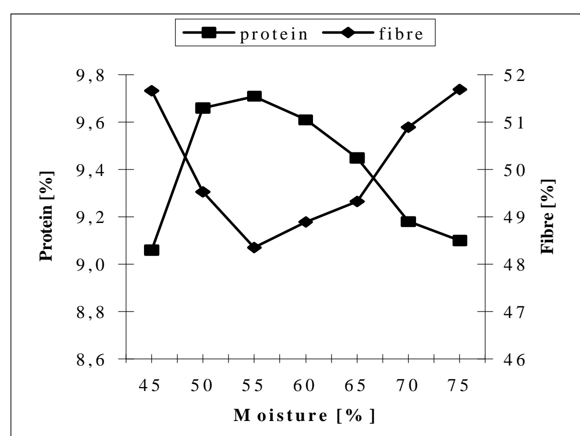

Figure 2b - Effect of moisture on SSC of coffee spent ground after 20 days growth.

Figure 2a shows the SSF using coffee spent-ground as the substrate. It demonstrated that the protein content increased and fibres content decreased with the time of cultivation during 25 days. The ideal moisture for mycelial growth was 55%, which resulted in maximum content of protein and lowest content of fibres in the substrate (Fig. 2b). It indicated that the variation of ideal moisture depended on the substrate. In this case, the 55% moisture was appropriate for SSC.

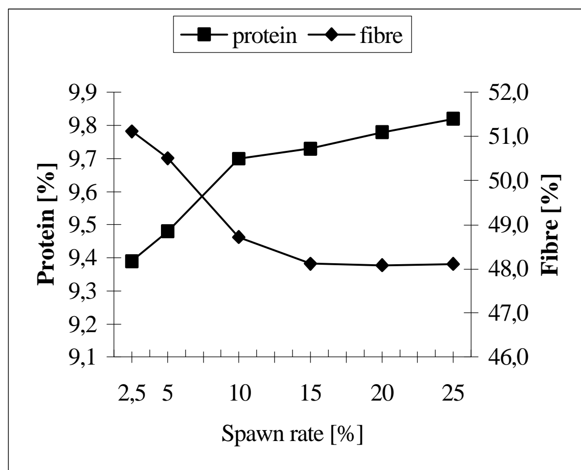

Figure 2c - Effect of spawn rate on SSC of coffee spent ground after 20 days growth.

In case of spawn rate, although 25% spawn rate resulted in highest content of protein and lowest content of fibres in the substrate, there was not much difference in their contents with 10% spawn rate (Fig. 2c). Thus, from economics point of view we recommended 10% spawn rate as appropriate.

Fructification on the coffee husk and spent-ground

When coffee husk was used as the substrate, the primodia appeared after 25 days of inoculation; the biological efficiency reached at about 56% with two flushes in 40 days. There is no report on the production of *Flammulina* using coffee husk. Thus, our findings are very important. With spent ground as substrate, first primodia of fructification occurred 21 days after inoculation and the biological efficiency reached about 78% with two flushes in 40 days. Thielke (1989) who first reported the fructification of *F. velutipes* supplemented the medium with yeast extract while Song et al. (1993) who also obtained the fruit body from spent-ground, supplemented it with corn flour. In the present studies, we did not provide any nutrients or supplemented the medium with any other ingredients.

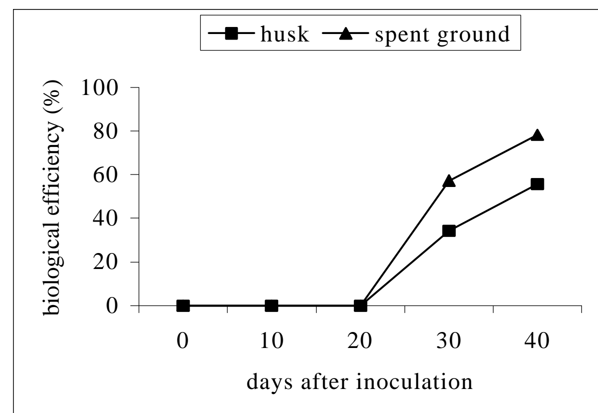

Figure 3 - Biological efficiency of *F. velutipes* LPB 01 on the coffee husk and spent ground.

Figure 3 shows the biological efficiency of *F. velutipes* LPB 01 on coffee husk and spent-ground.

Change of protein and fibre in the substrates before and after fructification

Table 1 shows the initial and final contents of protein and fibres in the substrates. Although the mushroom body containing higher content of protein than the substrate took out majority of protein in the substrate, the content of protein in the substrate increased because of consuming relatively a lot of carbohydrates. The content of fibre increased in the final residue of coffee husk, being 10.70% while it decreased in the coffee spent ground after fructification (–7.25%). The increase rate of protein was between 24.68 and 27.05%. These modifications of protein and fibre contents in the substrates could be attributed to the weight loss during SSC, degradation of ligno-cellulose and liberation of CO_2. It indicated that *Flammulina* has capability to degrading the ligno-cellulosic residues.

Table 1 - The contents of protein and fibre in the substrate before and after fructification of *F. velutipes* LPB 01

Parameters	Coffee husk		Spent-ground	
	protein	Fibre	protein	fibre
Initial	8.14	34.11	8.06	49.24
Final	10.15	37.76	10.24	45.67
Increase or decrease(%)	+24.68	+10.70	+27.05	-7.25

Content of caffeine and tannins in the fruit body, initial and final coffee residues

Table 2 shows the contents of caffeine and tannins in the fruit body of *Flammulina* and coffee residues. The fruit body of *Flammulina* did not contain caffeine and tannins when grown on coffee husk or spent-ground. The contents of caffeine and tannins were decreased at 10.2 and 20.4%, respectively in the fermented husk, which indicated that the fungal strain was able to degrade it partially. In spent-ground there was no caffeine detected after fermentation. This probably was due to its low initial concentration, which could have been degraded completely, but tannins concentration decreased by 28%. There is no literature report about action of *Flammulina* on caffeine and tannins.

Table 2 - Contents of caffeine and tannins in the fruit body and final substrates after fructification of *F. velutipes* LPB 01.

Parameters	Coffee husk (%)		Spent ground (%)	
	caffeine	tannins	caffeine	tannins
Fruit body	0	0	0	0
Initial	0.65	3.65	0.05	0.25
Final	0.58	2.91	0	0.18
Increase or decrease	-10.21	-20.37	--	-28.00

Due to the presence of these anti-physiological and anti-nutritional factors, coffee husk is not considered an adequate material as feed for cattle and other livestock, or substrate for bioconversion processes. Consequently, most of the husk remains unutilised or poorly utilised. If these toxic constituents could be removed, or at least degraded to a reasonably low level, it would open new avenues in their utilisation as feed. It will also improve its value to be used as substrate for bioprocesses (Fan et al. 2000a,b). Attempts have been made to degrade caffeine present in coffee pulp (which is generated by wet-processing of coffee cherries) and use it for the production of enzymes etc (Roussos et al., 1995; Hakil et al., 1998; Hakil et al 2000).

CONCLUSIONS

The studies showed the feasibility of using coffee husk and spent-ground without any nutrients supplementation for cultivation of *F. velutipes* LPB 01 in solid state cultures. Coffee spent-ground could be a more suitable substrate for its cultivation. There is no report on the production of *Flammulina* using coffee husk. Thus, our findings are very important. SSC offers a potential way to utilize these residues economically.

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RESUMO

Cultivo no estado sólido foi utilizado para avaliar as possibilidades de utilizar a casca e a borra de café como substrato para a produção do cogumelo comestível do gênero Flamulina. A cepa de *F. velutipes* LPB foi adaptada em um meio contendo extrato de casca de café. Os melhores resultados em termos de produção do cogumelo foram obtidos com taxas de inoculação de 25%, embora não tenha sido observadas diferenças significativas quando taxas inferiores foram utilizadas (10-20%). O teor de umidade ideal para o crescimento micelial foi de 60% e 55% para a produção com casca e a borra de café.

Utilizando a casca de café como substrato, a primeira frutificação ocorreu após 25 dias de inoculação e a eficiência biológica foi de aproximadamente 56% com duas colheitas após 40 dias. Utilizando-se a borra de café como substrato, a primeira frutificação ocorreu 21 dias após a inoculação e a eficiência biológica alcançada foi de 78% em 40 dias de cultivo. Houve uma redução nos teores de cafeína e taninos da ordem de 10,2 e 20,4%, respectivamente na casca de café após 40 dias. Na borra de café, os índices de taninos foram reduzidos em 28% após 40 dias. Esta redução foi atribuída à degradação da cafeína e taninos pela cultura. Os resultados mostraram a praticabilidade de usar a casca e a borra de café como substrato sem nenhum suplemento nutritivo para o cultivo sólido desse fungo comestível. A borra apresentou melhores resultados do que a casca de café.

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