

SUPPLEMENTATION OF SUGARCANE BAGASSE WITH RICE BRAN AND SUGARCANE MOLASSES FOR SHIITAKE (*LENTINULA EDODES*) SPAWN PRODUCTION

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ABSTRACT

The purpose of this study was to assess the myceliation rate, mycelial vigor and “estimated biomass” of *Lentinula edodes* (Berk.) Pegler, grown on a sugarcane bagasse substrate enriched with rice bran and sugarcane molasses for spawn production. The proportions of rice bran used were 0, 10, 15, 20, 25, 30 and 40% (dry weight/dry weight of bagasse) and the sugarcane molasses concentrations tested were 0, 10, 20, 30, 40, 50 and 60 g/kg (dry weight/dry weight of bagasse plus rice bran). The myceliation rate was decreased by the addition of the higher quantities of rice bran. The 25 and 30% rice bran proportions induced the highest stimulation of mycelial vigor. The addition of sugarcane molasses did not change myceliation rate or mycelial vigor. The “estimated biomass” values were similar when intermediate rice bran proportions were used and for all sugarcane molasses concentrations. Based on response surface obtained for the “estimated biomass” data, higher values were obtained with substrates containing 20 to 25% rice bran combined with 10 to 30 g sugarcane molasses, although the latter supplement was not considered to stimulate *L. edodes* growth.

Key words: *Lentinula edodes*, shiitake, sugarcane bagasse

INTRODUCTION

The production of shiitake, *Lentinula edodes*, on sterilized and enriched artificial substrates has increased over the last few years (2,13). Although sawdust has been traditionally used as the basis for these substrates, shiitake is also produced on supplemented sugarcane bagasse (4,6,15,18) since it can grow on different agricultural residues rich in lignin and cellulose (1).

Shiitake spawn is normally produced on sawdust enriched with rice bran at the 4:1 proportion (25). *L. edodes* grown on this material, when used as spawn for sugarcane bagasse substrates, may present a very slow initial growth due to the adaptation phase (lag phase) to the new substrate, with a consequent impairment of the profitable development of a production system based on this residue. The use of spawn

obtained on substrates of the same nature as that to be used for fungus cultivation may provide a more rapid and vigorous growth of the microorganism.

Supplementation of bagasse with nutrients is necessary since this is the residue obtained after different types of treatments and washes applied to sugarcane. Bagasse is rich in cell wall material with a low cell content and low density, and is therefore poor in proteins and minerals (20).

Molasses is a promising source of sugars and nutrients and is used to improve mycelial development, resulting in good biomass production by different *L. edodes* strains (22,24). Sources of carbon such as lignin, glucose and fructose among others are important energy suppliers for the metabolic activity of the mushrooms and represent the basis for the synthesis of different molecules (26). Another source of nutrients is rice bran,

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traditionally used to supplement sawdust in the culture (7,8,19) and production of shiitake spawn (25), stimulating mycelial growth, mycelial vigor and the biomass of various mushroom species (5,25). In addition, rice bran is normally used to enrich sugarcane bagasse in the culture of both *L. edodes* (4,6,15,18) and *Pleurotus* spp. (10,12).

On this basis, the objective of the present study was to assess the effects of sugarcane bagasse supplementation with different amounts of rice bran and sugarcane molasses on some developmental parameters of *L. edodes* to establish the best substrate composition for the production of spawn.

MATERIALS AND METHODS

We used a culture of isolate JAB-K1 grown on minimal medium (17), from basidiocarps of *Lentinula edodes* (Berk.) Pegler, obtained from growers of the northeast region of the São Paulo State, Brazil. The culture was multiplied in the same medium in order to obtain a sufficient initial amount of spawn.

The experimental design consisted of supplementing sugarcane bagasse with nondefatted rice bran, sugarcane molasses and mixtures of the two supplements. The amount of water necessary to correct the moisture content of the substrate to 50% was calculated considering the amount of natural humidity of each material. Bran was mixed with bagasse before the increase in moisture content and molasses was diluted in the water used for correction of humidity.

The substrates were placed in autoclavable cylindrical glass containers externally measuring 120 mm in height and 62 mm in diameter. The different substrates were distributed into each flask without weight determination and with a similar extent of compaction, and then autoclaved at 121°C for 90 min.

The experimental treatments were organized according to a 7 x 7 factorial scheme [seven levels of rice bran: 0, 10, 15, 20, 25, 30 and 40% (dry weight/bagasse dry weight) and seven levels of molasses: 0, 10, 20, 30, 40, 50 and 60 g/kg (dry weight/dry weight of bagasse plus rice bran)]. The experiment was arranged as a completely randomized design with different number of replicates (3 to 6 flasks per treatment), since any flask presenting the slightest suspicion of contamination was immediately eliminated.

After inoculation, which consisted of transfer of aliquots of mycelium grown in minimal medium (17) to the surface of the substrates, the flasks were placed in an environment at a temperature of 25 to 28°C for mycelial growth during 45 days. Four strips of graph paper were vertically attached to each flask in opposite positions and used for the measurement of mycelial growth towards the bottom of the flask (18). The measurements were recorded in mm under the strips in the region where the flask presented a uniform diameter, which corresponded to approximately 70.0 mm in length (18). The mean mycelial growth rate was estimated on the basis of the ratio between the total distance covered by the mycelium and the time needed for this

growth to occur. Mycelial vigor was arbitrarily scored from 1 to 5 by five different persons as follows: 1, very low mycelial density; 2, low mycelial density; 3, regular density; 4, high density; 5, very high density. The values resulted from the comparison of the various treatments to one another. "Estimated biomass" was calculated as the product of mycelial growth rate by mycelial vigor in each flask.

RESULTS AND DISCUSSION

Myceliation rate

Myceliation rate decreased significantly with increasing proportions of rice bran. The addition of this supplement promoted a gradual decline in the C:N ratio of the substrate (0% of rice bran, C:N = 107:1; 40% of rice bran, C:N = 38:1), suggesting that a large amount of rice bran inhibits the growth of *L. edodes* (Table 1). In agreement with this result Singh and Verma (21) reported that the limiting factor for *Lentinula lateritia* growth was a low C:N ratio (25 and 50:1). According to Kamra and Zadrazil, cited by Maziero (11), high N concentration in the medium repress the lignin degradation by edible mushrooms. Nevertheless, Song *et al.* (23) obtained higher mean growth rates with 30:1 C:N ratio.

The addition of rice bran to bagasse alters the structure of the substrate and therefore its gas exchange ability. Substrates with larger particles present higher O₂ and lower CO₂ levels (3), and higher O₂ and CO₂ levels stimulate and inhibit growth, respectively (2). Leatham (9) showed the importance of O₂ supply during mycelial growth by obtaining higher lignocellulolytic enzyme activities during the first 20 days, a time during which the concentration of this element is higher. Thus, the exchange capacity of the substrate decreases with increasing rice bran amounts due to the reduction of its empty spaces. On this basis, it can be seen that a large amount of rice bran added to bagasse reduces the degrading activity of the fungus and the capacity for gas exchange of the substrate, reducing the mycelial growth rate.

Sugarcane molasses had no effect on the myceliation rate of *L. edodes* (Table 1), a result different of that obtained by Song *et al.* (22) who observed better mycelial growth with 30 g molasses/l solution. A sugar source is required to increase the lignocellulolytic activity of the fungus (9). *L. edodes* removes cellulose and hemicellulose, more than lignin (14). Sugarcane bagasse contains larger quantities of cellulose (44.7%) and hemicellulose (22.9%) than of lignin (14.9%), but apparently degradation of this material by the fungus was not stimulated by the addition of any amount of sugarcane molasses.

Mycelial Vigor

Treatments with 25 and 30% rice bran proportions supported a more vigorous mycelial growth in *L. edodes* (Table 1). Similar results was showed by the fungus grown on sawdust

Table 1. Effect of rice bran and sugarcane molasses levels on some parameters of mycelial development of *Lentinula edodes* grown on sugarcane bagasse substrates.

Rice bran (%)	Assessment of mycelial development		
	Rate (mm/day)	Mycelial vigor ^a	“Estimated biomass” ^b
0	1.51 a ^c	1.44 f ^c	2.16 b ^c
10	1.50 a	2.95 de	4.41 a
15	1.40 b	3.45 bc	4.83 a
20	1.27 c	3.30 cd	4.39 a
25	1.21 cd	3.94 a	4.74 a
30	1.14 d	3.82 ab	4.44 a
40	0.93 e	2.65 e	2.47 b
Molasses (g/kg)			
0	1.25 a	3.12 a	3.90 a
10	1.29 a	3.00 a	3.91 a
20	1.31 a	3.07 a	4.09 a
30	1.29 a	3.16 a	4.08 a
40	1.29 a	3.12 a	3.93 a
50	1.28 a	2.99 a	3.85 a
60	1.24 a	3.08 a	3.70 a

^a Scores attributed as described in the text; ^b Values obtained by multiplying growth rate by vigor; ^c Means followed by the same letter in a column did not differ by the Tukey test at the 5% level of probability.

supplement with 20% rice bran (25). Although, *Lentinus subnudus* presented greater mycelial growth with *Andropogon tectorum* straw enriched with 50% rice bran (5). An important factor to be considered is the capacity for gas exchange of the substrate. If aeration stimulates the rate of hyphal growth, it also does so with their ramifications. Thus, the substrate with the highest bran level would have the lowest possibility of gas exchange with the medium since its internal empty spaces would be reduced. On the other hand, a substrate without rice bran would have good conditions for gas exchange but its vigorous growth would be impaired by the lack of nutrients. This event is probably also due to the fact that the large amount of N present in bran reduces the degradation of lignin present in bagasse [Kamra and Zadrazil, cited by Maziero (11)], impairing the formation of hyphal ramifications (21). Similarly, when N is scarce there is little hyphal accumulation, i.e., there is inhibition of the formation of hyphal ramifications since the fungus must rapidly look for another source of nutrients in the substrate.

The supplement of sugar cane molasses had no effect on the mycelial growth vigor (Table 1) a result different of that reported by Song *et al.* (22). The addition of sugar to the substrate stimulates the lignin degradation (9), but Moyson e Verachttert (14) observed that the fungus uses less lignin and more cellulose and hemicellulose, present in large amounts in

bagasse. So, the enrichment with molasses do not stimulate vigorous development of *L. edodes*.

“Estimated Biomass”

Any amount of rice bran added to sugarcane bagasse increased the “estimated biomass” of *L. edodes*, except for the 40% proportion (Table 1). Similar results was reported by Okeke *et al.* (16), who showed that the biomass was greater when high levels of soluble proteins were present in the medium. The stimulating effect of rice bran is due to the carbohydrates, amino acids and minerals present in the supplement (5). Our results suggest that rice bran improved the nutrient content of the substrate. On the other hand, the reduced “estimated biomass” with higher rice bran proportions was probably associated with the growth-inhibiting effect on *L. edodes* caused by the high nitrogen levels discussed earlier.

Sugarcane molasses did not alter “estimated biomass” (Table 1), in contrast with Song *et al.* (22) and Tan and Moore (24), who observed greater *L. edodes* biomass values in media containing molasses compared to other media.

The results of “estimated biomass” (EB) were used to determine a second order response surface as a function of the proportions of rice bran and of the concentrations of sugarcane molasses according to the following equation: $EB = 1.946256 + 0.268768F + 0.020100M - 0.006191F^2 - 0.000255M^2 - 0.000445F \times M$ ($R^2 = 0.59$; $F = 64.77^{**}$; $P < 0.01$) (Fig. 1). The figure shows that intermediate amounts of rice bran and molasses led to greater “estimated biomass”. These results can be better visualized in

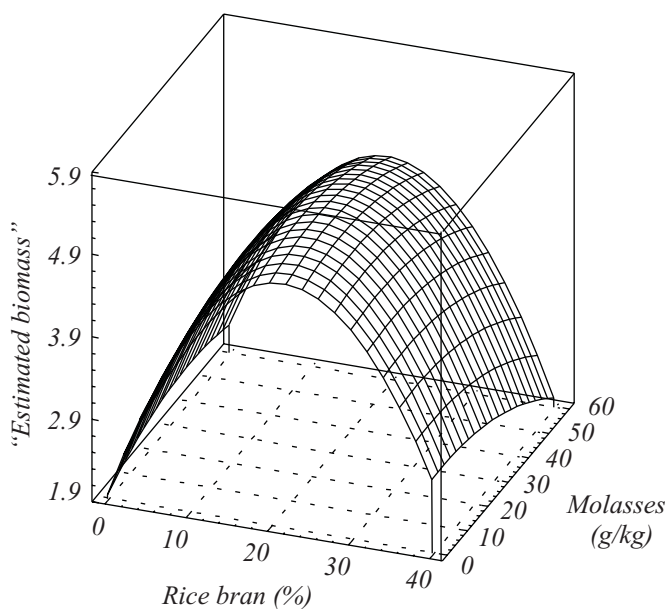


Figure 1. Response surface of “estimated biomass” as a function of rice bran and sugarcane molasses.

the isoline graph in Fig. 2 representing cuts of the same “estimated biomass” on the response surface. It can be seen that the effect was greater when the amount of rice bran was changed and was lower when the molasses concentration was changed. The highest “estimated biomass” values were obtained with 20 to 25% rice bran and with 10 to 30 g sugarcane molasses.

Since “estimated biomass” values represent the product of mean growth rate and mean mycelial vigor, it is clear that these parameters have combined effects leading to a new variable that differs in behavior from the previous ones but represents better the desired objective, i.e., supplement combinations that provide ideal growth rate and vigor. Thus, we may assume that 20 to 25% rice bran proportions can be used for the production of *L. edodes* spawn on sugarcane bagasse and that sugarcane molasses practically has no stimulating effect on the mycelial development of the fungus but, when used at concentrations of 10 to 30 g/kg substrate in combination with rice bran, it stimulates “estimated biomass”.

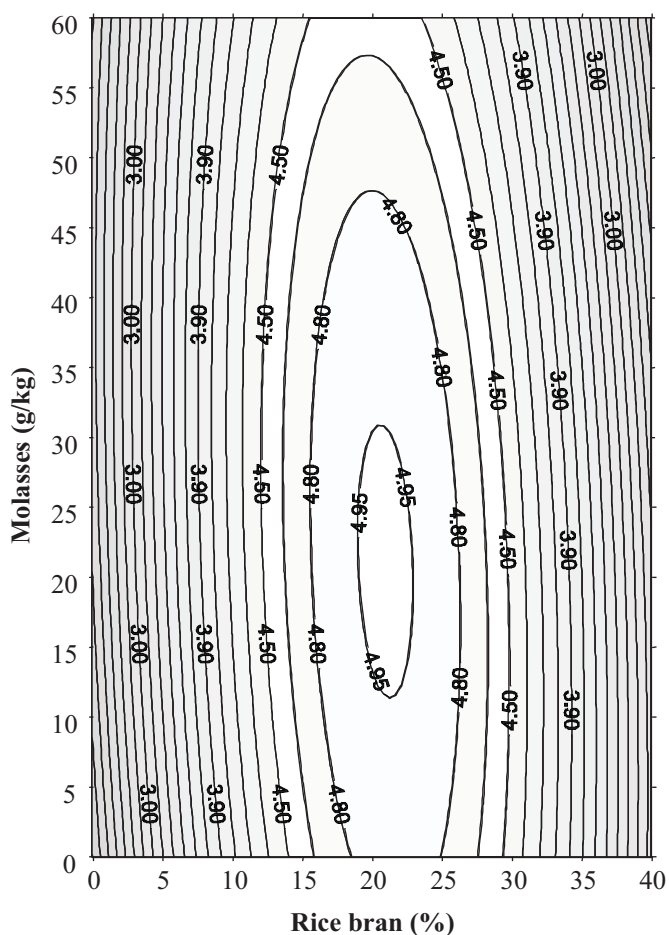


Figure 2. Isoline graph for “estimated biomass” as a function of rice bran and sugarcane molasses.

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RESUMO

Suplementação de bagaço de cana-de-açúcar com farelo de arroz e melaço de cana-de-açúcar para a produção de “semente” de shiitake (*Lentinula edodes*)

O objetivo deste trabalho foi avaliar a velocidade de miceliação, o vigor do micélio e a “biomassa estimada” de *Lentinula edodes* (Berk.) Pegler cultivado em substrato à base de bagaço de cana-de-açúcar, suplementado com farelo de arroz e melaço de cana-de-açúcar, para a produção de “semente” do fungo. As proporções de farelo de arroz usadas foram 0, 10, 15, 20, 25, 30 e 40% (peso seco/peso seco de bagaço) e as concentrações de melaço de cana-de-açúcar empregadas foram 0, 10, 20, 30, 40, 50 e 60g/kg (peso seco/peso seco de bagaço mais farelo de arroz). A velocidade de miceliação diminuiu quando se adicionou as maiores quantidades de farelo de arroz. As suplementações com 25 e 30% de farelo de arroz proporcionaram maior estimulação do vigor micelial. A adição de melaço de cana-de-açúcar não estimulou tanto a velocidade de miceliação como o vigor micelial. Os valores de “biomassa estimada” foram similares quando se usou as proporções intermediárias de farelo de arroz e para todas as concentrações de melaço de cana-de-açúcar. Através da superfície de resposta obtida com os dados da “biomassa estimada” verificou-se que os valores mais altos foram observados quando os substratos continham 20 a 25% de farelo de arroz combinado com 10 a 30g de melaço de cana-de-açúcar, embora o último suplemento não tenha, isoladamente, estimulado o crescimento de *L. edodes*.

Palavras-chave: *Lentinula edodes*, shiitake, bagaço de cana-de-açúcar.

REFERENCES

1. Buswell, J.A.; Cai, Y.J.; Chang, S.T. Lignolytic enzyme production and secretion in edible mushroom fungi. In: Royse, D.J. (ed.). *Mushroom Biology and Mushroom Products*. University Park, Pennsylvania, 1996, p. 113-122.
2. Donoghue, J.D.; Denison, W.C. Shiitake cultivation: gas phase during incubation influences productivity. *Mycologia*, 87: 239-244, 1995.
3. Donoghue, J.D.; Denison, W.C. Commercial production of shiitake (*Lentinula edodes*) using whole-log chip of *Quercus*, *Lithocarpus*, and *Acer*. In: Royse, D.J. (ed.). *Mushroom Biology and Mushroom Products*. University Park, Pennsylvania, 1996, p. 265-275.
4. Eira, A.F.; Minihoni, M.T.A. *Manual Teórico-Prático do Cultivo de Cogumelos Comestíveis*. FEPAF, Botucatu, 1996, 96p.

5. Fasidi, I.O.; Kadiri, M. Use of agricultural wastes for the cultivation of *Lentinus subnudus* (Polyporales: Polyporaceae) in Nigeria. *Rev. Biol. Trop.*, 41: 411-415, 1993.
6. Iizuka, H. Production of *Lentinus edodes* mycelia extracts (LEM). *Food Rev. Int.*, 13: 343-348, 1997.
7. Ikegaya, N. Breeding. *Food Rev. Int.*, 13: 335-343, 1997.
8. Kawai, G.; Kobayashi, H.; Fukushima, Y.; Yamada, S.; Fuse, H.; Ohsaki, K. Continuous manufacturing system of solid culture media packed in film bags for cultivation of shiitake. *Food Rev. Int.*, 13: 349-356, 1997.
9. Leatham, G.F. The lignolytic activities of *Lentinus edodes* and *Phanerochaete chrysosporium*. *App. Microbiol. Biotechnol.*, 24: 51-58, 1986.
10. Macaya-Lizano, A.V. Cultivo de *Pleurotus ostreatus* y especies afines (Fungi: Pleurotacea) sobre medios naturales semi-estériles. *Rev. Biol. Trop.*, 32(2A): 255-260, 1988.
11. Maziero, R. *Substrats alternativos para o cultivo de Pleurotus spp.* São Paulo, 1990, 136p. (M.Sc. Dissertation. Instituto de Biociências. USP).
12. Maziero, R.; Bononi, V.L.; Capelari, M. Cultivo e produtividade de *Pleurotus ostreatus* var. Florida em Mogi das Cruzes, SP, Brasil. *Hoenea*, 10: 1-7, 1992.
13. Miles, P.G.; Chang, S.T. *Mushroom biology: concises basics and current developments*. World Scientific, Singapore, 1997, 194p.
14. Moyson, E.; Verachttert, H. Growth of higher fungi on wheat straw and their impact on the digestibility of the substrate. *App. Microbiol. Biotechnol.*, 36: 421-424, 1991.
15. Oei, P. *Manual on Mushroom Cultivation. Techniques, Species and Opportunities for Commercial Application in Developing Countries*. TOOL Foundation, Amsterdam, 1991, 249p.
16. Okeke, B.C.; Paterson, A.; Smith, J.E.; Watson-Craik, I.A. The relationship between phenol oxidase activity, soluble protein and ergosterol with growth of *Lentinus* species in oak sawdust logs. *App. Microbiol. Biotechnol.*, 41: 28-31, 1994.
17. Pontecorvo, G.; Roper, J.A.; Hemmons, L.M.; McDonald, K.D.; Bufton, A.W.J. The genetics of *Aspergillus nidulans*. *Adv. Genet.*, 5: 141-238, 1953.
18. Rossi, I.H. *Suplementação de bagaço de cana para cultivo axênico do cogumelo shiitake [Lentinula edodes (Berk.) Pegler]*. Jaboticabal, 1999, 120p. (M.Sc. Dissertation. Faculdade de Ciências Agrárias e Veterinárias. UNESP).
19. Royse, D.J. Specialty mushrooms. In: Janick, J. (ed.). *Progress in New Crops*. ASHS Press, Arlington, 1996, p. 464-475.
20. Santos, F.A.P. *O bagaço de cana-de-açúcar tratado sob pressão de vapor como alternativa para a alimentação de bovinos na entressafra das pastagens*. SBZ, Campinas, 1990, 203p.
21. Singh, T.G.; Verma, R.N. Studies on carbon and nitrogen of *Lentinula lateritia* (Berk.) Pegler strains from northeastern India. In: Royse, D.J. (ed.). *Mushroom Biology and Mushroom Products*. University Park, Pennsylvania, 1996, p. 345-354.
22. Song, C.H.; Cho, K.Y.; Nair, N.G. A synthetic medium for the production of submerged cultures of *Lentinus edodes*. *Mycologia*, 79: 866-876, 1987.
23. Song, C.H.; Cho, K.Y.; Nair, N.G.; Vine, J. Growth stimulation and lipid synthesis in *Lentinus edodes*. *Mycologia*, 81: 514-522, 1989.
24. Tan, Y.H.; Moore, D. Convenient and effective methods for *in vitro* cultivation of mycelium and fruiting bodies of *Lentinus edodes*. *Mycol. Res.*, 96: 1077-1084, 1992.
25. Teixeira, E.M. *Efeito da suplementação de serragem de Eucalyptus grandis (Hill ex Maiden), na velocidade e intensidade de colonização do substrato para produção de "Semente" de Lentinula edodes (Berk.) Pegler e sua eficiência na produtividade*. Jaboticabal, 1996, 37p. (M.Sc. Dissertation. Faculdade de Ciências Agrárias e Veterinárias. UNESP).
26. Zanetti, A.L.; Ranal, M.A. Suplementação da cana-de-açúcar com gandu no cultivo de *Pleurotus* sp. 'Florida'. *Pesq. Agrop. Bras.*, 32: 959-964, 1997.