

Full Length Research Paper

Cultivation and bromatological analysis of the medicinal mushroom *Ganoderma lucidum* (Curt.: Fr.) P. Karst cultivated in agricultural waste

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Received 3 July, 2014; Accepted 22 January, 2015

The objective of the study was to evaluate the production of two strains of *Ganoderma lucidum* on agricultural waste and carry out bromatological analyses of the basidiomata obtained from the cultivation. The experiment was carried out at the Mushroom Module at the School of Agronomic Sciences of the São Paulo State University (FCA/UNESP - Botucatu, SP, Brazil) and two strains were used (GLM-09/01 and GLM-10/02) which were cultivated on waste, oat straw, bean straw, brachiaria grass straw, Tifton grass straw and eucalyptus sawdust under two situations: with (20%) and without (0%) supplementation with wheat bran. All the waste was taken from dumps of agricultural activities in Botucatu-SP. Both treatments were carried out in 10 repetitions, totaling 200 packages. The mushrooms cultivation took 90 days. Next, the biological efficiency of the treatments and the bromatological analysis of the basidiomata were evaluated. The biological efficiency (BE) values (%) varied from 0.0 to 6.7%. In the mushroom bromatological analyses, the results ranged from 8.7 to 13.7%, from 2.0 to 6.7%, from 0.83 to 1.79% and from 38.8 to 54.5%, for total protein, ethereal extract, ash and crude fiber, respectively. Thus, we conclude that the substrates which presented the greater yield were the brachiaria straw, 20% in both strains tested (GLM-09/01 and GLM-10/02) and the bean straw, 20% in the strain GLM-10/02. The mushrooms showed high levels of ethereal extract, fibers and ashes and a low level of proteins.

Key words: *Ganoderma lucidum*, bromatological analyses, mushroom.

INTRODUCTION

The mushroom consumption is a millennial habit in many cultures. Such interest is explained by the fact that these

macrofungi present several medicinal and nutritional characteristics. These beings were even considered as

deities by some civilizations, and were often used in religious ceremonies.

Nowadays, they have a guaranteed market in many places around the world, not only because of their importance in medicine, but also because they are obligatory elements in various cuisines, mainly in the Italian and French.

Among the most appreciated mushrooms in the world, *Lentinula edodes* (shiitake), *Pleurotus ostretatus* (shimeji, oyster mushroom, hiratake) and *Agaricus blazei* (Paris champignon) stand out mainly because of their nutritional characteristics and pleasant taste.

Among the mushrooms with a pharmacological value, the *Ganoderma lucidum*, (known as *orelha-de-pau*; *Reishi* by the Japanese and *Ling Zhi* by the Chinese) raised much interest on such potential, as they are reported mainly by their medicinal power among their numerous properties, and they can also be used for preventing and treating various diseases, including cancer and AIDS (Russell and Paterson, 2006).

This species presents many pharmacologically active compounds, such as: triterpenoids, steroids, polysaccharides, proteins, alkaloids, nucleosides and nucleotides (Boh et al., 2007). They occur in different types of colors, such as Chinese red, bright yellow and white. The fruiting body is initially white; it becomes yellow with its ripening and acquires a brownish varnished aspect in its adult phase (Perumal et al., 2009).

At first, the mushrooms were extracted mainly from forests. Afterwards, they began to be cultivated in artificial environments. Several ways were employed to carry out such activity, such as the traditional cultivation (substrate sterilization through composting) and the axenic cultivation (sterilization at a temperature of 121°), which is considered the most common in many yields, as it is faster and more practical.

The substrates used in the cultivation are usually formulated with straw and sawdust, which allows the use of many agricultural and agroindustrial raw materials and are considered of low or none aggregate value. This practice is also an income option for growers who generate a great amount of waste, as it represents an efficient alternative to enable the use of organic material for bioconversion into products with a high added value: mushrooms. In addition, this raw material is rich in lignocellulosic fibers, which makes it a favorable substrate for the development of edible fungi.

Considering the great abundance of these residues generated by various segments (agricultural, agroindustrial, forest and wood) and the possibility of using them as

substrate to cultivate edible fungi, we must also consider the several fungi species present in nature and their affinity with the residues. The *G. lucidum* species presents many distinct strains and also nutritional requirements which vary regarding the place where it is collected and the type of substrate. This study aimed to evaluate the production of two strains of *G. lucidum* on agricultural waste and carry out bromatological analyses of the initial and residual substrates and of the basidiomata obtained in cultivation.

MATERIALS AND METHODS

The experiment was carried out at the Mushroom Module facilities at FCA/UNESP-Botucatu, SP, Department of Plant Production, Plant Defense, in a Dalsem Mushroom climatic chamber ("Reefer" container type with dimensions of 12 m in length X 2.25 in height and 2 m in width).

Obtainment of strains

The strains of *G. lucidum* used in the experiment were GLM-09/01 and GLM-10/02, which are preserved in mineral oil at the Mushroom Module Bank matrix, + Department of Plant Production, Plant Defense - FCA/UNESP, Botucatu.

Residues used

The residues used in the experiment were: oat straw, bean straw, brachiaria grass straw, Tifton grass straw and eucalyptus sawdust. The eucalyptus sawdust was considered as testimony, once it constitutes a residue the mushrooms have affinity with, according to many studies observed in literature. All the waste was taken from dumps of agricultural activities from the city of Botucatu-SP.

Residues processing

The residues of oat, bean, brachiaria grass and tifton grass straws were dried at environmental temperature and later stored in raffia bags until their use. The same procedure was applied to the eucalyptus sawdust.

Inoculum "Spawn" preparation

Sorghum grains, which were used to prepare the inoculums, were cooked for 40 min in boiling water. Afterwards, they were left resting for 40 min to drain the excess of water. Next, 20 g Kg⁻¹ of calcitic limestone and 160 g Kg⁻¹ of plaster were added, considering the weight of the cooked grains when wet. The homogenization was carried out in a construction mixer (capacity of 420 L).

After that, 250 g of substrate were transferred to the HDP bags. Later, the secondary matrix of the two strains of *G. lucidum* (GLM-

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Table 1. Treatments used to evaluate the yield of *Ganoderma lucidum* in substrates based on agricultural waste and supplemented with wheat bran (proportion based on dry matter).

Strain	Substrates
GLM-10/02 and GLM-09/01	Tyfton straw 0% of wheat bran
	Tyfton straw 20% of wheat bran
	Brachiaria straw 0% of wheat bran
	Brachiaria straw 20% of wheat bran
	Oat straw 0% of wheat bran
	Oat straw 20% of wheat bran
	Bean straw 0% of wheat bran
	Bean straw 20% of wheat bran
	Eucalyptus sawdust 0% of wheat bran
	Eucalyptus sawdust 20% of wheat bran

10/02 and GLM- 09/01) grown in petri dishes containing malt agar medium were divided into 8 triangular fragments with the same size. Each package received a fragment of inoculum and was sealed using heat sealer and incubated at 25°C until they were totally colonized by the fungi.

Substrate preparation

The experiment was entirely randomized in a 2 × 10 factorial scheme, corresponding to two strains and 10 types of substrates. Both treatments were carried out in 10 repetitions, totaling 200 packages.

The substrates were prepared according to their formulations (Table 1). The residue was inserted into a construction mixer with the CaCO₃ and the supplement of wheat bran. Next, water was added until it reached 65% of humidity. The mixture was homogenized until acquiring uniformity.

Afterwards, 1 kg of the mixture was added to high-density polyethylene (HDP) bags. The properly identified packages were sealed in heat sealer and sterilized at a temperature of 121°C for 180 min.

Inoculation

After the sterilization period, the packages were taken to an inoculation room, where they were maintained under environmental temperature and inoculated with their referred strains, according to the treatments. For so, side cuts were made in each bag with a pair of scissors sterilized with alcohol; the scissors were also sterilized in the flame of a Bunsen burner at every cut. 12 g of inoculum seed previously prepared was inserted in each substrate through these openings. The packages were sealed in heat sealer, identified and distributed in a room which was adapted for incubation with adjusted temperature of 25°C and humidity of 75%, until the full colonization of the substrate.

Thirty (30) days after incubation, the substrates had been completely colonized by the fungi and were taken to the Dalsen Mushroom climatic chamber, where they were kept until the end of the cultivation cycle, by keeping the same temperature and humidity used in the previous incubation.

The primordia began to appear after 85 days of incubation and the first harvests were initiated ten days later. The harvest was

carried out manually in the stage prior to sporulation by removing all the basidiomata.

Basidiomata processing

The mushrooms collected were put in aluminum foil and dehydrated in a greenhouse with forced ventilation and temperature adjusted to 40°C until they reached a constant weight. Afterwards, the samples were stored in high-density polyethylene (HDP) bags, properly identified according to each treatment. The grinding was carried out in a knife mill with a 30-mesh sieve. Next, all samples were packed in properly sealed plastic bags and identified until their use in the later analyses.

Variables analyzed

Biological efficiency (BE)

The yield was expressed by biological efficiency (BE), which represents the percentage of conversion of the substrate into fungi biomass (mushrooms).

$$BE (\%) = \frac{\text{Total dry mass of mushrooms (g)}}{\text{Dry mass of the initial substrate (g)}} \times 100$$

Chemical and bromatological characterization

The ground samples of the substrates (initial and residual) and mushrooms were taken to the Bromatology Laboratory of the Faculdade de Medicina Veterinária e Zootecnia - FMVZ/UNESP, Botucatu, SP, Brazil, to be analyzed for crude protein, ash, ethereal extract and crude fiber, according to the methodology recommended by Silva and Queiroz (2002). The conversion factor of 4.38 (Furlani and Godoy, 2007) was used to determine the proteins level in the mushrooms.

Statistical analysis

The experiment data were submitted to the ANOVA variance

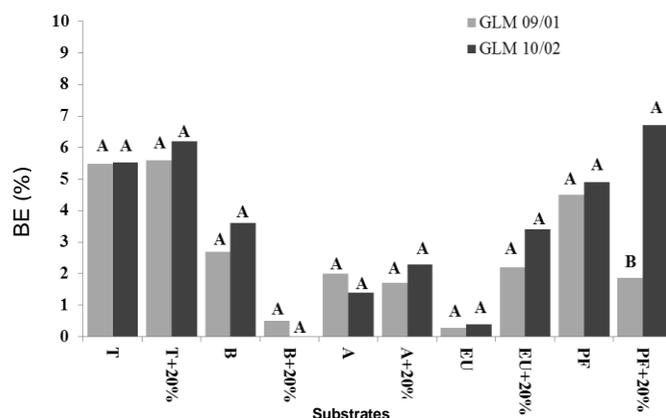


Figure 1. Biological Efficiency (EB), % of the strains GLM-09/01 and GLM-10/02 of *Ganoderma lucidum*, cultivated on substrates based on agroindustrial residues. T, Tifton straw without supplementation; T + 20%, Tyfton straw supplemented with 20% of wheat bran; B, Brachiaria straw without supplementation; B + 20%, Brachiaria straw supplemented with 20% of wheat bran; A, oat straw without supplementation; A + 20%, oat straw supplemented with 20% of wheat bran; EU, eucalyptus sawdust without supplementation; EU + 20%, eucalyptus sawdust supplemented with 20% of wheat bran; PF, bean straw without supplementation; PF+ 20%, bean straw supplemented with 20% of wheat bran. The means followed by the same letters in each treatment do not differ from each other (Tukey, 5%) (mean of two repetitions). CV (%), 58.89.

analysis using the SISVAR 4.2 statistics software, which was developed by the Departamento de Ciências Exatas at the Universidade Federal de Lavras, Minas Gerais, Brazil (UFLA). The means were compared by the Tukey's test at 5% probability.

RESULTS AND DISCUSSION

The biological efficiency is a parameter used to measure the yield of a substrate in the cultivation of mushrooms. After 90 days of cultivation, it was possible to estimate these values expressed as percentages. According to the data obtained in the production of two strains of *G. lucidum*, it is possible to observe that the values of BE (%) varied from 0.0 to 6.7%, and the best results were obtained in the substrates T + 0% (BE - 5.5%) in the two strains, T + 20% in both strains (GLM-09/01 - BE 5.6% and GLM-10/02 - BE 6.2%), and BS + 20% (Figure 1), only for the strain GLM-10/02 - (BE - 6.7%).

Similar results to the highest values obtained in this experiment were found by González-Matute et al. (2002), who reported a biological efficiency of 7.6% using sunflower seed supplemented with 2.5% of malt extract as substrate for the cultivation of *G. lucidum*. In the same experiment, using other substrates [sunflower seed (100%); sunflower seed + 5% of malt; sunflower seed + 2.5% of wheat bran and sunflower seed + 5% of malt] the authors obtained higher values of BE (8.8, 9.9, 8.6 and 10%,

respectively). Gurung et al. (2012) also found similar values to the experiment (BE 7.81%) using *Aulnus nepalensis* sawdust without any supplementation in the cultivation.

Higher percentages than the referred experiment were observed by Erkel (2009) when he used poplar sawdust (a European tree used in the paper industry) supplemented with gluten and sugar cane molasses in the proportions of 1, 2 and 3% as substrate in the cultivation of *G. lucidum*. Higher results were obtained and the most satisfactory were verified in the treatment sawdust + sugar cane molasses 1% (BE 20.3%), followed by the treatment sawdust + gluten 1% (BE 19%). The treatments with the highest supplementation levels (2 and 3%) obtained lower values of BE (%), showing that the ideal supplementation was 1% for both supplements. For Aysun and Gokcen (2009), who used substrates based on sawdust supplemented with residues of green tea in the proportions of 75:25, 80:20, 85:15 and 90:10, the highest results were obtained in the proportions 80:20, (BE 34.90%) and 75:25 (BE 31%).

Triratana et al. (1991) obtained a biological efficiency of 17% when using rubber tree sawdust (*Hevea brasiliensis*) supplemented with rice bran in the cultivation of *G. lucidum*. Similar data were observed by Veena and Pandey (2006), who used sawdust supplemented with rice bran in the proportion of 9:1 obtaining indices of BE equivalent to 20%.

Rolim et al. (2014) obtained higher results than the others (BE - 72%) by cultivating *G. lucidum* in substrate based on elephant grass + mango tree sawdust, supplemented with 10% of wheat bran and 10% of crushed sugar cane. Percentages close to the lowest result obtained in the experiment were reached by Gurung et al. (2012), who cultivated this experiment fungus in substrate based on Sal sawdust (*Shorea robusta*) supplemented with 10% of wheat bran and Sal sawdust + 10% of rice bran, and obtained BE of 0.0 and 0.81%, respectively. The authors also obtained 0% of BE when they used mango tree sawdust supplemented with 20% of wheat bran as substrate for the cultivation.

The biological efficiency obtained in the experiment was not satisfactory regarding most data observed in literature. Such fact might probably be associated with the nitrogen sources present in the substrate (Table 2). According to Hsieh and Yang (2004), the species *G. lucidum* requires a 70:1 to 80:1 C/N ratio for a satisfactory growth and the average C/N ratio in this experiment was of 53:1.

The substrates based on eucalyptus sawdust without supplementation and brachiaria straw with 20% of supplementation obtained a lower BE value (%) (Figure 1). It was possible to verify by means of the analyses carried out in the initial substrate (Table 2) that such substrate presented a lower concentration of nitrogen and a broader C/N ratio (121:1 and 35:1, respectively),

Table 2. Centesimal composition, pH and C:N ratio of initial substrates (raw material supplemented with wheat bran).

Treatment	C (%)	N (%)	C:N	pH
T	17.52	0.50	35/1	6.5
T+20%	19.93	0.60	34/1	5.8
B	19.22	0.47	41/1	7.1
B+20%	20.50	0.59	35/1	6.3
A	18.64	0.30	63/1	6.8
A+20%	19.49	0.42	48/1	5.7
EU	21.77	0.18	121/1	3.5
EU+20%	19.83	0.35	58/1	4.4
PF	20.10	0.47	43/1	6.9
PF+20%	20.32	0.44	47/1	6.9

T, Tifton straw without supplementation; T + 20%, Tyfton straw supplemented with 20% of wheat bran; B, Brachiaria straw without supplementation; B + 20%, Brachiaria straw supplemented with 20% of wheat bran; A, oat straw without supplementation; A + 20%, oat straw supplemented with 20% of wheat bran; EU, eucalyptus sawdust without supplementation; EU + 20%, eucalyptus sawdust supplemented with 20% of wheat bran; PF, bean straw without supplementation; PF+ 20%, bean straw supplemented with 20% of wheat bran.

Table 3. Bromatological values of *G. lucidum* cultivated on agricultural waste.

Substrate	Bromatological parameters							
	Protein (%)		Ethereal extract (%)		Crude fiber (%)		Ash (%)	
	Strain							
	GLM 09/01	GLM 10/02	GLM 09/01	GLM 10/02	GLM 09/01	GLM 10/02	GLM 09/01	GLM 10/02
T	12.5A*	12.4 A	4.8A	2.9B	48.2A	48.4A	1.6A	1.4A
T+20%	15.2A	13.8B	3.5B	4.9A	51.7A	51.4A	1.8A	1.7A
B	11.6A	12.2A	4.7A	4.5A	48.8A	50.2A	1.3A	1.6A
B+20%	13.8A	13.6A	6.7A	6.7A	48.8A	48.1A	1.8A	1.7A
A	9.9A	9.3A	5.2A	5.4A	56.2A	50.7B	1.0A	0.8A
A+20%	10.6B	12.5A	4.8A	4.8A	57.7A	47.8B	1.3A	1.5A
EU	9.6A	8.7B	5.7A	5.8A	41.2A	38.8B	1.1A	0.8A
EU+20%	11.9A	11.2B	3.1A	2.0B	50.7A	51.2A	1.5A	1.6A
PF	12.3A	11.6B	4.5A	4.9A	57.3A	51.3B	1.4A	1.4A
PF+20%	14.8A	12.5B	4.8A	2.7B	50.1B	54.3A	1.8A	1.2B

*Averages followed by equal letters in each line and among strains, within each bromatological parameter, are not statistically different among each other (Tukey, 5%). T - Tifton straw without supplementation; T + 20% - Tyfton straw supplemented with 20% of wheat bran; B - Brachiaria straw without supplementation; B + 20% - Brachiaria straw supplemented with 20% of wheat bran; A - oat straw without supplementation; A + 20% - oat straw supplemented with 20% of wheat bran; EU - eucalyptus sawdust without supplementation; EU + 20% - eucalyptus sawdust supplemented with 20% of wheat bran; PF - bean straw without supplementation; PF + 20% - bean straw supplemented with 20% of wheat bran.

far from the one recommended for the cultivation of *G. lucidum*.

It was also observed that the substrates which provided a lower biological efficiency (EU and EU + 20%) were the ones that presented pH of 3.5 and 4.4 respectively, considered inappropriate for the cultivation of *G. lucidum*

as it requires 5.0 to 6.9 for its development (Nawawi and Ho, 1990; Gurung et al., 2012; Kamara and Bhatt, 2013).

The nutritional values of the basidiomata of the two species of *G. lucidum* (GLM-09/01 and GLM-10/02) cultivated in substrates based on agricultural waste can be verified in Table 3. In the comparison of the total protein

levels present in the basidiomata of strains GLM-09/01 and GLM-10/02, the obtained values varied from 8.7 (EU) to 13.7% (T+20%) for the strain GLM-10/02 and from 9.5 (EU) to 15.1% (T+20%) for the strain GLM-09/01.

Significant differences between the two strains were not observed in the treatments T, B, O and B + 20%. In the treatments T + 20%, EU, EU + 20%, BS, BS + 20%, the basidiomata of the strain GLM-09/01 presented the highest levels of total proteins, whereas the basidiomata of the strain GLM-10/02 obtained the highest percentages of total proteins in the treatment O + 20%. Higher results were obtained by Rawat et al. (2012), who obtained 20.6% of total proteins in the basidiomata of *G. lucidum* collected in the Himalaya forest.

By comparing the highest protein value obtained with the nitrogen values of the substrates used, we observed that the residues which provided mushrooms rich in proteins (T + 20%) presented the highest N levels (0.60%). According to Silva et al. (2002), the level of crude protein of the basidiomata is influenced by the level of nitrogen present in the initial substrate. Those authors cultivated *P. sajor caju* on substrates supplemented with different concentrations of nitrogen and noticed that the greater the concentration, the greater the amounts of proteins present in the mushrooms. Substrates with higher N levels were not colonized by the fungus. The values of ethereal extract for the basidiomata of the strain GLM-09/01 varied from 2.0 (EU + 20%) to 6.75% (B + 20%).

By analyzing the strains separately in relation to 09/01, the treatments T, EU + 20%, BS + 20% WB presented a higher level of ethereal extract. Regarding the strain GLM-10/02, the highest values were obtained from the treatment T + 20%. Lower results than the ones obtained in this experiment (1.7%) were observed in *G. lucidum* samples sold in markets in Vietnam (Hung and Nhi, 2012). Ogbe and Obeka (2013) also obtained lower results (1.5%) in *G. lucidum* collected in Nigerian forests. Aremu et al. (2009) carried out the bromatological analyses of the fruiting body of *G. lucidum* also collected in Nigerian forests, but found results similar to those of this experiment (6.9%).

The crude fiber values present in the basidiomata of *G. lucidum* varied from 38.8 (EU) to 54.5% (BS+20%) in the strain GLM-10/02 and from 41% (EU) to 57.7% (O+20%) in the GLM-09/01. When analyzing each strain separately in relation to the GLM-09/01, the highest concentrations of fibers were obtained in the basidiomata provided from the treatments O (56.2%), O + 20% (57.7%), BS (57.3%) and EU (41.2%). BS+20% was the treatment which provided a higher result (54.5%) for strain GLM-10/02.

All the results observed in literature were lower than the ones obtained in this experiment, such as Ogbe and Obeka (2013), who analyzed mushrooms *G. lucidum* collected in the campus of the University of Nassarawa-Nigeria and found 7.7% of crude fiber present in the

basidiomata. However, Aremu et al (2009), who also collected *G. lucidum* in Nigerian forests, obtained 6.9% of fibers in the analyses. Nagaraj et al. (2013) obtained 14.4% of fibers in mushrooms *G. lucidum* found in Indian forests.

Considering the ash levels present in the basidiomata of the strains GLM-09/01 and GLM-10/02 in each specific substrate in comparison with GLM-09/01, we observed that the greater concentrations of ash were originated from treatments BS + 20% WB (1.8%). The two strains did not present significant differences among each other in the other treatments. The lowest values in both strains were verified in the treatments O and EU.

The values oscillated from 0.83 (O) to 1.79% (T + 20%) for the strain GLM-10/02 and from 1.0% (O) to 1.8% (B+20%) for the strain GLM-09/01. The ash values corresponded to about 5 to 12% of dry matter of the basidiomata and it estimates the amount of micro and macro elements present (Kalac, 2009).

Similar results were observed by Hung and Nhi (2012) in *G. lucidum* mushrooms sold in Vietnamese markets (1.4%). Higher values than those of the experiment were observed by Singh et al. (2014) analyzing basidiomata of *G. lucidum* (8.3%) and *G. philippii* (7.6%) found in Indian forests. Higher results were also obtained by Ogbe and Obeka (2013) and Aramu et al. (2009) in *G. lucidum* mushrooms collected in Nigerian forests, who found ash values of 8.4 and 7.8%, respectively. Higher results to those obtained in the experiment were also observed by Rangunathan and Swaminathan (2003), by cultivating *P. citrinopileatus* on residues of coconut husk and corn cob (6.10 to 6.30%) and Sales-Campos (2008), by using wood and agroindustrial residues in the cultivation of *P. ostreatus*.

Conclusions

Out of the substrates analyzed, the ones based on 20% of brachiaria straw presented the greater yield in both strains tested (GLM-09/01 and GLM-10/02) and the substrate based on 20% of bean straw presented the greater yield in the strain GLM-10/02. The mushrooms showed high levels of ethereal extract, fibers and ash and a low level of proteins. The analyzed residues are within the standard observed in the literature, regarding both the yield and the bromatological analyses of the basidiomata obtained.

Conflict of interests

The authors have not declared any conflict of interest.

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