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Viability of the use of grass in the cultivation of the medicinal mushroom *Ganoderma lucidum*

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The objective of this work was to evaluate the use of ten types of grasses as base substrate for the cultivation of strain GLM-10/02 of *Ganoderma lucidum*, considering the chemical characterization of the substrates, the biological efficiency (BE), the quantification of fresh and dry basidiomata and the number of basidiomata as evaluation criteria. Ten treatments were outlined, made up with napier or elephant grass (*Pennisetum purpureum*), marandu grass (*Brachiaria brizantha* cv. Marandu), aruana grass (*Panicum maximum* cv. Aruana), massai grass (*Panicum maximum* cv. Massai), mombaça grass (*Panicum maximum* cv. Mombaça), brachiaria grass (*Brachiaria decumbens* cv. Basilisk), humidícola (*Brachiaria humidicola* cv. humidicola), xaraés grass (*B. brizantha* cv. Xaraés), tifton (*Cynodon* species cv. Tifton 85), piatã grass (*B. brizantha* cv. BRS Piatã) and a control treatment based on eucalyptus sawdust. All the treatments had 80% of grass, 18% of wheat bran and 2% of limestone, with humidity adjusted to 60%. The treatments based on *B. brizantha* cv. Aruana (aruana) and *Cynodon* spp. cv. Tifton 85 (tifton) showed the best results, with 22.9 and 25% of BE, respectively. These data showed that not all grasses used in the experiment have the same fungal biomass conversion and it was concluded that *B. brizantha* cv. Aruana (aruana) and *Cynodon* spp. cv. tifton 85 (tifton) were the most indicated grasses for the cultivation of *G. lucidum*.

Key words: Productivity, biological efficiency, substrates, fungi.

INTRODUCTION

The appropriate selection of the substrate is very important for the success of every kind of mushroom production. Agro-industrial residues, such as grasses, coffee pulp, cereals bran, crushed sugar cane, processed fruit peels, potato, cereals flour, cassava and others, are widely used substrates in these processes (Alquati et al., 2016; Carvalho et al., 2015; Roy et al., 2015; Erkel, 2009). The material with potential for sale in the agricultural production corresponds to 5%, while the remaining has great potential for biotransformation and

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Table 1. Experimental treatments tested in this experiment.

Treatment	Type of straws (popular name)
1	*Pennisetum purpureum* (napier or elephant grass)
2	*Brachiaria brizantha* cv. Marandu (marandu)
3	*Brachiaria brizantha* cv. Aruana (aruana)
4	*Panicum maximum* cv. Massai (massai)
5	*Panicum maximum* cv. Mombaça (mombaça)
6	*Brachiaria Decumbens* cv. Basilisk (brachiaria)
7	*Brachiaria humidicola* cv. Humidícola (humidícola)
8	*Brachiaria brizantha* cv. Xaraés (xaraés)
9	*Cynodon* spp. cv. tifton 85 (tifton 85 or Bermuda grass)
10	*Brachiaria brizantha* cv. Piatã (piatã)
11 (Control)	*Eucallyptus* spp. (eucalyptus sawdust)

All the substrates were added with 18% of wheat bran and 2% of limestone (dry weight). Humidity was adjusted to 60%.

bioconversion into organic matter that can be applied to the soil and serve as animal food, or even used for the production of fungi and bacterial protein biomass.

The *Ganoderma* species fungi are called Reish by the Chinese and Ling Zi by the Japanese. In face of their innumerous properties, they are mainly known for their medicinal power (Lin, 2009).

In China, their cultivation was originally carried out in logs and sawdust from tree species (*Pinus* species, *Eucalyptus* species and others). This type of substrate has gradually been replaced by several grass species, for ecological reasons and preservation of native species (Lin, 2009). Moreover, the conversion of sun energy in grass species is 6 to 8 times higher than in the tree species used in conventional cultivation. Fungi grown on grasses have higher productivity than those grown in sawdust, possibly being 30% higher. Also, the nutritional quality of the mushrooms is equal or higher (Zhanxi and Zhanhua, 2001).

Although, the production of mushrooms in Brazil is continuously expanding due to the findings of their medicinal and cooking properties; it still needs the development of a suitable cultivation technology to match the conditions of the country. For many years, the technology used was adapted from developed countries which have different weather conditions and raw materials (Dias, 2010).

The *Ganoderma lucidum* mushroom is classified as lignocellulosic because it develops naturally in substrates rich in lignin and cellulose (Erkel, 2009). Consequently, it has an affinity with a wide variety of residues, including grass species. However, *G. lucidum* is still little grown in Brazil and the most used substrate is based on eucalyptus sawdust.

The grass species grown in pastures are potential high-quality substrates for mushroom cultivation. Limited data and reference texts are available about the use of grass species for the cultivation of *G. lucidum*. It is necessary to compare the response of several mushroom strains in several types of straws in terms of productivity and quality of the mushrooms obtained. Weather conditions and the level of the cultivation technology should also be part of these studies.

The replacement of traditional wood based substrates (logs and sawdust) by grasses, cereal straws and other agricultural residues might dispense the nitrogen supplementation and reduce the production cost. An investigation made by Zhanxi and Zhanhua (2001) revealed that some grass species can replace sawdust and partially rice bran in the cultivation of *Pleurotus ostreatus*. Dias et al. (2003) reported that the bean husk for the cultivation of *Pleurotus sajor-caju* dispenses nitrogen supplementation. In addition to the high productivity and easy adaptation in Brazil, the protein, nitrogen, fat, phosphorus, potassium and magnesium contents in grasses are higher than those in sawdust (Zhanxi and Zhanhua, 2001).

The aforementioned advantages of the *G. lucidum* cultivation have increasingly attracted interest by investors and rural producers as an extra alternative to the family income, as well as an environmentally friendly option in the use of residues. Thus, the objective of the present research was to evaluate the viability of the use of different types of grass species in the cultivation of the medicinal mushroom *G. lucidum*.

MATERIALS AND METHODS

The experiment was conducted in the Mushrooms Module, at the School of Agronomic Sciences, Universidade Estadual Paulista (UNESP), in the city of Botucatu, state of São Paulo.

The spawn of strain GLM-10/02 of *G. lucidum* was obtained from the Fungi Herbarium of the Mushrooms Module, FCA/UNESP and was wrapped in High Density Polyethylene (HDPE) packs containing the substrates previously prepared (Table 1) and sterilized in an autoclave at a temperature of 121°C for 4 h.

After being sterilized and under room temperature, the packages were taken to a laboratory with a laminar flow chamber, in order to inoculate the Spawn of *G. lucidum*. Then, they were stored under the controlled temperature of 25°C for two weeks, until they reached the ideal point (total colonization of the grains – spawn) to be inoculated in the treatments with the grass species.

The second step consists of obtaining the grass for the production of the substrates. The grass species were obtained from the experimental beds of the Department of Animal Improvement and Nutrition of the School of Veterinary Medicine and Animal Science (FMVZ) of UNESP with the use of a grass cutting machine.

After being cut, the grass species were organized and identified with their respective names in common raffia bags and stored in an open sides and covered environment (shed) to prevent rainwater from hindering the drying process, which took two weeks. Later, the packages were taken to a masonry stove set to a temperature of 40°C, allowing an homogeneous drying.

Right after drying, the straws were ground with a conventional grinder to reach the particle size required for the development of the fungus. The ground material was weighed with the following proportions to be wrapped in HDPE packs before passing through the sterilization process. The formulations of the experimental treatments (composition of the substrates) followed the same procedure for all treatments, made up with (dry weight) 80% of grass, 2% of limestone and 18% of wheat bran. Water was added until substrate was moistened to 60%.

The experimental design was totally randomized, in factorial scheme, corresponding to the 10 types of cultivation substrates based on different grass species and a control (eucalyptus sawdust) (Table 1), with 6 repetitions each (700 g substrate block), totaling 66 experimental units.

Next, the material was homogenized in a concrete-mixer used in the building industry, pressed and had PVC pipe cork and cotton placed on the top of the package to allow gas exchange and the development of *G. lucidum*. All packages were submitted to the sterilization process at 121°C for 4 h so that only the desired fungus was developed in the production, thus preventing the contamination of the packages.

The inoculation of the packages with the strain GLM-10/02 of *G. lucidum* was carried out after they were cooled to room temperature in the laboratory, by using a laminar air flow chamber, under appropriate aseptic conditions, thus avoiding contamination by other microorganisms.

After this procedure, they were taken to a climatized room and were kept at the temperature of 25°C until the complete colonization of the substrates, which lasted for approximately two weeks. Once colonized, the packages were moved to a rustic greenhouse, made with a bamboo structure and covered with transparent plastic, installed on a farm named Estância Saad (Saad Ranch), in the city of Botucatu, São Paulo. The packages were placed in the greenhouse and randomly arranged. At this time, the cotton plugs were removed.

The average temperature in the greenhouse was maintained at 25 ± 5°C and the relative humidity at 60 to 85%, with irrigation whenever the ground floor was dry. After 30 days, the mushrooms began to develop.

After 60 days, the color of the mushrooms was observed by their different tones and texture so as to quantify their maturation and development as parameters in the harvest decision. The harvest procedure was quite simple, by just twisting and pulling them from the packages. All the mushrooms harvested were weighed with a precision scale and placed in plastic containers identified according to the treatment.

In this step, the number of basidiomata present in each treatment was quantified, as well as the mass of the fresh basidiomata. The productivity of each treatment was expressed by means of the biological efficiency (BE) (Das and Mukherjee, 2007; Tisdale et al., 2006) and is described according to Equation 1:

BE (%) = Total fresh mass of mushrooms (g) / Dry mass of the initial substrate (g) × 100 (1)

Chemical characterization of the substrates

The collection of the samples of the cultivation substrates was made soon after the substrate sterilization process (initial substrate) and the end of the cultivation cycle (exhausted substrate). Two samples of the different types of substrates were separated and sent to the laboratory for chemical analysis of fertilizers and correctives at the Department of Natural Resources, Soil Science, FCA/UNESP for their chemical characterization (N, organic matter, C, C/N, humidity and pH), according to Lanarv's methodology (1988).

Statistical analysis

The data obtained were submitted to variance analysis and averages were compared by the Tukey's test (5%) (Snedecor and Cochran, 1972) using the SISVAR 4.2 software developed by the Department of Exact Sciences of the Federal University of Lavras (UFLA).

RESULTS AND DISCUSSION

The use of grass species for the preparation of substrates in the cultivation of *G. lucidum* is a promising alternative, especially in our country, which has a large amount of species and cultivars that can be used as a raw material in the formulation of substrates. Vieira (2012) highlights that grass species have a solar energy conversion factor 6 to 8 times higher than the tree species, which are frequently used as sawdust for the conventional cultivation of mushrooms. According to Zhanxi and Zhanhua (2001), the fungi cultivated in grass species have a higher productivity; possibly being 30% higher than in the substrates produced using sawdust composts.

The results of the chemical analysis of substrates were evaluated in the experiments, such as nitrogen, organic matter, carbon, C/N ratio, humidity, pH prior to the inoculation with *G. lucidum* (initial substrate) and at the end of the cultivation cycle (final or exhausted substrate). The mass of fresh basidiomata (MFB), the number of basidiomata (BN) and their BE were also evaluated.

Chemical analysis

In order to reach a favorable C/N ratio for the production of mushrooms, the supplementation of these substrates with a nitrogen source has been commonly adopted, especially wheat bran. Philippoussis et al. (2007) reported that the carbon and nitrogen content of the substrate influences the precocity of “fruiting” and productivity. Boyle (1998) reports that the degradation of lignin is also important for the growth, since it can make the access to nitrogen contained in the wood components

Table 2. Chemical analysis before the inoculation of *Ganoderma lucidum* (initial substrate).

Treatment	N%	MO%	C%	C/N	Humidity (%)	pH
Pennisetum purpureum (napier or elephant grass)	0.4	32	18	45/1	56	5.7
Brachiaria brizantha cv. Marandu (marandu)	0.5	32	18	36/1	57	6
Brachiaria brizantha cv. Aruana (aruana)	0.7	34	19	27/1	55	5.7
Panicum maximum cv. Massai (massai)	0.7	33	18	26/1	56	5.9
Panicum maximum cv. Mombaça (mombaça)	0.6	35	19	32/1	54	6
Brachiaria Decumbens cv. Basilisk (brachiaria)	0.6	35	19	32/1	57	6.3
Brachiaria humidicola cv. Humidícola (humidícola)	0.7	40	22	31/1	50	6
Brachiaria brizantha cv. Xaraés (xaraés)	0.6	35	19	32/1	56	5.8
Cynodon spp. cv. tifton 85 (tifton 85 or bermuda grass)	0.6	35	19	32/1	56	5.9
Brachiaria brizantha cv. Piatã (piatã)	0.5	36	20	40/1	55	5.8
Eucallyptus spp. (eucalyptus sawdust)	0.3	35	19	63/1	60	6.4

Table 3. Chemical analysis of the final substrate (exhausted).

Treatment	N%	MO%	C%	C/N	Humidity (%)	pH
Pennisetum purpureum (napier or elephant grass)	0.5	30	17	34/1	57	4.6
Brachiaria brizantha cv. Marandu (marandu)	0.6	34	19	32/1	57	6.5
Brachiaria brizantha cv. Aruana (aruana)	0.5	32	18	36/1	57	7.5
Panicum maximum cv. Massai (massai)	0.7	26	14	20/1	62	5.2
Panicum maximum cv. Mombaça (mombaça)	0.6	30	17	28/1	61	5.0
Brachiaria Decumbens cv. Basilisk (brachiaria)	0.6	27	15	25/1	64	5.6
Brachiaria humidicola cv. Humidícola (humidícola)	0.8	36	20	25/1	56	5.6
Brachiaria brizantha cv. Xaraés (xaraés)	0.3	17	9	30/1	78	6.8
Cynodon spp. cv. tifton 85 (tifton 85 or bermuda grass)	0.7	28	16	23/1	64	5.4
Brachiaria brizantha cv. Piatã (piatã)	0.4	25	14	35/1	63	5.9

available for fungi. According to Hsieh and Yang (2004), the *G. lucidum* species requires an optimal C/N ratio of 70/1 to 80/1 for an efficient growth and low production cost. For Gurung et al. (2012), this fungus species requires an optimal pH of 5.0 to 7.0.

The average contents obtained by the chemical analysis of the initial and final substrates are described in Table 2.

An important and relevant characteristic in the C/N ratio occurred in the treatment using eucalyptus sawdust (63/1). Our findings are confirmed by Hsieh and Yang (2004), who reported that *G. lucidum* species requires an optimal C/N ratio of 70/1 to 80/1 for an efficient growth and low production cost.

Treatment with *Brachiaria brizantha* cv. Piatã (piatã grass) suffered the action of other contaminant fungi during the production process and had to be discarded to avoid the contamination of the other treatments. Therefore, the analysis of the exhausted compound could not be performed.

In relation to the C/N ratio of the exhausted compound (Table 3), it was observed that some treatments suffered degradation by the fungus during the production cycle. This is due to the metabolism (mycelial growth) of the fungus, which is considered lignocellulosic (Urben, 2011).

Biological efficiency

The biological efficiency of each treatment represents the conversion of the substrate into biomass for the mushroom development. This index is the most used by researchers because it makes the comparison of the results with the literature easier (Das and Mukherjee, 2007; Tisdale et al., 2006).

Figure 1 presents the percentage of biological efficiency of each treatment. The substrates containing *B. brizantha* cv. Aruana (aruana grass), with 22.9% of BE and *Cynodon* species cv. tifton 85 (tifton 85), with 25%, presented better statistical results in relation to the other substrates, including the traditionally used control with eucalyptus sawdust, with biological efficiency of 4.8%.

Rolin 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

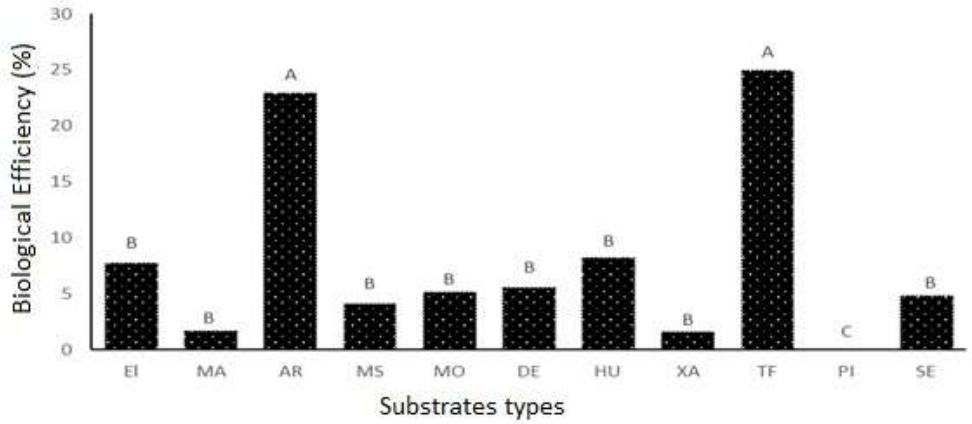

Figure 1. Biological efficiency of strain GLM-10/02 of *Ganoderma lucidum*, cultivated on substrates based on grass species. Averages followed by the same letters do not differ from each other, (Tukey, 5%). CV (%) 67.20. Averages of 6 repetitions. EL: Elephant Grass (napier); MA: Marandu; AR: Aruana; MS: Massai; MO: Mombaça (Colonião); DE: Decumbens; HU: Humidícola; XA: Xaraés; TF: Tifton; PI: Piatã; SE: Eucalyptus Sawdust. All treatments were added with 18% of wheat bran and 2% of limestone. The humidity was adjusted to 60%.

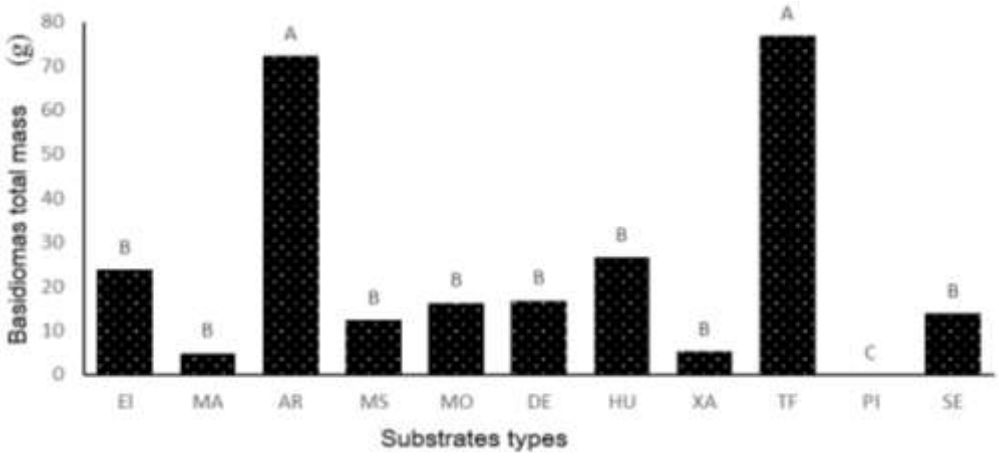

Figure 2. Total mass of the fresh basidiomata from the strain GLM-10/02 of *Ganoderma lucidum*, cultivated in substrates based on grass species. Averages followed by the same letters do not differ from each other, (Tukey =, 5%). CV (%) 69.07. Averages of 6 repetitions. Subtitle: EL: Elephant grass (napier); MA: Marandu; AR: Aruana; MS: Massai; MO: Mombaça; DE: Decumbens; HU: Humidícola; XA: Xaraés; TF: Tifton; PI: Piatã; SE: Eucalyptus sawdust. All treatments were added with 18% of wheat bran and 2% of limestone. The humidity was adjusted to 60%.

crushed sugar cane.

The piatã grass obtained BE of 0% because all the treatments were contaminated.

Total mass of the fresh basidiomata

The total mass of the fresh basidiomata from each treatment was obtained by means of the harvest carried out during the experiment, in a total of 90 days of production (Figure 2).

The total mass varied from 76.9 g (Tifton 85) to 72.2 g (Aruana), showing that both types of substrates have a great lignocellulosic potential for the conversion of fungal biomass for the mushroom formation.

Number of basidiomata

The number of basidiomata is the total mushrooms produced by each treatment during the experiment. The sum of the basidiomata corresponds to the harvest of the *G. lucidum* production (Figure 3).

The substrates that provided the highest amounts of

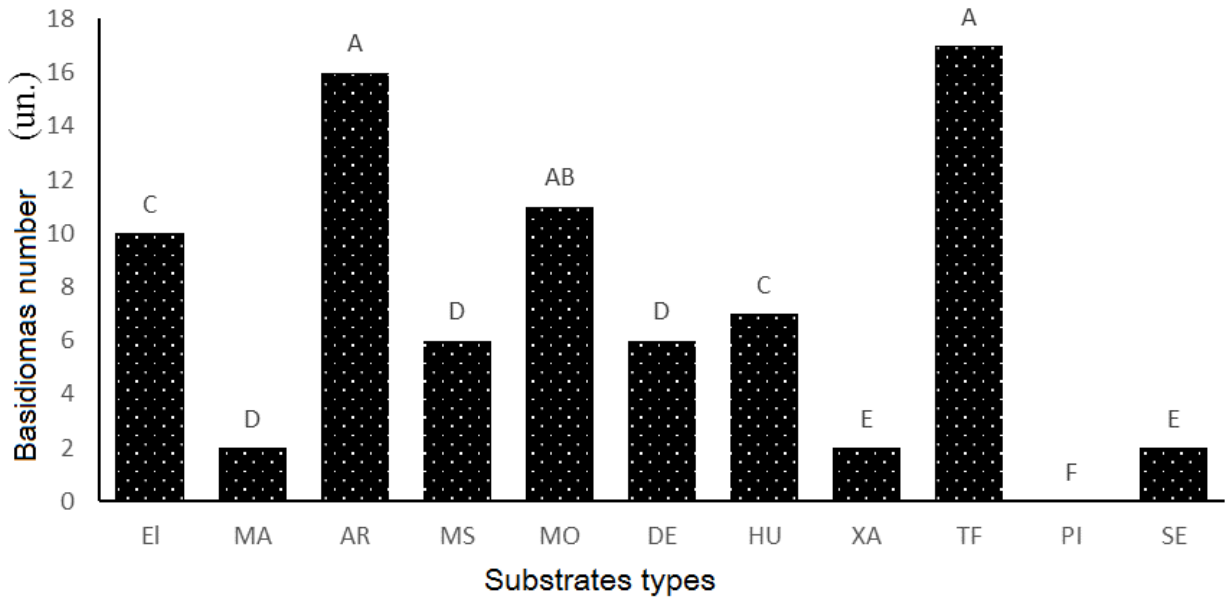

Figure 3. Total number of Basiodiomata from the strain GLM-10/02 of *Ganoderma lucidum*, cultivated in substrates based on grass species. Averages followed by the same letters do not differ from each other, (Tukey =, 5%). CV (%) 58.10. Average of 6 repetitions. EL: Elephant grass (napier); MA: Marandu; AR: Aruana; MS: Massai; MO: Mombaça; DE: Decumbens; HU: Humidícola; XA: Xaraés; TF: Tifton; PI: Piatã; SE: Eucalyptus sawdust. All treatments were added with 18% of wheat bran and 2% of limestone. The humidity was adjusted to 60%.

basidiomata were the treatments based on Aruana and Tifton 85 grass species, with averages of 16 to 17 units per treatment, showing that the choice of the appropriate substrate is fundamental for the mushroom formation and its biological efficiency. The substrate based on piatã grass was contaminated by another fungus and had to be discarded.

Conclusion

The *B. brizantha* cv. Aruana (aruana) and the *Cynodon* spp. cv. Tifton 85 (tifton 85) grass species were the most suitable for the cultivation of *G. lucidum*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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