

## Application of Chinese Jun-Cao Technique for the Production of Brazilian *Ganoderma lucidum* Strains

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### ABSTRACT

*Ganoderma lucidum* is a medicinal mushroom traditionally used in China against a wide range of diseases such as cancer and also for its prevention. In this work, commercial Chinese strains *G. lucidum* were compared to wild Brazilian strains aiming to determine the cultivation potential through the use of Jun-Cao. Six formulations were tested and the strains presented good response to the applied method. In general, the mixture between the grass and wood was well suited for the basidiomycetes, contributing to the preparation of substrates that generated better results in Jun Cao.

**Key words:** mushrooms, *Ganoderma lucidum*, Jun-Cao technique

### INTRODUCTION

*Ganoderma lucidum* (Fr.) Karst (Ganodermataceae), a medicinal mushroom named “Lingzhi” in China, is widely used there as a traditional medicine. The basidiocarp is popularly used against a wide number of diseases such as cancer, hepatic problems, cardio pathologies, inflammations, hypertension, some neurological problems, and others (Petrova et al. 2005; Yuen and Gohel 2005; Xie et al. 2006; Boh et al. 2007; Roupas et al. 2012; Zhou et al. 2012; Zong et al. 2012). *Ganoderma lucidum* is a very rare mushroom in nature and the collect of its wild samples is not being enough to supply for the commercial exploitation. Studies have been carried out in order to balance the demand with an increasing international market (Heleno et al. 2012; Liu et al. 2012; Pan et al. 2012; Manavalan et al. 2013).

The cultivation methods of this fungus were developed both for solid substrate, using wood or fodder, and for liquid cultures, which is significant only for the free mycelial growth. The concentration of the active principles of *G. lucidum*, which give it the status of “the immortality mushroom” vary according to the strain, and also depend on the place of origin and substrate where the mushroom is grown (Liang et al. 2011; Xu et al. 2011; Greenlee 2012). Among the various cultivation methods that have been developed, the Chinese Jun-Cao technique (Jun = mushroom; Cao = grass) has been considered a significant one. Developed in the 1980’s by the Chinese researchers, this technique represented a revolution in the mushroom production system, increasing the amount of biomass produced and reducing the fructification time (Zhanxi and Zhanhua 2001). The main characteristic in the Jun-Cao technique is the substitution of wood, or

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fodder by the grass and agricultural residues. The nutritive quality of the mushrooms cultivated through the Jun-Cao overcame the nutritional value of mushrooms cultivated through the classical methods (Urben 2004).

As with other mushrooms of commercial interest, the cultivation of *G. lucidum* through Jun-Cao could be an alternative for the producers willing to make recyclable cultivations for which the prime matter may be obtained from the materials that are easily found, such as grass, sugar cane bagasse, plaster, sugar, etc. Besides that, after mushrooms are harvested, the substrate may be used as feed for the animals or fertilizer (Urben 2004). In this context, the Jun-Cao technique represents an important tool in which new and unexploited strains may have their physiological potential evaluated. This work aimed to compare the development of Brazilian and Chinese *G. lucidum* strains, evaluating the best substrate and the best abiotic conditions for its cultivation to determine which strain could present the best response to the different growing conditions.

## MATERIALS AND METHODS

### *Ganoderma lucidum* Strains

Four *G. lucidum* strains were used: two Brazilian strains (Loguercio-Leite et al. 2005), identified as CC-144 and CC-157, and two Chinese strains (CC-22 and CC-63) (Table 1).

**Table 1** - Origin of *Ganoderma lucidum* strains used this study.

Strain	Place	Date of Collection
CC-22	China	19/feb/2004
CC-63	China	10/jan/2005
CC-144	Brazil	10/feb/2005
CC-157	Brazil	25/jan/2006

**Legend:** CC= Cenargen Collection

### Cultivation of Strains

The cultures were seeded on Petri dishes containing PDA medium (Potato-Dextrose-Agar) and incubated at 28 to 30°C until mycelia covered the agar surface in 7 to 10 days. In this period, the development of strains was evaluated for different conditions of temperature (20 to 35°C), pH (4.0 to 8.0) and luminosity (light and dark). Then strains were transferred to high-density polypropylene bags containing 300g of sterilized sorghum grains (*Sorghum bicolor*). Each bag was kept at dark at

28°C and relative humidity (RH) 80% for 15 days, until full colonization of strains occurred. Six different substrates formulations were used:

**Formulation 1:** Elephant-grass (*Pennisetum purpureum*) 70%, wheat bran 17%, rice bran 10%, agricultural plaster 2% and dark brown sugar 1%.

**Formulation 2:** Elephant-grass (*Pennisetum purpureum*) 25%, sugar cane bagasse 53%, wheat bran 20% and agricultural plaster 2%.

**Formulation 3:** Elephant-grass (*Pennisetum purpureum*) 73%, wheat bran 15%, rice bran 10%, agricultural plaster 1% and dark brown sugar 1%.

**Formulation 4:** Elephant-grass (*Pennisetum purpureum*) 77.6%, wheat bran 20%, agricultural lime 1%, calcium superphosphate 1%, dark brown sugar 0.2% and urea 0.2%.

**Formulation 5:** Elephant-grass (*Pennisetum purpureum*) 39%, sawdust of mango tree (*Mangifera indica*) 39%, wheat bran 10%, sugar cane bagasse 10% and agricultural plaster 2%.

**Formulation 6:** Sawdust of mango tree (*Mangifera indica*) 78% wheat bran 20% and agricultural plaster 2%.

The grass and/or organic residue were ground with the use of a grass grinder. Then the respective inputs were added to the grinder of each formulation. The substrate was moistened and put into polypropylene bags, resistant to high temperature. Each bag was filled with 700 g of substrate. The substrates in the bags were autoclaved at 121°C for 1 h, and after cooling to room temperature, they were inoculated with the sorghum grains with fungal mycelium and incubated in dark at 28°C, RH 80%, until it was completely colonized. After that, the substrates were taken to a greenhouse where they were buried on sterilized sand. The sand was moistened in between and the development periods were defined. Five repetitions were made, and for statistical analyses the Repeated Measures ANOVA (Tukey's Test with  $\alpha=0.05$ ) using Sisvar 5.3 for Windows was used.

## RESULTS

### Abiotic Conditions

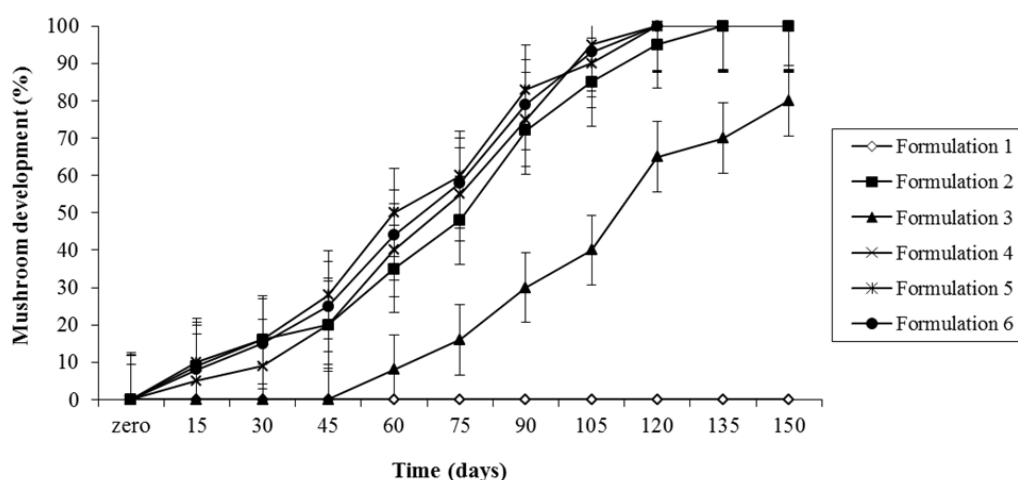
Variation in pH did not affect the strains developed on dishes containing PDA. The absence of light, however, was important for the fast mycelial growth. The best temperature was 28°C, just as proposed by Urben (2004). At temperatures

below 20°C and above 35°C, the growth of fungus was compromised. The commercial Chinese strain CC-22 and the Brazilian strain CC-144 presented the best development, with fast growth and a wide spectrum of tolerance to different temperatures. The strains CC-63 and CC-157 had less tolerance to temperature variations, necessitating this to be near 28°C.

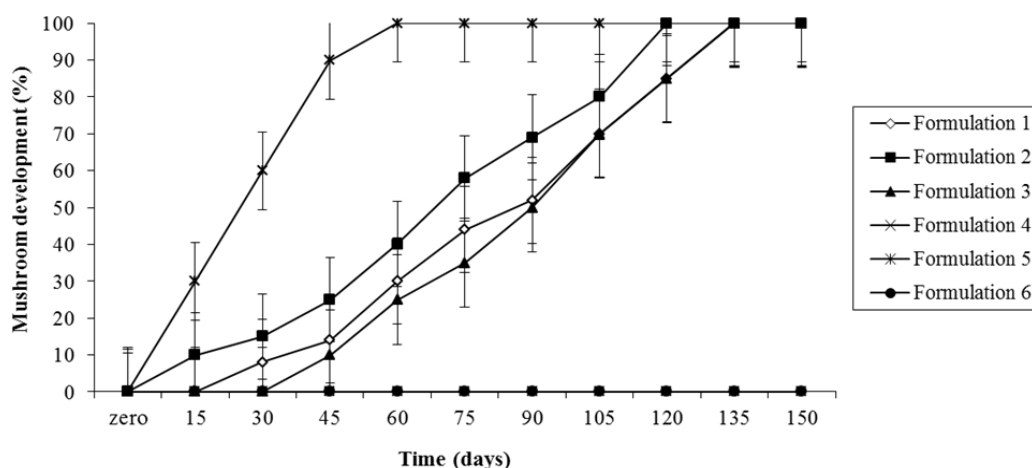
### Cultivation

During the development on sorghum seeds, the growth was faster for CC-22 and CC-144, while

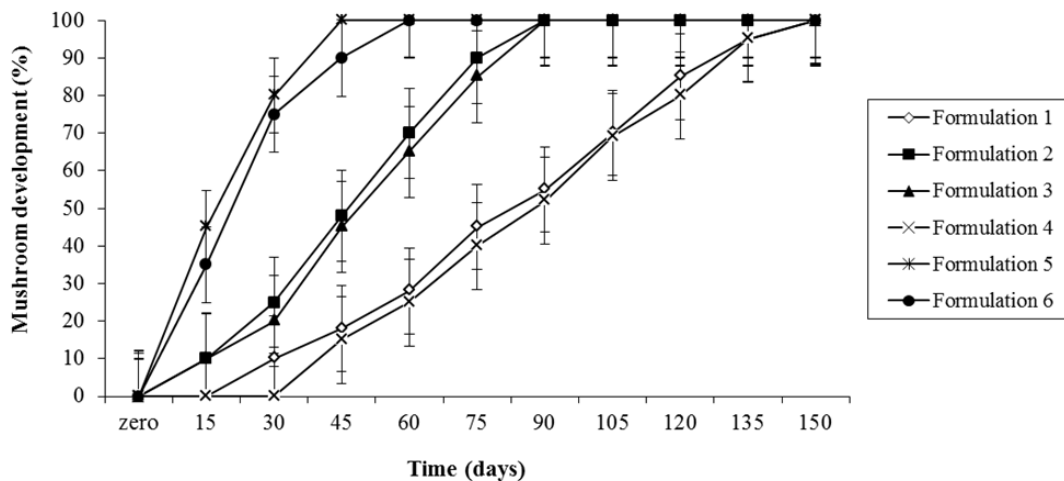
CC-63 and CC-157 presented slower growth. On the different substrates used, the strain CC-22 had its fructifications well developed on the formulations 2, 3, 4, 5 and 6, with no result on formulation 1. The strain CC-63 showed growth on substrates 1, 2 and 5, with late growth on formulation 3 and no growth on formulations 4 and 6. The strain CC-144 had good response to all the formulations, the formulations 5 and 6 showed better development. The strain CC-157 produced mushrooms only on substrates 5 and 6 (Figs. 1 to 4).



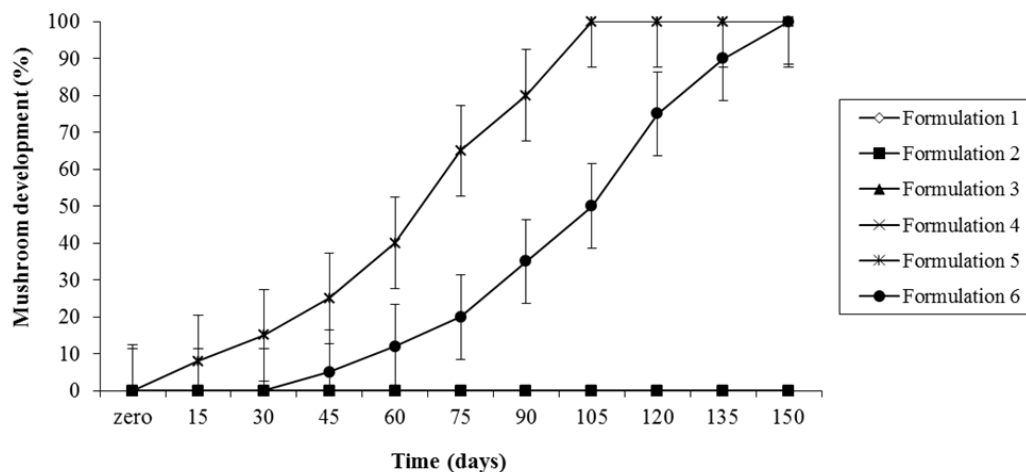
**Figure 1** - Percentage of development of the reproductive phase (mushroom) of *Ganoderma lucidum* (Strain CC-22) on different substrate formulations, during 150 days, in which 100% of development represents the best moment for harvesting.



**Figure 2** - Percentage of development of the reproductive phase (mushroom) of *Ganoderma lucidum* (Strain CC-63) on different substrate formulations, during 150 days, in which 100% of development represents the best moment for harvesting.



**Figure 3** - Percentage of development of the reproductive phase (mushroom) of *Ganoderma lucidum* (Strain CC-144) on different substrate formulations, during 150 days, in which 100% of development represents the best moment for harvesting.



**Figure 4** - Percentage of development of the reproductive phase (mushroom) of *Ganoderma lucidum* (Strain CC-157) on different substrate formulations, during 150 days, in which 100% of development represents the best moment for harvesting.

The Brazilian strain CC-144 was the one, which produced the fastest fructifications. The Chinese strains CC-22 and CC-63 were less efficient, while the Brazilian CC-157 presented the lowest results. The statistical analysis carried out through Repeated Measures ANOVA with Tukey's Test showed that there was significant difference among the strains on the period of development. On the average yield among the tested strains, CC-144 had highest gains than the other ones,

followed by the Chinese strain CC-22. The strain CC-157 had low growth, while CC-63 presented intermediate response. For CC-22, there was no significant difference between the results obtained in the formulations 3 and 4, or between the formulations 2 and 6. The treatment which presented the best result was the formulation 5, showing significance different from the others (Table 2).

The biological efficiency was calculated through the formula  $BE = (\text{fresh weight of mushrooms}) \times 100 / (\text{dry weigh of substrate})$ . For the CC-22 strain, best biological efficiency was observed in formulations 2, 5 and 6, especially the 5. The same occurred with the strains CC-144 and CC-157. The

CC-63 had best responses between treatments 2, 4 and 5. Statistical analysis showed significant difference between the formulation 5 and the others. All statistical comparisons have been shown in Table 3.

**Table 2** - Average weight obtained by *Ganoderma lucidum* strains for the 6 different formulations. Results measured in g/kg of substrate.

Strains	Formulations					
	F1	F2	F3	F4	F5	F6
CC-22	0 (a1)	180 (a3)	98 (a2)	108 (a2)	320 (a4)	200 (a3)
CC-63	60 (a2)	130 (a4)	90 (a2, a3)	110 (a3, a4)	204 (a5)	0 (a1)
CC-144	102 (a1)	200 (a2, a3)	115 (a1, a2)	150 (a1, a2, a3)	360 (a4)	212 (a3)
CC-157	0 (a1)	0 (a1)	0 (a1)	0 (a1)	160 (a3)	122 (a2)

The formulations 1, 2, 3, 4 and 5 were named F1, F2, F3, F4 and F5, respectively. The terms a1, a2, a3, a4 and a5 (in parentheses) denote similarity or difference between the treatments for each strain, according to Sisvar software. Significant difference can be seen whenever a term differ from others in the row. Formulations with equal terms indicate statistically similar results.

**Table 3** - Biological efficiency of *Ganoderma lucidum* strains grown on different substrates. The results were measured in %.

Strains	Formulations					
	F1	F2	F3	F4	F5	F6
CC-22	0 (a1)	36 (a3)	19,6 (a2)	21,6 (a2)	64 (a4)	40 (a3)
CC-63	12 (a2)	26 (a4)	18 (a2, a3)	22 (a3, a4)	40,8 (a5)	0 (a1)
CC-144	20,4 (a1)	40 (a2)	23 (a1)	30 (a1, a2)	72 (a3)	42,4 (a2)
CC-157	0 (a1)	0 (a1)	0 (a1)	0 (a1)	32 (a3)	24,4 (a2)

The formulations 1, 2, 3, 4 and 5 were named F1, F2, F3, F4 and F5, respectively. The terms a1, a2, a3, a4 and a5 (in parentheses) denote similarity or difference between the treatments for each strain, according to Sisvar software. Significant difference can be seen whenever a term differ from others in the row. Formulations with equal terms indicate statistically similar results.

## DISCUSSION

According to Urben (2004), the temperature is important factor for the development of mushrooms and when high, it could affect the development of the mycelium and formation of basidiome. The mycelium grew at temperatures 24-30°C and temperatures between 20-25°C favored fruiting, ensuring thick texture and golden color to the mushroom. The level of humidity in the environment should be between 80-90% for the basidiomes to grow well. According to Zhanxi and Zhanhua (2001), the absence of light is important for the development of mycelium, whereas the presence of light stimulates the formation of basidiocarps. In general, it takes up to 45 days to complete the colonization of the mycelium on the substrate and 5-6 months to harvest the mushrooms, but in this study complete colonization of substrate was obtained in 15 days

and harvest in 60 days (CC-144). The other strains began to be collected between 90 and 105 days. Urben (2004) reported that *G. lucidum* tolerated pH between 4.0 and 6.0, but in the present work the strains showed no problems in development at pH 8.0. The yield was in accordance with the results obtained for other mushrooms developed through Jun-Cao technique (Urben 2004), but it should be possible to improve these results optimizing the carbon-nitrogen (C:N) rate. This showed how necessary it would be to make a detailed chemical analysis of the formulation compounds.

In the treatments with rice bran (Formulations 1 and 3), there was a reduction in the productivity, when compared to the ones without it. This could be due to the fact that the addition of rice bran increased the nitrogen (N) concentration, as was with soy bran, which contained 7.38% of N (Eira and Minhoni 1997). The excess of nitrogen tends

to disable the degradation of lignin, retarding, or even inhibiting the mycelial growth, lessening the production of basidiomes (Urban 2004; Heleno et al. 2012). According to Regina (2001), in some cases, the most simple sources of N increase the protein concentration in the cultures, lessening mycelial growth and lignin degradation. This might have also been the case for the formulation 4 due to urea in its composition.

Cultivating *Lentinula edodes*, Rossi et al. (2003) observed that the mycelium growth lessened significantly with the use of increasing concentrations of rice bran. The addition of rice bran resulted in gradual decline in the C:N ratio in the substrate. The treatment with the addition of 20% of wheat bran accelerated the growth when compared to other brans (rice, corn, oatmeal and soy) in the same concentration. Contrary to the satisfactory results obtained with wheat bran in this work, Dias et al. (2003) observed a negative effect when wheat bran was added to bean straw for the cultivation of *Pleurotus sajor-caju*. In this case, the bran affected the colonization on the substrate. However, its addition to corn straw did not affect the mycelial growth, enabling an increase in the biological efficiency. Probably the reason might have been the addition of bean straw (high N) and not due to the wheat bran itself.

Donini et al. (2006) found that the addition of soy and rice bran caused lower growth rates in *Agaricus brasiliensis*. The cultivation medium which showed higher growth rate was the one incremented with 20% of wheat bran. Moda et al. (2005), cultivating *P. sajor-caju* on sugar cane bagasse supplemented with corn bran and mineral solution, observed lower biological efficiency. In this work, however, sugar cane bagasse enabled the increase of production for *G. lucidum* (formulations 2 and 5). Formulation 5 presented even better response, probably due to the addition of fodder to the substrate. The hypothesis for this was based on the fact that *G. lucidum* consumed quickly the nutrients from brans and grass. Sawdust could act as a later reserve of nutrients, which could be used by the mushroom when it was already established on the substrate.

The choice for substrates for cultivating mushrooms did not depend only on their compositions and C:N rates, but also on their compaction and moisture levels, since the substrates which were easily compacted, or that were excessively hygroscopic were difficult for aeration. It would also important to check the

nature of the substrate used for setting the C:N rate, because it could “cement” the substrate, resulting in the loss of aeration efficiency. In this study, aeration showed to be determinant on mycelial development, since without a good aeration, there was no growth on the substrate. This was also observed by Dias et al. (2003) when they worked with *Pleurotus* using coffee husk, which when compacted, reduced the aeration in the substrate.

## CONCLUSIONS

Jun-Cao could be an efficient technique for the production of *G. lucidum*, being flexible and allowing the use of different formulations of substrates. Results showed its growth at substantially reduced developmental time of the *G. lucidum* and 28°C as the best temperature. The variations of pH (4.0 to 8.0) had no harmful impact. The Chinese strains and Brazilian CC-144 responded better to the tested substrates. In general, the formulation 5 was best for the productivity. The combination of elephant grass (*Pennisetum purpureum*) and sawdust showed good nutritional balance for the fungal development.

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