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Residues from urban vegetable pruning in the production of the medicinal mushroom *Ganoderma lucidum*

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Every month, about 40 tons of waste prunings of trees and grasses are wasted in Bauru, São Paulo, Brazil, occupying unimaginable volumes in the municipal landfill. This rich material has been tested for cultivation of medicinal mushroom *Ganoderma lucidum*, rather unknown in Brazil, compared to commonly use eucalyptus sawdust. Thus, the objective of this study was to evaluate the use of wasted urban vegetable pruning for the production of the *G. lucidum* mushroom. For so, these wastes were collected and tested with five different substrates with mixtures of tree pruning and pruning of grasses, changing the percentage content, plus a control of eucalyptus sawdust base (T) in two strains of *G. lucidum*, a total of 12 experimental treatments (substrates × strains) with 7 replicates each totaling 84 packages with 600 g the unit. The experimental cultivation was from August to December with 21 days of incubation over 67 days of production. Through the statistics of the Carbon/Nitrogen relation, biological efficiency, loss of organic matter and mass of fresh and dried mushroom, only the control treatment maintained a good performance. Pruning substrates trees and grasses have varied low to medium fungal biomass conversion potential.

Key words: Fungi, sustainable development, solid waste.

INTRODUCTION

A great amount of organic residues is monthly generated in the city of Bauru, São Paulo, Brazil, which compromises the capacity and the life span of the city sanitary landfill. Most of those residues does not have an appropriate reuse, generating a significant amount in the environment.

Due to such environmental situation and the careless behavior of the human beings by discarding residues in an irrational way, the present work is based on one of the topics registered in the Agenda 21 document signed by

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world leaders during the ECO-92 conference in Rio de Janeiro.

Once the way these residues are discarded in the environment is harmful for the sustainable development, the efficient use of the resources rejected and the proposal of new practices are urgent. Science must strengthen the correct use of those residues, in answer to the emerging necessities (Furlan de Jesus et al., 2015; Carvalho et al., 2015). Besides the environmental aspect, the use of residues for the formulation of substrates aims to minimize the production costs of the mushrooms.

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) arouses 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 (Russell and Paterson, 2006).

The substrates used in the mushrooms 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 (Carvalho et al., 2015).

One of the main factors of mushrooms cultivation is the selection of substrates for the production. Biologically and economically appropriate materials are essential for a successful cultivation (Roy et al., 2015, Thakur and Sharma, 2015).

Due to its nutritional and medicinal benefits, the production of mushrooms in Brazil is growing, but its conditions lack appropriate cultivation technologies, once the technology used for years was adapted from the ones practiced in developed countries, with different weather conditions and raw material (Dias, 2010, Sales-Campos et al., 2012). Therefore, the objective of this study was to study the reuse of pruning residues in urban areas for the cultivation of *G. lucidum* as a viable option for the agricultural producer.

MATERIALS AND METHODS

The “Jun-cao” cultivation technology was used for the production of *G. lucidum*. “Jun” means “mushroom” and “Cao” means “grass”. So, this process is characterized by using grass and other agricultural residues as substrates for the cultivation of mushrooms, which are packed in appropriate and sterilized bags inoculated with the mycelium of the edible or medicinal fungus. Afterwards, the fruiting bodies are produced (Rolin et al., 2014; Carvalho et al., 2015).

The preparation of the substrate and the experiment was initially carried out at the Mushrooms Module of the School of Agronomic Sciences of the São Paulo State University – UNESP, Botucatu, São Paulo, Brazil. Next, the experiment was transported to the Universidade do Sagrado Coração (USC), Bauru, São Paulo, Brazil, for the cultivation stage.

Greenhouse mounting

A greenhouse was built at the experimental bed of the Agronomic Engineering course of the Universidade do Sagrado Coração, in order to propitiate a system similar to the natural one.

The place suffers many squalls of wind that increased the difficulties and is open to sky, that is, without natural or artificial ceiling during the hours of the day when sun rays strike directly, with full exposition to the sun.

A 30 cm deep, 4 m long and 1 m wide trench was made. The internal structure was made with bamboo arches, 150 µ wide transparent plastic cover and two layers of 70% shade cloth were placed 40 cm away from each other in order to decrease temperature.

Preparation of the substrates

After collection, the pruning residues were taken to the USC and dried in a sheltered environment, with natural ventilation, and afterwards sent to the Mushrooms Module of the São Paulo State University (UNESP), Botucatu, São Paulo, where they were submitted to a conventional forage grinder.

The experimental design was totally randomized, in 6×2 factorial scheme, corresponding to six types of cultivation substrates based on organic residues and two *G. lucidum* strains (Table 1), with 7 repetitions each (block with 600 g of substrate), adding up 84 experimental units.

G. lucidum strains used were GLM-09/01 and GLM-10/02, which are kept at the Mushrooms Module, School of Agronomic Sciences, UNESP, Botucatu, São Paulo. The preparation of the inoculum followed the methodology proposed by Minhoni et al. (2005).

After being homogenized and moistened, the substrates were placed in special HDPE (high density polyethylene) packs, capable to resist the sterilization process.

Each pack was compressed and a PVC segment and a piece of cotton cloth were added to the top of the pack, after involving their openings with aluminum foil. Next, the packs were submitted to the sterilization process at 121°C for 4 h.

Then, samples of the cultivation substrates were obtained soon after sterilization. Three samples of the different kinds of substrates were collected and sent to chemical characterization analysis (N, organic matter, C, C/N, humidity and pH), according to the Lanarv (1988) methodology.

The inoculation of packs with strains GLM-09/01 and GLM-10/02 of *G. lucidum* was performed after they were cooled until the environment temperature in the lab by using a laminar flow chamber in appropriate aseptic conditions, in order to avoid the contamination by other microorganisms.

Next, the packs were taken to incubation in an acclimatized room and kept at 25°C for three weeks, corresponding to the colonization period of the substrate by *G. lucidum* strains.

After the colonization period, the packs were transferred to the rough greenhouse built in the experimental area of mushrooms cultivation of the Sagrado Coração University, Bauru, São Paulo. The average temperature was kept at 25 ± 5°C and relative humidity of 60 to 85%. The packs were arranged totally at random.

Harvesting and data collection

Finally, mushrooms were harvested after their development, weighed, dehydrated and weighed again for obtaining the dry weight. The cultivation substrates were also weighed and samples were removed for chemical characterization of the substrates, loss of organic matter and biological efficiency.

A total of four harvestings were performed, beginning in October and ending in December, 2014. Data were registered in Microsoft

Table 1. Experimental treatments used in this research.

Treatments	*G. lucidum* strains	Formulation of the substrates[1]
1	GLM 09/01	100% tree pruning (TP)
2		100% grass pruning (GP)
3		75% TP + 25% GP
4		50% TP + 50% GP
5		25% TP + 75% GP
6		Witness (100% eucalyptus sawdust)
7	GLM 10/02	100% tree pruning (TP)
8		100% grass pruning (GP)
9		75% TP + 25% GP
10		50% TP + 50% GP
11		25% TP + 75% GP
12		Witness (100% eucalyptus sawdust)

[1]All substrates were added with 18% of wheat bran and 2% of limestone (dry weight). Humidity was adjusted to 65%.

Excel® spreadsheet to provide the statistical calculations.

Loss of organic matter

This evaluation was performed according to Rajarathman and Bano (1989). The loss of organic matter (LOM) is the index that evaluates the decomposition of the substrate by the fungus during cultivation. It is determined by the difference between the dry mass of the initial substrate and the dry mass of the residual substrate (post-harvest):

$$LOM\ (\%) = \frac{Dry\ mass\ of\ the\ initial\ substrate\ (g) - Dry\ mass\ of\ the\ residual\ substrate\ (g)}{Dry\ mass\ of\ the\ initial\ substrate\ (g)}\ X\ 100$$

Biological efficiency

Yield was expressed by means of the biological efficiency (BE), which represents the conversion percentage of the substrate into fungic biomass (mushrooms).

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

Statistical analysis

The data analyses were submitted to variance analysis. Averages were compared by Tukey's test (5%) by using the SISVAR 4.2 software developed by the Departmento of Exact Sciences of the Federal University of Lavras, Minas Gerais, Brazil (UFLA).

RESULTS AND DISCUSSION

According to the variation factors obtained in the Tukey's test observed in Table 2, we noticed the outstanding relations that influenced statistically the results of this research.

Contaminations with other microorganisms occurred in all the substrates, especially in the ones almost without production, such as 100% grass pruning and 25% tree pruning + 75% grass pruning, due to the low colonization of the substrate after the transference to the rough greenhouse, when the cotton plugs were removed from all the treatments.

C/N ratio

Regarding C/N ratio, Philippoussis et al. (2007) reported that carbon and nitrogen contents of the substrate influence on fruiting precociousness and yield. Boyle (1998) reports that lignin degradation is also important for growth, as long as fungi may have access to the nitrogen contained in the wood components.

According to Hsieh and Yang (2004), the *G. lucidum* species requires an optimal C/N ratio of 70/1 and 80/1 for efficient growth and low production cost. For Gurung et al. (2012), most of medicinal fungi require optimal pH of 5.5 to 6.5.

The average contents obtained by analyses in initial and final C/N ratio are described in Table 3.

We observed that all the initial substrates obtained optimal pH (between 5.7 and 6.3) for the production and development of *G. lucidum* (Table 3).

The substrates had average C/N ratio varying from 35/1 (100% tree pruning) to 70/1 (Control - eucalyptus sawdust) in the initial substrate (Table 3). All pruning results obtained unfavorable C/N ratio compared to the ones obtained by Hsieh and Yang (2004) because all the substrates were formulated considering the proportions of the materials used, that is, 80% of prunings (residue), 18% of bran and 2% of limestone for all the substrates, different from other works in literature.

Many mushroom producers in Brazil use the proportion-based formulation. If they chose the

Table 2. *ƒ* values expressed in percentages obtained in the variance analysis of LOM, FMB and BE provided by different substrates during the cultivation of strains of GLM 10/01 and GLM 09/01 of *G. lucidum.*

Variation factors	LOM	FMB	BE
Substrate (S)	5.8**	69.0**	77.7**
Strain (L)	10.3**	0.2^{ns}	0.7^{ns}
S × L	9.4**	1.5^{ns}	1.2^{ns}

LOM = Loss of organic matter; FMB = Fresh mass of Basidioma; BE = Biological Efficiency; ** significant at 1% level; ^{ns} = not significant.

Table 3. C/N ratio and pH of the initial and final substrates, according to strains, used during the cultivation cycle of *G. lucidum.*

Treatments	Initial substrate			
	N%	C%	C/N	pH
T - ES				
1 - 100% TP	0.5	19	35/1	6.3
2 - 100% GP	0.5	20	36/1	5.8
3 - 75% TP 25% GP	0.6	22	37/1	6.1
4 - 50% TP 50% GP	0.6	22	37/1	6
5 - 25% TP 75% GP	0.5	20	41/1	5.9
	Final substrate			
Strain GLM-10/02				
T - ES	0.4	15.5	35/1	4.2
1 - 100% TP	0.8	20	23/1	5.5
2 - 100% GP	0.5	12.5	25/1	8.7
3 - 75% TP 25% GP	0.7	18.5	25/1	4.3
4 - 50% TP 50% GP	0.8	18.5	23/1	4.3
5 - 25% TP 75% GP	0.9	18.5	19/1	4.3
Strain GLM- 09/01				
T - ES	0.5	16	29/1	4.2
1 - 100% TP	0.8	14.5	18/1	4.3
2 - 100% GP	0.4	10	22/1	8.3
3 - 75% TP 25% GP	0.8	19	24/1	4.5
4 - 50% TP 50% GP	0.6	11.5	19/1	4.7
5 - 25% TP 75% GP	0.6	12.5	19/1	4.5

ES = Eucalyptus sawdust; TP = tree pruning; GP = grass pruning. All treatments were added with 18% of wheat bran and 2% of limestone in their composition. Humidity was adjusted to 60%.

recommended C/N-based formulation, a previous analysis of the formulation would be necessary, followed by C/N calculation.

Once these analyses represent a cost, many of the producers use proportions-based formulations. Thus, the idea of this research was to simulate what most of producers do.

Biological efficiency

The biological efficiency of each treatment corresponds to the transformation of the substrate matter into mushroom biomass. This index is the most used by the researchers, what makes the comparison of the results with literature easier (Tisdale et al., 2006). Figure 1 characterizes the percentage of biological efficiency reached for each treatment. For the tested residues mixed, the best results for biological efficiency were for substrates 100% tree pruning, 75% tree pruning + 25% grass pruning and 50% tree pruning + 50% grass pruning.

The average of the biological efficiency varied from 0% for the substrate containing 100% of grass pruning (lowest) to 36% for the control substrate containing eucalyptus sawdust (Figure 1). Similar results to the highest values obtained in this experiment were found by Erkel (2009) when he used poplar sawdust (a European

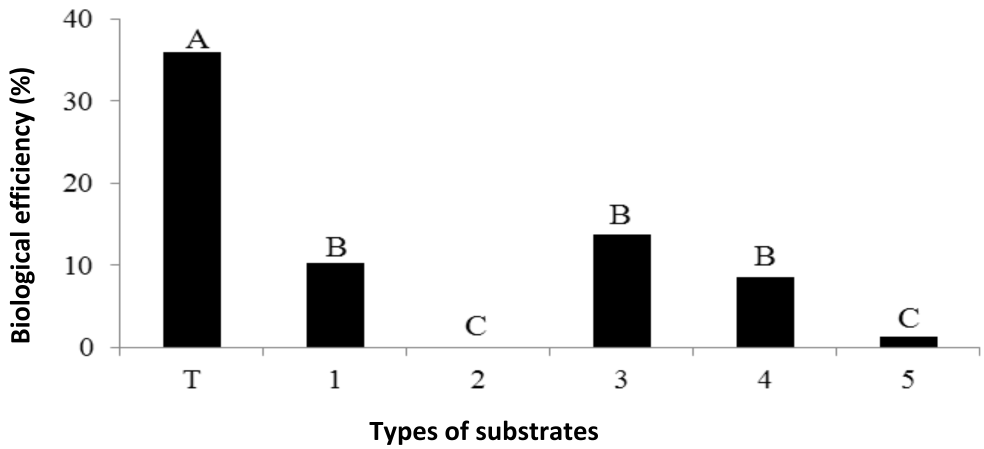

Figure 1. Biological efficiency (%) in the treatments of *G. lucidum* cultivated in substrates based in organic residues of prunings in urban areas. Averages followed by the same letters are not statistically different among each other, according to the Tukey's test (5%). Coefficient of variation: 77.7%. Values are average of 7 repetitions. T = Witness - eucalyptus sawdust; 1 = 100% tree pruning (TP); 2 = 100% grass pruning (GP); 3 = 75% TP + 25% GP; 4 = 50% TP + 50% GP; 5 = 25% TP + 75% GP. All treatments were added with 18% of wheat bran and 2% of limestone in their composition. Humidity was adjusted to 60%.

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. As well as 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%).

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 initial substrate (Table 3). 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 35:1 to 41:1 to the treatments utilizing tree pruning and/ or grass pruning.

Therefore, his substrates containing grass pruning mostly were not biologically efficient as an alternative for the cultivation of *G. lucidum* in Bauru, São Paulo, resulting in higher preference for tree pruning.

Fresh mass of basidiomata

The results of total fresh mass of the basidiomata (Figure 2) were obtained by adding up the production of each treatment in the four harvestings carried out during the experiment, in a total of 67 days of production in greenhouse, preceded by 21 days of incubation, totalizing 88 days of experiment.

It was observed that the Control treatment obtained mushrooms with the highest mass and the best combination tested was the substrate with 50% of tree pruning + 50% of grass pruning, with some statistical differences from the others.

During the cultivation cycle, the sum of the basidiomata fresh mass obtained the lowest average of 0.0 g for 100% grass pruning and the highest one of 88.5 g for the Control treatment containing eucalyptus sawdust.

With an yield of 51.6 g, the substrate made up with 50% of tree pruning + 50% of grass pruning was statistically the best residue mix tested. This result shows that this substrate produced a little more than half of the mass yield of the Control substrate.

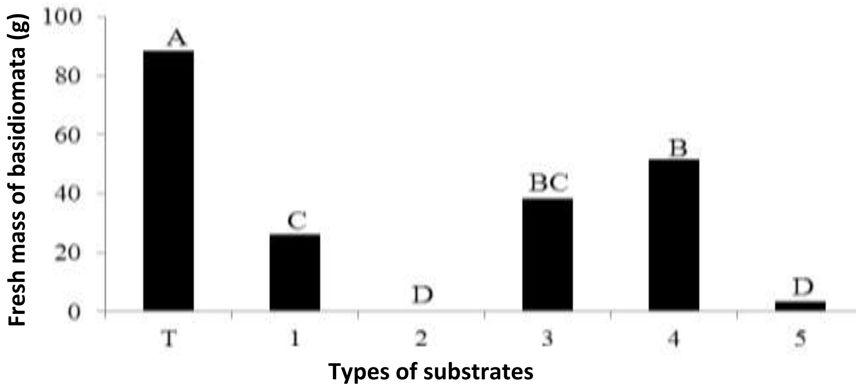

Figure 2. Total fresh mass of basidiomata of the substrates used in the cultivation of *G. lucidum*, based on organic residues of prunings in urban areas. Averages followed by the same letters are not statistically different among each other, according to the Tukey's test (5%). Coefficient of variation: 69.0%. Values are average of 7 repetitions. T = Witness - eucalyptus sawdust; 1 = 100% tree pruning (TP); 2 = 100% grass pruning (GP); 3 = 75% TP + 25% GP; 4 = 50% TP + 50% GP; 5 = 25% TP + 75% GP. All treatments were added with 18% of wheat bran and 2% of limestone in their composition. Humidity was adjusted to 60%.

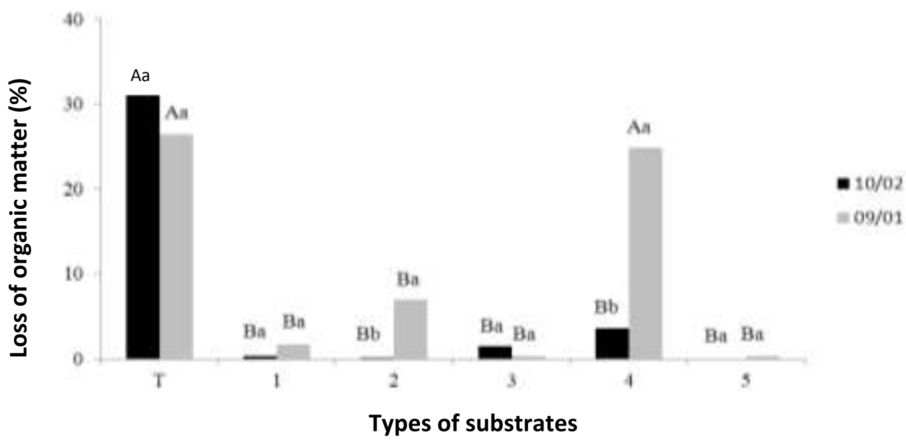

Figure 3. Loss of organic matter of strains GLM-10/02 and GLM-09/01 of *G. lucidum*, cultivated in substrates based on organic residues of pruning in urban areas. Averages followed by equal letters are not statistically different among each other, according to the 5% Tukey's test. Uppercase letters compare the substrates among each other and lowercase letters compare the strains inside the same substrate. Coefficient of variation is 9.4%. Values are average of 7 repetitions.T = Witness – eucalyptus sawdust; 1 = 100% tree pruning (TP); 2 = 100% grass pruning (GP); 3 = 75% TP + 25% GP; 4 = 50% TP + 50% GP; 5 = 25% TP + 75% GP. All treatments were added with 18% of wheat bran and 2% of limestone in their composition. Humidity was adjusted to 60%.

Loss of organic matter

The loss of organic matter (LOM) evaluates the reduction of the decomposed matter by the action of the fungus, as a consequence of the production process.

The best result was obtained by the Control treatment with eucalyptus sawdust, with 31.1% (strain GLM-10/02) and 26.4% (strain GLM-09/01). The substrate with 50% of tree pruning + 50% of grass pruning distinguished itself with strain GLM-09/01, reaching 24.6% (Figure 3).

By observing the data of the fresh mass of basidiomata produced (Figure 2) and the loss of organic matter (Figure 3), we notice that the Control treatment, eucalyptus sawdust, obtained the best yields. Moreover, the Control treatment obtained the best performance: 31.3% (strain GLM-10/02) and 26.4% (strain GLM-09/01) in relation to the loss of organic matter.

The substrates and the strains of the treatments 4 (50% tree pruning + 50% grass pruning) and 2 (100% grass pruning) distinguished themselves in loss of organic matter, with 24.9 and 7.0%, respectively, especially for strain GLM-09/01, expressed in the statistic comparison (lowercase letters in Figure 3).

The curious fact in the analysis of the fresh mass of basidiomata (Figure 2) and the loss of organic matter (Figure 3) of Treatment 2 is the absence of basidiomata production (0 g). However, there was 7% of loss of organic matter. According to Zadrazil (1978), it is justified by the loss of CO_2 and H_2O during the metabolism of the microorganisms and not only by removing materials for the formation of the basidiomata.

Conclusions

1. The alternative use of the urban organic residues tree and grass pruning is little viable for the cultivation of *G. lucidum*.
2. The strains GLM-09/01 and GLM-10/02 of *G. lucidum* have good results with eucalyptus sawdust. Only strain GLM-09/01 stands out with the residues substrate, in the ratio of 50% tree pruning + 50% grass pruning.
3. This research shows another possibility to produce mushrooms and improves the studies in the area of medicinal mushrooms production, with hypothesis for new tests for edible mushrooms also.
4. New proposals of solutions appear to reduce organic residues discharge by using them to produce food, generate income and improve their use instead of sending them to a sanitary landfill.

Conflict of Interests

The authors have not declared any conflict of interests.

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