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Use of spent compost in the cultivation of *Agaricus blazei*

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Two compost formulations, based on Braquiaria straw (*Brachiaria sp.)*, a conventional one and a spent one, were tested in the cultivation of ABL 99/30 and ABL 04/49 strains of *Agaricus blazei*. The experimental design was in a completely randomized factorial scheme with four treatments (two strains of *A. blazei* x two types of compost) and 30 repetitions. Each experimental unit consisted of a box with 10 to 10.5 kg of moist fresh compost. According to the results obtained, the loss of organic matter of the composts was affected by the *A. blazei* strain and the type of compost used. The traditional compost lost a higher organic matter content compared to the spent compost, and the ABL 99/30 strain caused a higher loss of organic matter in the composts compared to the ABL 04/49 strain. Yield, biological efficiency, mass and number of basidiomata produced were similar between the conventional and the spent compost, as well as the chemical analysis of the produced basidiomata. However, the *A. blazei* strains showed some differences among each other, the basidiomata of strain ABL 04/49 obtained a higher percentage of crude protein in their composition, compared to the ABL 99/30, in both composts. Thus, the utilization of spent compost in the cultivation of *A. blazei* did not impair the basidiomata yield nor their nutritional value, demonstrating it to be a good option to be used as an ingredient in the compost formulation for the *A. blazei* cultivation.

INTRODUCTION

The use of spent compost (substrate resulting in the end of the production cycle) for the production of new mushroom cultivation cycles is a promising alternative, which aims at replacing the soil and organic substrates

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commonly used in mushroom production, and whose advantages are the reduction of the cost production and the environmental impact caused by these materials extraction from the environment (Pardo-Giménez and Pardo-González, 2009; Pardo-Giménez et al., 2010).

Mushrooms have a great commercial importance due to their nutritional and medicinal properties. Mushrooms are considered food of a high nutritional value as they have low lipid content, a considerable amount of phosphorus and present a high level of proteins and dietary fibers (Furlani and Godoy, 2007).

Shibata and Demiate (2003), while carrying out the nutritional analysis of two strains of *A. blazei*, obtained the following basidiomata chemical composition means: 37.4% protein, 8.82% fiber, 7.49% ash, 0.99% lipid and 45.30% carbohydrate.

The composting process and the appropriate chemical composition of the substrates and supplements used in the compost are fundamental to reach a desirable yield in the mushroom cultivation. The use of agroindustrial residues for the formulation of composts is intended to minimize the cost of mushrooms production (Silva et al., 2009).

In Brazil, the cultivation of *A. blazei* occurs in a very similar way to the cultivation of *A. bisporus*. Athough the species present certain similarities, it is necessary to develop specific technologies for the cultivation of *A. blazei* in order to increase its yield, considering the low yield reached in this mushroom cultivation when compared to the one obtained by the cultivation of *A. bisporus* (Kopytowski Filho, 2006; Dias, 2010).

According to Kopytowski Filho (2006), the cultivation of *A. blazei* can be divided as follows: composting phase I, which corresponds to the period of composting process in yard; composting phase II, which is the process including the compost pasteurization and conditioning, and the composting phase III, which corresponds to the stages of inoculation and colonization of the compost; the covering and harvesting are carried out afterwards. The composting process is generally critical to obtaining good quality compost; however the high yield reached depends significantly on phase II (Sánchez, 2004).

In Brazil, materials such as cereal straws (wheat, rice, and barley), grasses (brachiaria, coast-cross and tifton), animal bedding (horses and poultry), nitrogen sources (organic and/or mineral), limestone and plaster are used as substrates for compost formulation in *Agaricus* cultivation (Minhoni et al., 2005; Kopytowski Filho, 2006).

Usually, the material supply for composts formulation varies according to its availability depending on the season of the year (Andrade et al., 2008). Several materials have been used in the compost preparation for the cultivation of *A. blazei*; the uses vary according to their availability in the different country regions and season of the year.

However, little is known about the reutilization of these materials for a new cycle of *A. blazei* production, yield and nutritional composition of basidiomata produced. Thus, this study aims at assessing the yield, biological efficiency, number of mushrooms, mass of mushrooms and the bromatological analysis of mushrooms produced, using two strains of *A. blazei* and two formulations of composts based on brachiaria straw (*Brachiaria* sp.): conventional compost and a spent one.

MATERIALS AND METHODS

The experiment was carried out in the facilities of the Mushrooms Module, Plant Production Departament, FCA/UNESP, Botucatu-SP, with two types of composts (traditional and spent) (Table 1) and two strains of *A. blazei* ABL 99/30 and ABL 04/49.

Seeds production

The strains ABL 99/30 and ABL 04/49 of *A. blazei* used were both kept in the Mushrooms Module Matrix Bank, Plant Production Department, FCA/UNESP, Botucatu-SP. Initially, 0.5 cm diameter disks were transferred from the primary matrix, under aseptical conditions, to other Petri dishes with compost - agar (CA). After inoculation, the Petri dishes were transferred to an incubator where they were kept for 10 days in darkness at 28 ± 1°C for colonization. The Petri dishes colonized were split in eight equal parts; each part of this segment was inoculated in flasks containing sorghum grains (400 g), plaster, and calcium carbonate. The sorghum grains were initially boiled in water for 40 minu. After draining the excess of water, 20 g kg^{-1} calcium carbonate and 160 g kg^{-1} of plaster were added relative to the moist weight of grains cooked. The lower part of the flasks cap were fitted with filter paper in order to allow aeration and prevent contaminations after autoclaving. The flasks were incubated in an incubator, in darkness at 28 ± 1°C for 12 days. The inoculum was produced by packing the substrate prepared in high density polyethylene (HDPE) bags, using about 1200 g of sorghum grains per plastic. The plastic bags contained Tyvek® filters in the upper parts, thus, allowing the gas exchanges.

The substrates prepared were autoclaved at 121°C for 3 h. Then, the bags were kept at rest for 24 h in order to reduce the temperature to about 25°C. Then, the inoculation of each plastic bag was undertaken at temperature of 28 ± 1°C for 15 days. By the end of the incubation period, the substrates were colonized by the fungus, and then called spawn, and ready to be inoculated in the compost.

Composting

Composting phase I was carried out on concrete floor, with open sides and natural ventilation. Before forming the furrows, brachiaria straw was moistened and overturned every two days for a total period of 10 days. The furrows were formed by a layer of straw (20 cm high), followed by a layer of sugarcane bagasse (20 cm high) until they reached 1.8 × 1.8 m, height and width respectively. Limestone, urea and soy bran were added to both furrows according to each treatment. Table 2 presents the amount of each ingredient added in the formation of the furrows for the 2 types of composts.

The composts were overturned, and water was added manually with a hose in order to keep the moisture between 70 to 75%. Altogether, six overturns were carried out, totaling 14 days in

Table 1. Content of moisture, mass and percent of carbon and nitrogen, and the C/N relation of the ingredients used in the traditional and spent composts.

Ingredient	Moisture (%)	Carbon (%)	Carbon (Kg)	Nitrogen (%)	Nitrogen (Kg)	C/N
Traditional compost						
Sugarcane bagasse	64.90	50.00	70.20	0.52	0.73	96.15
Brachiaria	18.36	48.10	62.83	1.26	1.65	38.17
Soy Bran	12.83	50.00	3.92	7.80	0.61	6.41
Urea	0	27.00	0.41	45.00	0.68	0.60
Spent compost						
Sugarcane bagasse	64.90	50.00	52.65	0.52	0.55	96.15
Brachiaria	18.36	48.10	47.12	1.26	1.23	38.17
Soy Bran	12.3	50.00	2.62	7.80	0.41	6.41
Urea	0	27.00	0.27	45.00	0.45	0.60
Spent Compost	71.6	9.35	5.31	0.50	0.28	18.70

C/N = Carbon/nitrogen relation. Traditional Compost = substrate used in the mushrooms production, consisting of sugarcane bagasse, brachiaria straw, soy bran and urea. Spent compost = substrate used in the mushrooms production, consisting of sugarcane bagasse, brachiaria straws, soy bran and urea added of spent compost (substrate obtained by the end of the cultivation cycle).

Table 2. Traditional and spend composts formulation.

Ingredient (kg)	Compost			
	Traditional		Spent	
	Moist weight	Dry weight	Moist weight	Dry weight
Sugarcane bagasse	400.00	140.40	300.00	105.30
Brachiaria straw	160.00	130.62	120.00	97.97
Soy bran	9.00	7.85	6.00	5.23
Urea	1.50	1.50	1.00	1.00
Plaster	-	8.00	-	8.00
Limestone	-	9.00	-	9.00
Spent compost	-	-	200.00	56.80
Total Mass of Compost	570.50	297.37	627.00	283.30
Total Mass of Carbon	-	137.36	-	107.97
Total Mass of Nitrogen	-	3.67	-	2.92
Initial C/N relation	-	37.42	-	37.00

phase I. In phase II, the composts were transferred into perforated plastic boxes, which measured 56.5 × 46.5 × 28.5 cm (length, width and height respectively). The boxes were randomly placed inside a climate-controlled chamber (Dalsem mushrooms) for the pasteurization (8 h at 62 ± 2°C) and conditioning (8 days at 48± 2°C). In the end of phase I and II (Table 3), three samples of each compost were collected and dehydrated at 65°C for 48 h to analyze carbon, nitrogen, organic matter and pH. The results are presented in Table 4.

Inoculation of composts

The inoculation of composts was carried out manually by adding 1.5 g of *A. blazei* seed per kg^{-1} of moist compost. The composts were split and transferred (10 to 10.5 kg of moist compost) to other polyethylene boxes internally covered with polyethylene transparent plastic containing orifices in the lower part. The boxes were randomly placed in an incubator (Dalsem Mushrooms) and kept for 16 days at 28 ± 1°C.

The soil used in the covering layer was classified as Dystrophic Red Nitosol (Carvalho, 1983) from the Fazenda Lageado (FCA / UNESP). The soil pH was corrected to 7.0 by adding calcium carbonate, 20 days before the compost covering. Altogether, 840 L of soil were used, and 30% (360 liters) of charcoal (1 to 2 cm thick) was added. The soil pasteurization was carried out at 62°C for 8 h, in an incubator (Dalsem Mushrooms). About 15 kg of soil were added to each box to act as cover layer. The soil was previoulsly moistened with the assistance of a hose to keep the moisture at about 70%. After the addition of the cover layer, the compost was covered with transparent plastic, and incubated for six days at 22 ± 1°C. After the cover layer had been colonized, the plastic was

Table 3. Composting process phases.

Days	Activity	Procedure	Phase
-10	Pre-moistening	Straw moistening	Pre-composting
-7		Overturn and moistening of straw	
-5		Overtun of straw	
-3		Overtun of straw and addition of bagasse	
0	Setting of furrows	Additon of 1st half of soy bran, urea and limestone.	Composting Phase I
2	1st Turn over	Additon of 2nd half of soy bran, urea and limestone.	
4	2nd Turn over	Additon of 1st half of plaster	
7	3rd Turn over	Additon of 2nd half of plaster	
9	4th Turn over	Eventual correction of moisture	
11	5th Turn over	Eventual correction of moisture	
14	6th Turn over	Eventual correction of moisture	
15	Pasteurization	Temperature of 62°C ± 2 for 8 h	Composting Phase II
16	Conditioning	Temperature of 48°C ± 2 for 8 days	
27		Compost with 25°C ready to be inoculated	

Table 4. Content of moisture, nitrogen, organic matter and carbon, C/N relation and pH of traditional and spent composts, in the end of composting phase I and II.

Compost	Traditional	Spent
End of phase I		
Moisture (%)	77.54	73.72
N (%)	0.33	0.32
C (%)	10.19	10.03
O.M (%)	18.34	18.04
C/N	32/1	32/1
pH	7.43	7.53
End of phase II		
Moisture (%)	72.60	71.07
N (%)	0.44	0.40
C (%)	11.38	9.85
O.M (%)	20.49	17.53
C/N	26/1	25/1
pH	7.65	7.67

N, Nitrogen; O.M, organic matter; C, carbon; C/N, carbon/nitrogen relation.

removed. During the production of basidiomata, water was added in the cover layer with the assistance of a hose to keep the moisture at about 75%.

By the end of the mushrooms harvesting period, the composts loss of organic matter was calculated by using 6 boxes of each tratment, from which the cover layers were removed, and the composts moisture and mass content was later determined in the end of the mushrooms production.

Variables analyzed

Number and fresh mass of mushrooms

The number and fresh mass of mushrooms were daily determined during harvest. A semi-analytical scale was used to determine the mushrooms fresh mass.

Yield and biological efficiency

Yield was expressed as the fresh mass of mushrooms / fresh mass of compost × 100, and the biological efficiency as the fresh mass of mushrooms / dry mass of compost × 100. The mushrooms fresh mass was determined in the end of harvest and the compost fresh mass was determined in the end of composting phase II.

Organic matter loss

The loss of organic matter was expressed as the compost dry mass in the end of composting phase II - compost dry mass in the end of the production / compost dry mass in the end of composting phase II × 100. The organic matter loss of the composts is presented in Table 5.

Nutritional analysis of *A. blazei* strains

Nutritional analyses of mushrooms were carried out at the Faculdade de Medicina Veterinária e Zootecnia - FMVZ/ UNESP, Laboratory of Bromatology, Botucatu-SP. Two samples of dehydrated mushrooms of each treatment were collected during the production and the contents of crude protein, ether extract, ash and crude fiber were determined according to Silva and Queiroz (2002). The conversion factor 4.38 is used to determine protein in mushrooms (Furlani and Godoy, 2007).

RESULTS AND DISCUSSION

The F values of variance of the organic matter loss of traditional and spent composts according to the *A. blazei* strain used are presented in Table 5. The type of compost and the strain of *A. blazei* used influenced the percentage of organic matter loss of composts. Table 6

Table 5. F values obtained in the analysis of variance of organic matter loss of traditional and spent composts according to the *A. blazei* strain used.

Parameter	Organic matter loss
Compost	12.99**
Strain	24.84**
Compost x strain	0.38^{ns}
Variation coefficient %	16.64

**Significance level < 1%; *significance level < 5%; ns: no significant difference.

Table 6. Organic matter loss of traditional and spent composts according to the *A. blazei* strain used.

Strain	Compost	
	Traditional	Spent
ABL 99/30	42.0^{Aa}	32.8^{Ab}
ABL 04/49	29.8^{Ba}	23.3^{Bb}

*Means followed by the same capital letters inside a column and small letters inside a row do not differ significantly (Tukey, 5%). Mean obtained from 6 repetitions.

Table 7. F values obtained in the analysis of variance for the fresh mass of basidiomata (MB), number of basidiomata (NB), yield (Y) and biological efficiency (BE) of ABL 99/30 and ABL 04/49 strains of *Agaricus blazei* , cultivated in two types of composts, traditional and spent.

Parameter	MB	NB	Y	BE
Compost	0.125^{ns}	0.095^{ns}	0.125^{ns}	1.163^{ns}
Strain	96.99**	57.57 0**	96.972**	96.965**
Compost x Strain	2.55^{ns}	1.797^{ns}	2.517^{ns}	3.433^{ns}
Variation coefficient %	20.28	23.69	20.27	20.37

**Level of significance < 1%; * level of significance < 5%; ns, no significant difference.

presents the organic matter loss of each compost according to the *A. blazei* srain used for the mushrooms production. Generally speaking, the ABL 99/30 strain caused a higher organic matter loss of composts (37.40%) than the ABL 04/49 strain (26.55%), while in the avarage, the traditional compost lost a higher organic matter content (35.90%) compared to the spent compost (28.05%).

In Table 7, the effect of the *A. blazei* strains over the variables, fresh mass of basidiomata, number of basidiomata, yield and biological efficiency of mushrooms produced was verified. The type of compost and the interaction compost x strain did not cause effects over the variables analyzed.

The values obtained of mass and number of basidiomata, yield and biological efficiency of ABL 99/30 and ABL 04/49 strains of *A. blazei*, grown in both traditional and spent composts, are present in Table 8. Differences were verified between the variables analyzed as for the *A. blazei* strain used, being that the ABL 99/30 strain was superior to ABL 04/49, regardless of the kind of compost grown, in all variables analyzed. The results show that ABL 99/30 strain was superior in 35.16, 32.55, 35.07 and 35.16% as for mushrooms mass, number of mushrooms, yield and biological efficiency, respectively, in relation to the ABL 04/49 strain when they were cultivated in the traditional compost.

When *A. blazei* strains were grown in the spent compost, the ABL 99/30 strain was superior to ABL 04/49 for the same variables in 26.35, 23.66, 26.40 and 26.33%,

Table 8. Total mean values for number and fresh mass of basidiomata, yield and biological efficiency of 04/49 and 99/30 strains of *Agaricus blazei*, obtained in function of the kind of compost used.

Strain	Compost	
	Traditional	Spent
Mass (g)		
ABL 99/30	1303.23^{Aa}	1253.43^{Aa}
ABL 04/49	845.03^{Ba}	923.17^{Ba}
Number of Basidiomata		
ABL 99/30	68.20^{Aa}	65.63^{Aa}
ABL 04/49	46.00^{Ba}	50.1^{Ba}
Yield (%)		
ABL 99/30	13.03^{Aa}	12.54^{Aa}
ABL 04/49	8.46^{Ba}	9.23^{Ba}
Biological efficiency (%)		
ABL 99/30	47.56^{Aa}	43.37^{Aa}
ABL 04/49	30.84^{Ba}	31.95^{Ba}

Means followed by the same capital letters inside a column and small letters inside a row do not differ significantly (Tukey, 5).

respectively. Mamiro et al. (2007) studied the use of the spent compost, mixtures of spent compost with non-composted substrate and the supplementation period of the compost in the cultivation of *A. bisporus* and obtained the lowest indexes of productivity (4.9 kg/m^2) and biological efficiency (25.7%), by using spent compost without compost supplementation and the highest indexes of productivity (27.2 kg/m^2) and biological efficiency (144.3%) were reached when a 50/50 mixture of spent compost with non-composted substrate was used and the Target® (commercial nutrient for mushrooms) supplement was added at the moment of laying the cover of the compost.

Giménez and González (2009) used a mixture of spent substrate of *P. ostreatus* with spent substrate of *A. bisporus* for new cultivation cycles of *P. ostreatus* and obtained the best behaviour for the production parameters when they used combinations of 9:1 and 8:2 (p/p) (spent substrate of *P. ostreatus* and spent substrate of *A. bisporus*, respectively).

The values for frutification precociousness, frutification index, yield and biological efficiency obtained were next to the ones reached by the control treatment carried out by the authors by using an approppriate commercial substrate. Mamiro and Royse (2008) evaluated the effect of mixtures of spent compost and non-composted substrate in different ratios for the cultivation of *A. bisporus* on yield, biological efficiency and mass of the mushrooms and obtained higher results when they used 50/50 and 75/25 mixtures of non-composted substrate and spend substrate, respectively. They reached values of 10.9 kg/m² for yield and 61.5% for biological efficiency when they used the materials in a 50/50 ratio. When the ingredients were mixed in a 75/25 ratio, the results were 67.3% of biological efficiency and 11.9 kg/m² of productivity.

The kind of compost used for the cultivation of mushroms did not influence the mass and number of mushrooms and did not affect their yield and biological efficiency. A similar fact occurred with Zied et al. (2009) who worked with different composts formulations for the cultivation of *A. blazei* and did not found significant differences in the variables studied (mass of the mushrooms, number of mushrooms, yield and biological efficiency) in relation to the kind of compost used for the cultivation of the mushrooms.

The F values obtained in the analysis of variance for the dry matter, crude protein, ether extract, ash and crude fiber of ABL 99/30 and ABL 04/49 strains of *A. blazei*, cultivated in both types of compost are presented in Table 9. The results show that the type of compost used for the mushrooms cultivation affected the contents of dry matter and ether extract of mushrooms produced. The content of ether extract and of mushrooms crude protein were influenced by the strain of *A. blazei* used, and the effect of the interaction compost x strain was also verified on the composition of ether extract of mushrooms produced.

Table 10 presents the results obtained in the bromatological analysis of the mushrooms produced, these results show the ABL 99/30 and ABL 04/49 strains were similar regarding the content of dry matter of mushrooms. However, the mushrooms cultivated in the spent compost presented a higher content of dry traditional compost.

In relation to the crude protein of mushrooms, it was verified that the mushrooms of ABL 04/49 strain were superior when compared to the mushrooms of 99/30 strain, regardless of where the composts were cultivated. The first ones presented mean values of 24.84% of crude protein, while the second ones presented 22.91% as mean. The type of compost used didn´t alters the content of crude protein of mushrooms produced.

Basidiomata of ABL 99/30 strain presented lower content of ether extract when cultivated in the traditional compost (0.68%), and higher content in the spent compost (1.21%). On the contrary, the basidiomata of ABL 04/49 strain presented higher content of ether extract (1.17%) when cultivated in the traditional compost, and the smallest content (0.96%) was obtained when they were cultivated in the spent compost.

There were no significant differences in the percentage of ash and crude fiber of mushrooms produced, no effect over these variables were verified regarding the strain of *A. blazei* adopted or the type of compost used for the cultivation of mushrooms. Andrade et al. (2008) using

Table 9. F values obtained in the analysis of variance of dry matter, crude protein, ether extract, ash and crude fiber of ABL 99/30 and ABL 04/49 strains of *A. blazei*, cultivated in two types of composts, a traditional and a spent one.

Variance cause	Dry matter	Crude protein	Ether extract	Ash	Crude fiber
Compost	60.098**	5.173^{ns}	82.843**	1.112^{ns}	0.144^{ns}
Strain	6.089^{ns}	59.677**	47.078**	1.954^{ns}	0.107^{ns}
Compost x Strain	6.753^{ns}	0.535^{ns}	423.706**	1.626^{ns}	3.400^{ns}
Variation coefficient %	0.18	1.48	2.52	4.53	6.29

**Significance level < 1%; *significance level < 5%; ns: no significant difference.

Table 10. Content of crude protein, ether extract, ash and crude fiber obtained in the bromatological analyzes of the basidiomata produced according to the strains of *Agaricus blazei* and the type of compost used.

Strain	Compost	
	Traditional	spent
Dry matter (%)		
ABL 99/30	91.6^{Ab}	92.79^{Aa}
ABL 04/49	92.18^{Ab}	92.77^{Aa}
Crude protein (%)		
ABL 99/30	23.1^{Ba}	22.72^{Ba}
ABL 04/49	25.21^{Aa}	24.46^{Aa}
Ether extract (%)		
ABL 99/30	0.68^{Bb}	1.21^{Aa}
ABL 04/49	1.17^{Aa}	0.96^{Bb}
Ash (%)		
ABL 99/30	5.98^{Aa}	6.46^{Aa}
ABL 04/49	6.53^{Aa}	6.48^{Aa}
Crude fiber (%)		
ABL 99/30	8.26^{Aa}	8.82^{Aa}
ABL 04/49	9.09^{Aa}	8.24^{Aa}

*Means followed by the same capital letters inside a column and small letters inside a row do not differ significantly (Tukey, 5%)

three formulations of composts for the production of four strains of *A. bisporus*, verified that the strain and the type of compost used influenced the production of mushrooms and also caused variations in the contents of crude protein, ash and crude fiber of mushrooms produced.

Conclusion

The use of the spent compost in the *A. blazei* cultivation can be considered a viable alternative since its use did not alter variables such as mass and number of mushrooms, yield and biological efficiency of mushrooms, and also did not compromise the nutritional composition of the mushrooms produced. Furthermore, according to the results obtained, the use of spent compost in new cultivation cycles of *A. blazei* is an alternative for the reduction of the production costs and the accumulation of these materials in the environment.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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