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Using of Appropriated Strains in the Practice of Compost Supplementation for *Agaricus subrufescens* Production

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The aim of this study was to analyse the viability of supplementation of *Agaricus subrufescens* compost with different organic materials, using three commercial strains. Compost was prepared by the traditional method and was used as a control (without supplementation). Six supplements were applied and can be separated into four categories: (i) commercial supplements (recommended to *Agaricus bisporus* and *Pleurotus ostreatus*); (ii) supplements based on agro-industrial waste (provided by peanut and acerola juice); (iii) supplements based on noble grains (a mix with bran of soybean, corn, and cotton); and (iv) a blend of supplements (ii) and (iii) (peanut waste, acerola juice waste, and noble grains—a mixture of 33.3% each). The results showed that the practice of supplementation is an important tool to improve the yield in the industrial production of *A. subrufescens*. Waste materials and noble grains can be selected as quality supplements. The use of appropriated strains is essential for the success of the supplementation practice.

Keywords: ***Agaricus blazei*, bioconversion, medicinal mushroom, sun mushroom, waste materials, yield**

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INTRODUCTION

An emerging *Agaricus* species, *Agaricus subrufescens* Peck, also named *Agaricus blazei* Murrill sensu Heinemann, *Agaricus rufotegulis* Nauta, or *Agaricus brasiliensis* Wasser, M. Didukh, Amazonas & Stamets, has been an actively cultivated mushroom in Brazil since 90's; when it was cultivated for first time in the Sao Paulo State and then extended to other regions close to the Atlantic coast (Farnet et al., 2014).

This ascend is because of the important medicinal proprieties of the mushrooms used for the treatment of cancer, leukemia, and hypertension, which are attributed to its active compounds, such as glycoproteins, β-d-glucans, saponins, steroids, tannins, polysaccharides, ergosterol, and fatty acids (Wang H. et al., 2013; Venkateshgobi et al., 2018). Previous studies reported that aqueous extracts of *A. blazei* offered the neuroprotective effect against the experimental model of Parkinson's disease and aging due to its anti-oxidant, anti-inflammatory, and anti-apoptotic functions (Nakanishi et al., 2014; Venkatesh Gobi et al., 2017, 2018).

During the last 5 years, various studies occurred involving several steps of production, with the objective of making commercial production more and more feasible, transforming family scale units to industrial scale production (Dias et al., 2013; Llarena-Hernández et al., 2014; Pardo-Giménez et al., 2014; Zied et al., 2014a; Carvalho Sousa et al., 2016).

Among the different steps of production, compost (formulation, phase I and II) is thought to be one of the most important, because it directly influences the productivity and quality of the mushrooms (Horm and Ohga, 2008; Matute et al., 2010; Llarena-Hernández et al., 2013; Wang J. T. et al., 2013; Souza et al., 2014). To improve the compost process, substrate supplementation has been used to provide nutrients to the developing mushroom, reducing the cultivation cycle and increasing productivity by up to 30% (Pardo-Giménez et al., 2012, 2016).

The practice of supplementation can be carried out at spawning, which does not demand additional production costs, except purchasing supplements that will be added to substrate. Currently, several companies commercialize supplements to be applied in the cultivation of *Agaricus bisporus* and *Pleurotus ostreatus*, although commercial supplement was not found for industrial scale of *A. subrufescens* production.

Therefore, the aim of this study was to analyse the efficiency of supplementation in *A. subrufescens* compost at spawning, with different sources of organic materials, using three commercial strains. In addition, this study considered the influence of the chemical characteristics of the supplements on the physiological development of the mushrooms.

MATERIALS AND METHODS

Strains

Three commercial strains were used, including ABL 16/01 and ABL 16/02 (provided by the company Funghi & Flora, Valinhos, Brazil) used by growers in Sao Paulo State and ABL 16/03 (codified as CS7—Carvalho Sousa et al., 2016) used by growers in Minas Gerais State. The strains are deposited in the public culture collection of Sao Paulo State University, Câmpus de Dracena (FCAT/UNESP), which allows access to interested researchers. Spawns of each strain were produced on sterile sorghum-based substrate (*Sorghum bicolor*) supplemented with gypsum (160 g kg^{-1}) and lime (20 g kg^{-1}). All preparation and sterilization procedures were done in accordance with Zied et al. (2010, 2014b).

Compost

The compost was prepared using the traditional method, lasting 22 days of phase I and 10 days of phase II, totalling 32 days. The formulation used had a dry weight of 1,000 kg of *Panicum maximum*, 1,500 kg of sugarcane bagasse, 50 kg of soybean, 5 kg of urea, 5 kg of ammonium sulfate, 10 kg of superphosphate, and 40 kg of limestone. The bulk materials (*P. maximum* straw and sugar cane bagasse) were moistened for 9 days and rotated after 2 days. The concentrated materials (soybean, urea, ammonium sulfate, simple superphosphate, and lime) were added after each turning operation throughout the composting phase I (**Table 1**). The compost remained for 18 h at 59 ± 1°C for pasteurization and 8 days at 47 ± 2°C for conditioning during phase II of composting. The chemical characteristics of the compost at the end of phase II are listed in **Table 1**.

Supplement

Six supplements were used in the research and can be separated into four categories: (i) commercial supplements (Spawn Mate II SE®–recommended to the production of *P. ostreatus* and Pro Mycel Gold®–recommended to the production of *A. bisporus*, both of Amycel and Spawn Mate company, Watsonville, US); (ii) supplements based on agro-industrial wastes (20% grain or nut and 80% hulls provided of peanut waste and acerola waste—special seeds, obtained after the extraction of the juice); (iii) supplement-based noble grains (bran of soybean, corn, and cotton—a mixture of 33.3% each); and (iv) a mix of supplements (ii) and (iii) (peanut waste, acerola juice waste, and noble grains—a mixture of 33.3% each), which was used in order to provide a balance of the chemical characteristics of the three supplements presented in category (ii) and (iii). Supplements (ii), (iii), and (iv) were dried at 68°C for 24 h (which served as a heat treatment) until reaching 4–6% moisture and then were crushed with a <0.5 mm sieve. Additionally, a substrate without supplement was used as a control. The chemical characteristics of each supplement used are listed in **Table 2**. The macro and micro nutrient content, organic matter (OM), C/N ratio, and pH were evaluated following the methodology presented by Bell and Ward (1984) and Sonneveld and van Elderen (1994).

Supplementation at Spawning

At spawning or at the end of the Phase II composting process, supplements were added to the compost in the amount of 1% wet weight of compost. At the same time, the strains were added to the compost in the amount of 1.5% of spawn wet weight of compost. Therefore, in each bag, we deposited 4 kg of compost with 40 g of supplement and 60 g of spawn. Spawn run occurred under controlled temperature (26°C), relative humidity (80%) and carbon dioxide content (3,000 ppm) conditions and was delayed 18 days.

Casing Layer

Soil with 350 g kg^{-1} of clay, 100 g kg^{-1} of silt, and 550 g kg^{-1} of sand was collected 2.0 m below surface, as recommended by Zied et al. (2011a), to produce *A. subrufescens* casing layer. Calcitic lime was added to the soil for pH correction as well as small fragments of charcoal in the proportion of 4:1 (v/v) to increase the porosity and reduce the density and compaction of the casing layer. When the mycelia were fully developed, the compost was pressed, and leveled to facilitate the addition of the casing layer to a height of 5 cm.

Production

The total production phase after casing addition was 90 days, with four flushes of mushroom harvested. The first one lasted 12 days, the second lasted 6 days, the third lasted 4 days, and finally, the fourth lasted 3 days. During the production, the air

TABLE 1 | Operations performed during the composting process (phase I) and chemical analysis of compost at the end of phase II.

Day	Procedures
01	Wetting of the straw and sugar cane bagasse (cord mounting)
04	1st turning of the compost and addition water
07	2nd turning of the compost and addition water
10	3rd turning and addition of soybean and water
13	4th turning and addition of urea, ammonium sulfate and simple superphosphate
16	5th turning and addition of lime and water
19	6th turning and addition of water
22	Filling the pasteurization tunnel (phase II)
32	Spawning and addition of supplements

Chemical characteristics of the compost

N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn	OM	C/N	pH
g kg^{-1}						mg kg^{-1}					%	ratio	
15 ± 0.7	2.9 ± 0.2	9 ± 0.6	50 ± 3.6	2.1 ± 0.4	6.4 ± 0.5	20 ±3	17 ± 1.2	7456 ± 17	141 ± 4,9	107 ± 9	53 ± 2	21 ± 1/7	6.1 ± 0.4

Each value is expressed as mean ± standard deviation (n = 3).

TABLE 2 | Chemical characteristics of the supplements.

Supplements	Chemical characteristics of the supplements													
	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn	OM	C/N	pH
	g kg^{-1}						g kg^{-1}					%	ratio	
Spawn mate II SE	44 ± 0.6	3.4 ± 0.5	17 ± 0.7	7 ± 1.1	2.4 ± 0.4	1.0 ± 0.2	26 ± 1	11 ± 1.1	334 ± 18	22 ± 2	48 ± 1.3	86 ± 1	11 ± 0.3/1	5.8 ± 0.5
Pro Mycel Gold	68 ± 0.4	3.9 ± 0.4	14 ± 0.8	6 ± 3	1.9 ± 0.5	2.8 ± 0.3	22 ± 3	11 ± 0.7	197 ± 8	18 ± 1	67 ± 1.4	87 ± 4	6 ± 1/1	5.7 ± 0.2
Waste of peanut	17 ± 0.3	0.5 ± 0.1	5 ± 1	19 ± 2	1.3 ± 0.2	0.7 ± 0.04	22 ± 2	15 ± 2	795 ± 57	52 ± 3	24 ± 1.6	85 ± 2	31 ± 2.8/1	6.1 ± 0.9
Waste of acerola juice	20 ± 0.7	1.8 ± 0.2	9 ± 2.4	21 ± 2.4	1.7 ± 0.7	0.9 ± 0.1	23 ± 2	11 ± 0.2	402 ± 34	16 ± 2	24 ± 0.8	82 ± 1	26 ± 0.7/1	6.8 ± 0.2
Noble grains[£]	55 ± 2	5.3 ± 2	16 ± 0.4	25 ± 4	3.2 ± 0.2	1.2 ± 0.5	21 ± 3	11 ± 0.4	174 ± 18	21 ± 1	49 ± 1.0	84 ± 1	11 ± 0.5/1	6.4 ± 0.4
Mix of supplements[ǂ]	26 ± 0.5	2.2 ± 0.4	11 ± 0.5	23 ± 2	1.8 ± 1.0	0.9 ± 0.2	22 ± 1	10 ± 0.3	394 ± 20	38 ± 1	29 ± 0.7	81 ± 2	18 ± 0.3/1	6.3 ± 0.1

[£]*A mixture of corn, soybean and cotton bran (33.3% each).*
[ǂ]*A combination of waste of peanut, waste of Acerola juice, and a mixture of noble grains (33.3% each). Each value is expressed as mean ± standard deviation (n = 3).*

temperature ranged from 18 to 30°C, the compost temperature ranged from 20 to 28°C, and the relative humidity was between 85 and 90%. For primordial induction, the temperature was reduced to 2°C per day until reaching 20°C following the methodology presented by Zied and Minhoni (2009). The basidiocarps were harvested manually, followed by scraping the base of the stipe to remove the casing layer residues. The analysis was subsequently performed to quantify (i) the yield calculated as 100 times the wet weight of the mushrooms divided by the wet weight of compost, expressed as a percentage (1st, 2nd, 3rd, and 4th flush and total yield); (ii) the number of mushrooms harvested; and (iii) the weight per mushroom, expressed in grams (total fresh weight harvested during the cycle) divided by the number of mushrooms by bag, as previously described by Zied et al. (2014b), Pardo-Giménez et al. (2016), and Zied et al. (2017).

Statistical Analyses

The experiment was carried out using a double factorial, completely randomized design, with seven supplementations (six supplements + control) × three strains, totalling 21 treatments, each with six replicates (represented by a box containing 4 kg of wet compost). The variations between the chemical compositions of the supplements were analyzed considering the similarity by using the Nearest Neighbor Method. The means of each variable were compared by the least significant difference (LSD) test at

$p < 0.05$ using SAS JMP software. Sigma Stat 3.5 software was used to calculate the linear correlations among the values for the yield (1st, 2nd, 3rd, 4th, and total), number, and weight of the basidiocarps and the chemical characteristics of the supplements.

RESULTS

The supplements formulated in this study had the highest Ca-values. Commercial supplements (Spawn Mate and Pro Mycel Gold) and noble grains showed the highest values of N, P, K, Mg, S, and Zn and, in this sense, are characterized as supplements with high nutritional content (**Table 2**). According to **Figure 1,** the macronutrient and micronutrient contents, OM, C/N ratio, and pH-values of the commercial supplements were considered similar, by using the Nearest Neighbor Method.

Supplements from the peanut and acerola juice waste showed the highest values of Fe and C/N ratio, and the peanut waste showed high Cu and Mn contents. The mix of supplements (33.3% peanut waste + 33.3% acerola juice waste + 33.3% noble grains) showed intermediate results, which were similar to the supplement formulated with the acerola juice waste, which had the highest proximity resulting in inferior distance in the Nearest Neighbor Method. The peanut waste supplement showed superior distance to the other supplements being the most different supplement from the chemical point of view when compared to the other supplements (**Figure 1**).

As the evaluation of the yield in the flushes were separately done, ABL 16/01 strain presented the highest results when the supplements were added, which can be checked comparing the

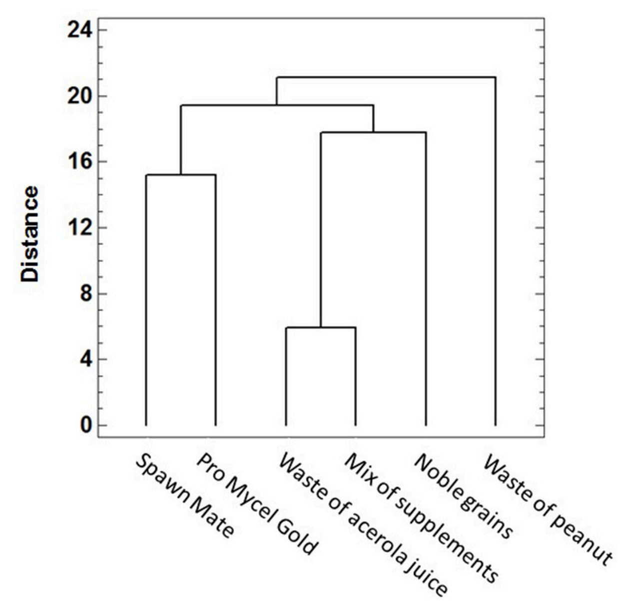

FIGURE 1 | Dendrogram (Nearest Neighbor Method, Squared Euclidean) generated from chemical characterizations of the supplements based on the macronutrients, micronutrients, organic matter, C/N ratio, and pH-values.

TABLE 3 | Results of yield in the 1st, 2nd, 3rd, and 4th flush, during the production phase of *A. subrufescens* cultivation.

Supplement/Treatment	1st flush (yield, %)			2nd flush (yield, %)			3rd flush (yield, %)			4th flush (yield, %)		
	ABL 16/01	ABL 16/02	ABL 16/03	ABL 16/01	ABL 16/02	ABL 16/03	ABL 16/01	ABL 16/02	ABL 16/03	ABL 16/01	ABL 16/02	ABL 16/03
Spawn mate II SE	4.28^{ABa}	2.70^{ABab}	1.81^{b}	7.36^{Ba}	2.85^{ABb}	4.02^{ab}	4.26^{Ca}	1.92^{ABb}	5.57^{ABa}	3.51^{Ba}	0.00^{Cb}	2.54^{Ba}
Pro Mycel Gold	6.05^{Aa}	1.84^{Bb}	2.17^{b}	9.57^{ABa}	4.92^{ABCb}	7.08^{ab}	3.38^{Cab}	2.02^{ABb}	5.14^{ABCa}	2.17^{CDb}	4.05^{Aa}	4.18^{Aa}
Waste of peanut	6.21^{Aa}	2.86^{ABb}	2.61^{b}	8.34^{AB}	6.21^{AB}	6.45	4.99^{BC}	3.12^{AB}	3.03^{CD}	7.45^{Aa}	0.67^{BCc}	2.31^{Bb}
Waste of acerola juice	3.52^{B}	2.00^{AB}	2.22	9.52^{AB}	6.33^{AB}	6.32	14.16^{Aa}	3.86^{Ab}	5.68^{Ab}	0.95^{Db}	0.00^{Cb}	2.74^{Ba}
Noble grains[£]	4.29^{Aba}	1.99^{ABb}	3.21^{ab}	8.19^{ABa}	1.71^{Cb}	6.96^{a}	4.11^{Ca}	1.58^{Bb}	3.38^{BCDab}	0.95^{Db}	1.86^{Bab}	2.71^{Ba}
Mix of supplements[‡]	4.97^{ABa}	2.22^{ABb}	2.78^{b}	11.73^{Aa}	4.44^{ABCb}	6.98^{b}	2.88^{C}	2.25^{AB}	2.54^{D}	3.03^{BCa}	0.00^{Cb}	2.13^{Ba}
Control (no supplemented)	4.55^{AB}	4.04^{A}	2.86	7.20^{B}	6.94^{A}	4.28	6.83^{Ba}	1.67^{ABb}	3.21^{CDb}	1.71^{CD}	0.85^{BC}	1.87^{B}
Media		3.29			6.54			4.08			2.17	

[£]*A mixture of corn, soybean, and cotton bran (33.3% each).* [‡]*A combination of waste of peanut, waste of acerola juice, and a mixture of noble grains (33.3% each). Spawn Mate II SE and Pro Mycel Gold are commercial supplements produced by the company Amycel. Values followed by different lower case letters within a line and upper case letters within a column are significantly different at t < 0.05.*

TABLE 4 | Results of total yield, number, and weight of mushroom, during the production phase of *A. subrufescens* cultivation.

Supplements/Treatments	Total yield, %			Number of mushrooms, u			Weight per mushroom, g		
	ABL 16/01	**ABL 16/02**	**ABL 16/03**	**ABL 16/01**	**ABL 16/02**	**ABL 16/03**	**ABL 16/01**	**ABL 16/02**	**ABL 16/03**
Spawn Mate II SE	19.42^{Ca}	7.48^{Bc}	13.95^{Bb}	19.5^{Ba}	5.83^{b}	14.00^{Ba}	30.41^{BCb}	48.44^{BCa}	32.55^{ABb}
Pro Mycel Gold	21.18^{Ca}	12.85^{Ab}	18.58^{Aa}	18.5^{Ba}	7.83^{b}	18.50^{Ba}	35.55^{ABCb}	65.79^{Aa}	34.89^{Ab}
Waste of peanut	27.00^{ABa}	12.87^{Ab}	14.42^{ABb}	31.33^{Aa}	9.16^{c}	16.00^{Bb}	25.70^{Cb}	53.38^{Ba}	28.02^{ABb}
Waste of acerola juice	28.16^{Aa}	12.20^{ABb}	16.97^{ABb}	21.83^{Bb}	8.33^{c}	28.33^{Aa}	40.19^{ABb}	53.61^{Ba}	23.71^{Bc}
Noble grains£	17.55^{Ca}	7.15^{Bb}	16.27^{ABa}	17.50^{Ba}	5.16^{b}	18.33^{Ba}	33.68^{ABCb}	48.86^{BCa}	31.18^{ABb}
Mix of supplementsǂ	22.63^{BCa}	8.92^{Bc}	14.45^{ABb}	20.00^{Ba}	7.16^{b}	13.66^{Ba}	34.94^{ABC}	39.72^{C}	33.73^{AB}
Control (no supplemented)	20.30^{Ca}	13.51^{Ab}	12.24^{Bb}	16.33^{B}	10.33	14.00^{B}	41.29^{Aa}	43.32^{BCa}	28.56^{ABb}
Media		16.10			15.34			38.45	

£*A mixture of corn, soybean and cotton bran (33.3% each).*
ǂ*A combination of waste of peanut, waste of acerola juice, and a mixture of noble grains (33.3% each). Spawn Mate II SE and Pro Mycel Gold are commercial supplements produced by the company Amycel. Values followed by different lower case letters within a line and upper case letters within a column are significantly different at $t < 0.05$.*

yield values in each flush (1st, 2nd, 3rd, and 4th) with the other strains. In the substrate control, there was no significant difference among yield values in each flush by the strains, except the third flush (**Table 3**).

Considering the 1st flush, the Pro Mycel Gold and peanut waste supplements provided the highest yields with ABL 16/01 strain. The substrate control provided the highest yield with ABL 16/02 strain, and ABL 16/03 strain showed no response to substrate supplementation. Although ABL 16/03 strain did not show a significant difference due to the supplementation, it was verified that Ca contents (21–25 g kg^{-1}) and B content (23–26 mg kg^{-1}) of the supplements positively and negatively influenced the yield values during the 1st flush (**Table 5**).

In the 2nd flush, the mix of supplement provided the highest yield with ABL 16/01. Once again, the substrate control provided the highest yield with ABL 02/16 strain, and ABL 16/03 strain showed no response to supplementation. Negative correlations were observed between the P (3.4–5.3 g kg^{-1}), K (14–17 g kg^{-1}), Mg (1.9–3.2 g kg^{-1}), and B (26 mg kg^{-1}) contents in the 2nd flush for ABL 16/02 and ABL 16/03 strains. The ABL 16/03 strain seems to be sensitive to the B contents of the supplements.

In the 3rd flush a particular results with the acerola juice waste provided the highest yield, significantly higher than control substrate in the three strains studied (**Table 3**), which may indicate a slow release of nutrients from this supplement to the mushroom, although correlation was not observed between any strains, chemical characteristics and 3rd flush (**Table 5**).

It is important to emphasize the influence of supplementation in the last flush (4th), to all stains used, when compared with the control. Some chemical characteristics may explain the positive contribution of the supplements in the high yield obtained: (i) the amount of Cu (close to 15 mg kg^{-1}), Fe (close to 795 mg kg^{-1}), and Mn (close to 52 mg kg^{-1}) for the ABL 16/01 strain; and (ii) the amount of S (close to 2.8 mg kg^{-1}) for the ABL 16/02 and the ABL 16/03 strain (**Tables 2**, **3**).

The only strain that did not respond to supplementation in the total yield was the ABL 16/02, for which superior yield was observed with the substrate control (**Table 4**). The addition of acerola juice waste to the substrate cultivated with the ABL 16/01 strain resulted in a significant superior total yield due to the low P (1.8 g kg^{-1}), K (9 g kg^{-1}), and Mg (1.7 g kg^{-1}) contents, which directly negatively influences the number of mushrooms harvested (**Table 5**). The high values of the C/N ratio (26) provided an increase in the total yield for ABL 16/01 strain. The amount of Cu and Mn provided a positive correlation with the number of mushrooms for the ABL 16/01 strain. Finally, the ABL 16/03 strain responded to the addition of supplements with high N content, providing significant superior yield.

Although correlation found among the chemical characteristics of the substrates and the weight of the mushrooms, ABL 16/02 strain provided mushrooms with high mushroom weight, as well as Pro Mycel Gold ones (**Table 4**).

DISCUSSION

The supplementation of the compost or substrate at spawning is an important and necessary practice used in *A. bisporus* and *P. ostreatus* cultivation to improve the quality of the mushroom and the efficiency of the crop during commercial production. Although supplements are not marketed around the world for the commercial production of *A. subrufescens*, upgrading the productive process can make a difference for the development of this important medicinal species.

Commercial supplements are marketed in different countries with a high potential for mushroom industry. Currently, the countries with higher mushroom production are China, USA, Poland, Netherlands, India, France, Spain, Canada, Mexico, and others (Royse et al., 2017). Some of these countries, like China and India, do not have companies that commercialize supplements to be applied in the substrate for mushroom production. In addition to these large mushroom-producing countries, other countries also produce mushrooms with a regular production scale, such as Brazil, Argentina, South Africa, Iran, Thailand, Vietnam, etc. Therefore, in our understanding, the practice of supplementation can also be performed in

TABLE 5 | Correlations between chemical characteristics of the supplements and the production parameters of the mushroom cultivation.

Person Correlation	Production parameters		
Chemical characteristics	**ABL 16/01**		
	4th flush	**Total yield**	**Number of mushroom**
P	–	−0.8925	−0.8492
		0.0167	0.0324
K	–	−0.9042	−0.8694
		0.0133	0.0245
Mg	–	−0.8577	–
		0.0291	
Cu	0.8345	–	0.9204
	0.0388		0.0093
Fe	0.8698	–	0.9762
	0.0243		0.0008
Mn	0.8839	–	–
	0.0194		
C/N	–	0.8138	–
		0.0482	

Chemical characteristics	**ABL 16/02**		
	2nd flush	**4th flush**	**Number of mushroom**
P	−0.8445	–	−0.8405
	0.0344		0.0362
K	−0.8577	–	−0.8895
	0.0289		0.0176
Mg	−0.9406	–	−0.9444
	0.0052		0.0045
S	–	0.9256	–
		0.0081	

Chemical characteristics	**ABL 16/03**			
	1st flush	**2nd flush**	**4th flush**	**Total yield**
N	–	–	–	0.8188
				0.0463
Ca	0.8211	–	–	
	0.0452			
S	–	–	0.9640	–
			0.0019	
B	−0.8257	−0.9616	–	–
	0.0429	0.0022		

First data correspond to r-values (degree of significance) and second data correspond to the P-values (probability). Values in bold mean: r-values above ± 0.7 and P-values below 0.05.

these countries using agricultural wastes or even noble grain meal.

In our literature review, two reports were found that utilized practice of substrate supplementation in the production of *A. subrufescens* (Kopytowski Filho et al., 2008; Dias et al., 2014). These studies had positive and negative results regarding the practice of supplementation. In both reports, the authors did not verify the influence of chemical characteristics of the supplements as a function of the physiological development of the mushrooms (1st, 2nd, 3rd, 4th, total yield, number, and weight of mushroom), beside, they did not use three different strains.

The present research confirms the importance of the supplementation practice, not just with commercial supplements but also with supplements based on agricultural wastes (peanut and acerola juice). The supplements based on noble grains may be used in some countries and have very similar chemical characterizations to the commercial supplements (**Figure 1**). The use of an appropriated strain is fundamental to supplementation practice. Subsequently, several companies that market supplements also market strains (i.e., Amycel and Lambert), providing greater safety in the production process.

The addition of acerola juice waste with the ABL 16/01 strain improved the total yield ∼39%, and the addition of Pro Mycel Gold in the ABL 16/03 strain improved yield ∼51% relative to the unsupplemented compost.

Due to the use of six different sources of organic supplements and three commercially grown strains in Brazil, it was possible to find some correlations that may be useful for the formulation or use of a waste as a substrate supplement to produce *A. subrufescens*. The most important elements that were verified in the two strains (ABL 16/01 and ABL 16/02) are P, K, and Mg, whose contents above 3.4, 14, and 1.9 g kg^{-1} can reduce the yield of the mushrooms (in the flush analyzed separately or in total yield). Sinden in 1949 already warned the mushroom producers about the problem of Mg toxicity, at this time the approach was carried out in the correction of the casing layer pH using dolomitic limestone for *A. bisporus* cultivation (Atkins, 1974). Later Zied et al. (2012) verified the same negative effect of Mg using dolomitic limestone in *A. subrufescens* cultivation.

S content was also verified as an important macronutrient in two strains (ABL 16/02 and ABL 16/03) in the 4th flush, whose content close to 2.8 g kg^{-1} provided high yield (**Tables 2**, **3**). The use of S in the form of calcium sulfate ($CaSO_4$) is well-documented in the cultivation of mushrooms, being added as an ingredient in the preparation of substrate of the species *Pleurotus* spp., *Lentunula edodes*, *A. bisporus* in different amounts for improving the physical characteristics of the substrate and provide Ca and S for mushroom nutrition (Liyama et al., 1994; Curvetto et al., 2002; Mandeel et al., 2005; Uddin et al., 2013). The macronutrient values found in the correlation can be used as reference for future studies involving dose response of an element and yield of mushroom, i.e., increasing doses of S in the substrate (2.0, 2.4, 2.8, 3.2, 3.6 g kg^{-1}).

The B content also reduced the harvest in the 1st and 2nd flush in the ABL 03/16 strain. Ca, S, Cu, Fe, and Mn were found to contribute positively to the physiological development of the mushroom. Estrada Rodrigues and Royse (2007) also verified the positive effect of these micronutrients (Mn and Cu) when applied to the substrate on the yield of *Pleurotus eryngii*.

The N content and the C/N ratio provided different reactions depending on the strain used (**Table 5**). Some studies reported the influence of the N content and the C/N ratio in the production of the compost for *A. subrufescens* production (Zied and Minhoni, 2012; Llarena-Hernández et al., 2014; Pardo-Giménez et al., 2014). In Brazil, the compost used for *A. subrufescens* production has a C/N ratio close to 27/1 (at the end of Phase II), and in Europe, the compost has a C/N ratio close to 18/1 (at the end of Phase II) (Andrade et al., 2007; Siqueira et al., 2011; Zied et al., 2011b; Llarena-Hernández et al., 2013, 2014).

The difference in the C/N ratio used in the different continents is related to the method of preparation of the compost (formulation, Phase I, and Phase II). In Europe, the compost used in the production of *A. subrufescens* is the same as the compost prepared to *A. bisporus* production, and in Brazil, a poorer compost with a higher C/N ratio is used specifically for *A. subrufescens* production. In this sense, we can consider not only the method of preparation of the compost but also the nutritional and specific demand of the macro and micronutrient of the strain used. There are strains more demanding in N content and others in C content.

Finally, the composting method used to produce *A. subrufescens* is still not ideal, despite advances and the publication of new information. It is not even possible to obtain the highest yield in the first flush of the harvest. In the present manuscript, the highest yields were obtained in the 2nd (media of 6.54%) and 3rd flushes (media of 4.08%) (**Table 3**). Other authors have also verified the low yield in the 1st flush and the long cycle of crop (Zied and Minhoni, 2009; Colauto et al., 2011; Zied et al., 2011b; Pardo-Giménez et al., 2014; Martos et al., 2017).

CONCLUSION

The practice of supplementation is an important tool for improving yield in the industrial production of *A. subrufescens*. Waste materials and noble grains can be selected as quality supplements. The use of appropriated strains is essential to the success of the practice of supplementation. We suggest that materials with high Mg content should be avoided on the selection of an ideal supplement, while materials with high S, Cu, and Mn contents should be selected as ideal supplements. Therefore, other studies should be performed to better understand this relationship in an experimental crop, to know, with safety, which macro and micronutrients should be present in a commercial supplement to be used in large scale by the mushroom industry.

AUTHOR CONTRIBUTIONS

DZ assisted in the experimental design, supervised the execution and the interpretation of the data, CC and MZ carried out the experiments, JP analyzed the results, ED and AP-G coordinated the statistical and wrote the manuscript. All authors read and approved the final manuscript.

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REFERENCES

Andrade, M. C. N., Kopytowski Filho, J., Minhoni, M. T. A., Coutinho, L. N., and Figueiredo, M. B. (2007). Productivity, biological efficiency, and number of *Agaricus blazei* mushrooms grown in compost in the presence of *Trichoderma* sp. and Chaetomium olivacearum contaminants. *Braz. J. Microbiol.* 38, 243–247. doi: 10.1590/S1517-83822007000200010

Atkins, F. C. (1974). *Guide to Growing Mushrooms*. London: Faber and Faber.

Bell, D. T., and Ward, S. C. (1984). Foliar and twig macronutrients (N, P, K, Ca and Mg) in selected species of *Eucalyptus* used in rehabilitation: sources of variation. *Plant Soil* 81, 363–376. doi: 10.1007/BF02323051

Carvalho Sousa, M. A., Zied, D. C., Marques, S. C., Rinker, D. L., Alm, G., and Dias, E. S. (2016). Yield and enzyme activity of different strains of almond mushroom in two cultivation systems. *Sydowia* 68, 35–40. doi: 10.12905/0380.sydowia68-2016-0035

Colauto, N. B., Silveira, A. R., Eira, A. F., and Linde, G. A. (2011). Pasteurization of brazilian peat for *Agaricus brasiliensis* cultivation. *Semin. Ciênc. Agrár.* 31, 1331–1336. doi: 10.5433/1679-0359.2010v31n4Sup1p1331

Curvetto, N., Figlas, D., and Delmastro, S. (2002). Sunflower seed hulls as substrate for the cultivation of shiitake mushrooms. *HortTechnology* 12, 652–655.

Dias, E. S., Zied, D. C., Alm, G., and Rinker, D. L. (2014). Supplementation of compost for *Agaricus subrufescens* cultivation. *Ind. Biotechnol.* 10, 130–132. doi: 10.1089/ind.2013.0040

Dias, E. S., Zied, D. C., and Rinker, D. L. (2013). Physiologic response of *Agaricus subrufescens* using different casing materials and practices applied in the cultivation of *Agaricus bisporus*. *Fungal. Biol.* 117, 569–575. doi: 10.1016/j.funbio.2013.06.007

Estrada Rodrigues, A., , and Royse, D. J. (2007). Yield, size and bacterial blotch resistance of *Pleurotus eryngii* grown on cottonseed hulls/oak sawdust supplemented with manganese, copper and whole ground soybean. *Bioresour. Technol.* 98, 1898–1906. doi: 10.1016/j.biortech.2006.07.027

Farnet, A. M., Qasemian, L., Peter-Valence, F., Ruaudel, F., Savoie, J. M., Roussos, S., et al. (2014). Do spawn storage conditions influence the colonization capacity of a wheat-straw-based substrate by *Agaricus subrufescens*? *C. R. Biol.* 337, 443–450. doi: 10.1016/j.crvi.2014.06.002

Horm, V., and Ohga, S. (2008). Potential of compost with some added supplementary materials on the development of *Agaricus blazei* Murill. *J. Fac. Agr. Kyushu Univ.* 53, 417–422.

Kopytowski Filho, J., Minhoni, M. T. A., Andrade, M. C. N., and Zied, D. C. (2008). Effect of compost supplementation (soybean meal and Champfood) at different phases (spawning and before casing) on productivity of *Agaricus blazei* ss. Heinemann (*A. brasiliensis*). *Mush. Sci.* 17, 260–270.

Liyama, K., Stone, B. A., and Macauley, B. J. (1994). Compositional changes in compost during composting and growth of *Agaricus bisporus*. *Appl. Environ. Microbiol.* 60, 1538–1546.

Llarena-Hernández, C. R., Largeteau, M. L., Ferrer, N., Regnault-Roger, C., and Savoie, J. M. (2014). Optimization of the cultivation conditions for mushroom production with European wild strains of *Agaricus subrufescens* and Brazilian cultivars. *J. Sci. Food Agric.* 94, 77–84. doi: 10.1002/jsfa.6200

Llarena-Hernández, R. C., Largeteau, M. L., Farnet, A. M., Foulongne-Oriol, M., Ferrer, N., Regnault-Roger, C., et al. (2013). Potential of European wild strains of *Agaricus subrufescens* for productivity and quality on wheat straw based compost. *World J. Microbiol. Biotechnol.* 29, 1243–1253. doi: 10.1007/s11274-013-1287-3

Mandeel, Q. A., Al-Laith, A. A., and Mohamed, S. A. (2005). Cultivation of oyster mushrooms (Pleurotus spp.) on various lignocellulosic wastes. *World J. Microbiol. Biotechnol.* 21, 601–607. doi: 10.1007/s11274-004-3494-4

Martos, E. T., Zied, D. C., Junqueira, P. P. G., Rinker, D. L., Silva, R., Toledo, R. C. C., et al. (2017). Casing layer and effect of primordia induction in the production of *Agaricus subrufescens* mushroom. *Agr. Nat. Resour.* 51, 231–234. doi: 10.1016/j.anres.2017.04.003

Matute, R. G., Figlas, D., and Curvetto, N. (2010). Sunflower seed hull based compost for *Agaricus blazei* Murill cultivation. *Int. Biodeterior. Biodegrad.* 64, 742–747. doi: 10.1016/j.ibiod.2010.08.008

Nakanishi, A. B., Soares, A. A., Natali, M. R., Comar, J. F., Peralta, R. M., and Bracht, A. (2014). Effects of the continuous administration of an *Agaricus blazei* extract to rats on oxidative parameters of the brain and liver during aging. *Molecules* 19, 18590–18603. doi: 10.3390/molecules191118590

Pardo-Giménez, A., Catalán, L., Carrasco, J., Álvarez-Ort,í, M., Zied, D. C., and Pardo, J. E. (2016). Effect of supplementing crop substrate with defatted pistachio meal on *Agaricus bisporus* and *Pleurotus ostreatus* production. *J. Sci. Food Agr.* 96, 3838–3845. doi: 10.1002/jsfa.7579

Pardo-Giménez, A., González, J. E. P., Figueirêdo, V. R., and Zied, D. C. (2014). Adaptabilidad de cepas brasileñas de *Agaricus subrufescens* Peck a la fructificación sobre diferentes capas de cobertura en cultivo comercial. *Rev. Iberoam. Micol.* 31, 125–130. doi: 10.1016/j.riam.2013.05.002

Pardo-Giménez, A., Zied, D. C., Álvarez-Ort,í, M., Rubio, M., and Pardo, J. E. (2012). Effect of supplementing compost with grapeseed meal on *Agaricus bisporus* production. *J. Sci. Food Agr.* 92, 1665–1671. doi: 10.1002/jsfa.5529

Royse, D. J., Baars, J., and Tan, Q. (2017). "Current overview of mushroom production in the world," in *Edible and Medicinal Mushrooms: Technology and Applications*, eds D. C. Zied and A. Pardo-Giménez (West Sussex: Wiley-Blackwell), 5–13.

Siqueira, F. G., Martos, E. T., Silva, E. G. D., Silva, R. D., and Dias, E. S. (2011). Biological efficiency of *Agaricus brasiliensis* cultivated in compost with nitrogen concentrations. *Hortic. Bras.* 29, 157–161. doi: 10.1590/S0102-05362011000200004

Sonneveld, C., and van Elderen, C. W. (1994). Chemical analysis of peaty growing media by means of water extraction. *Commun. Soil Sci. Plant Anal.* 25, 3199–3208. doi: 10.1080/00103629409369258

Souza, T. P., Marques, S. C., Santos, D. M. D. S., and Dias, E. S. (2014). Analysis of thermophilic fungal populations during phase II of composting for the cultivation of *Agaricus subrufescens*. *World J. Microbiol. Biotechnol.* 30, 2419–2425. doi: 10.1007/s11274-014-1667-3

Uddin, M. J., Haque, S., Haque, M. E., Bilkis, S., and Biswas, A. K. (2013). Effect of different substrate on growth and yield of button mushroom. *J. Environ. Sci. Nat. Res.* 5, 177–180. doi: 10.3329/jesnr.v5i2.14810

Venkateshgobi, V., Rajasankar, S., Johnson, W. M. S., Prabu, K., and Ramkumar, M. (2018). Neuroprotective effect of A*garicus blazei* extract against rotenone-induced motor and nonmotor symptoms in experimental model of Parkinson's disease. *Int. J. Nutr. Pharmacol. Neurol. Dis.* 8, 59–65. doi: 10.4103/ijnpnd.ijnpnd_20_18

Venkatesh Gobi, V., Rajasankar, S., Ramkumar, M., Dhanalakshmi, C., Manivasagam, T., Justin Thenmozhi, A., et al. (2017). *Agaricus blazei* extract abrogates rotenone-induced dopamine depletion and motor deficits by its anti-oxidative and anti-inflammatory properties in Parkinsonic mice. *Nutr. Neurosci.* 19,1–10. doi: 10.1080/1028415X.2017.1337290

Venkatesh Gobi, V., Rajasankar, S., Ramkumar, M., Dhanalakshmi, C., Manivasagam, T., Justin Thenmozhi, A., et al. (2018). *Agaricus blazei* extract attenuates rotenone-induced apoptosis through its mitochondrial protective and antioxidant properties in SH-SY5Y neuroblastoma cells. *Nutr. Neurosci.* 21, 97–107. doi: 10.1080/1028415X.2016.1222332

Wang, H., Fu, Z., and Han, C. (2013). The medicinal values of culinary-medicinal royal sun mushroom (*Agaricus blazei* Murrill). *Evid. Based Complement Altern. Med.* 8, 59–65. doi: 10.1155/2013/842619

Wang, J. T., Wang, Q., and Han, J.R. (2013). Yield, polysaccharides content and antioxidant properties of the mushroom *Agaricus subrufescens* produced on different substrates based on selected agricultural wastes. *Sci. Hortic.* 157, 84–89. doi: 10.1016/j.scienta.2013.04.006

Zied, D. C., and Minhoni, M. D. A. (2009). Influence of the environment of production in yield of mushroom *Agaricus blazei* ss. Heinemann (*A. brasiliensis*). *Energ. Agric.* 24, 17–34.

Zied, D. C., and Minhoni, M. T. A. (2012). Indoor composting methods for growing *Agaricus subrufescens* and the chemical characteristics of basidiomata. *Energ. Agric.* 27, 45–59. doi: 10.17224/EnergAgric.2012v27n4p45-59

Zied, D. C., Minhoni, M. T.A., Kopytowski-Filho, J., and Andrade, M. C. N. (2010). Production of *Agaricus blazei* ss. Heinemann (A. brasiliensis) on different casing layers and environments. *World J. Microbiol. Biotechnol.* 26, 1857–1863. doi: 10.1007/s11274-010-0367-x

Zied, D. C., Minhoni, M. T. A., Kopytowski-Filho, J., Barbosa, L., and Andrade, M. C. N. (2011a). Medicinal mushroom growth as affected by non-axenic casing soil. *Pedosphere* 21, 146–153. doi: 10.1016/S1002-0160(11)60112-4

Zied, D. C., Pardo-Gimenez, A., Savoie, J.M., Pardo, J.E., and Callac, P. (2011b). ""Indoor" method of composting and genetic breeding of the strains to improve yield and quality of the almond mushroom *Agaricus subrufescens*," in *7th International Conference on Mushroom Biology and Mushroom Products* (Arcachon, INRA), 424–432.

Zied, D. C., Pardo, J. E., Thomaz, R. S., Miasaki, C. T., and Pardo-Gimenez, A. (2017). Mycochemical characterization of *Agaricus subrufescens* considering their morphological and physiological stage of maturity on the traceability process. *Biomed. Res. Int.* 2017:2713742. doi: 10.1155/2017/2713742

Zied, D. C., Pardo-Giménez, A., de Almeida Minhoni, M. T., Boas, R. V., Alvarez-Orti, M., and Pardo-González, J. E. (2012). Characterization, feasibility and optimization of *Agaricus subrufescens* growth based on chemical elements on casing layer. *Saudi J. Biol. Sci.* 19, 343–347. doi: 10.1016/j.sjbs.2012.04.002

Zied, D. C., Pardo-Gimenez, A., Pardo, J. E., Dias, E. S., Carvalho, M. A., and Minhoni, M. T. A. (2014a). Effect of cultivation practices on the β-glucan content of *Agaricus subrufescens* basidiocarps. *J. Agric. Food Chem.* 62, 41–49. doi: 10.1021/jf403584g

Zied, D. C., Penachio, S. M., Dias, E. S., Minhoni, M. T. A., Ferraz, R. A., and Vieites, R. L. (2014b). Influence of productivity and processing method on physicochemical characteristics of white button mushrooms in Brazil. *J. Sci. Food Agr.* 94, 2850–2855. doi: 10.1002/jsfa.6624

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