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ORIGINAL ARTICLE

Characterization, feasibility and optimization of *Agaricus subrufescens* growth based on chemical elements on casing layer

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Abstract The aim of this study was to analyze yields, biological efficiency, earliness (expressed as days to first harvest), and precociousness and establish models for the mushroom growing according to these parameters. The experiment followed a double factorial design with four sources of calcium (calcitic limestone, calcitic limestone + gypsum, dolomitic limestone and dolomitic limestone + gypsum) and 2 application times (25 days before casing and at the moment of casing), with 4 replicates for each treatment. Different calcium sources influenced differently *Agaricus subrufescens* production, especially as regards earliness, which showed significantly higher values when dolomitic

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limestone with gypsum was applied. Yield and biological efficiency were negatively correlated with H + AL, organic matter and Mg amount. Furthermore, earliness was positively correlated with H + Al, organic matter, and the amount of Mg and Fe. Finally, negative correlations were observed between precociousness and the amount of Ca, SB (sum of base), CEC (cation exchange capacity) and V% (percentage of base saturation). The models presented in this work are extremely important for predicting the agronomic performance of *Agaricus subrufescens* on the basis of chemical analysis provided by the casing soil.

1. Introduction

The *Agaricus subrufescens*, a synonym of *Agaricus blazei* ss. Heinemann (*A. brasiliensis*), is a Basidiomycete fungus called the Medicinal Mushroom or Almond Mushroom. It has many medicinal properties such as the presence of glucan proteins with tumor-inhibition activity. It is grown in lignocellulosic composts with a C/N ratio of 37/1 (Kopytowski Filho et al., 2006). A critical step in the production of this mushroom is the addition of the casing layer, which is the material used as a top covering of the compost. Many materials can be used as casing layers, like soil, peat, coconut fiber, spent mushroom substrate, etc. The casing layer affects the metabolic stress, influencing the formation of basidiocarps due to its chemical properties (Hayes, 1981), such as pH, cation exchange capacity, concentration of soluble salts, non-decomposed organic matter, electrical conductivity, nitrogen and magnesium amount, and the level of V% "percentage of base saturation" (Couvy, 1972; Masaphy et al., 1989; Nair and Gokulapalan, 1994; Pardo et al., 2003; Zied et al., 2011).

In 2005–2006, a study conducted in the Piedade region (Brazil) found that the soil without additives was the preferred casing layer for the 50% of growers, followed by soil + charcoal (37.5%) and soil + vermiculite + charcoal (12.5%), to which calcitic or dolomitic limestone was added to correct the pH of soil (Andrade et al., 2006). Furthermore, the use of dolomitic limestone in the casing layer for *Agaricus bisporus* cultivation has been reported to delay fruiting and reduce total mushroom production as much as 50% when compared to the use of magnesium-free limestone (Atkins, 1974).

Magnesium was found to have a toxic effect on pure culture (Treschow, 1944; Gandy, 1953). In addition, Atkins (1974) observed that high magnesium content in the calcium source could lead to lower values of mushroom yield. Thus, calcium carbonate ($CaCO_3$) could be used to correct soil acidity as it is able to elevate pH and keep low Mg values (Oei, 2003). Other types of calcium sources used are spent lime (extract from sugar beet), gypsum ($CaSO_4$) and limestone (calcitic, dolomitic and magnesian) with several differences among them, including the MgO level.

The aim of this work was to find out the extent of these data that can influence *A. subrufescens* growth, a mushroom of the same family and genus of *A. bisporus*. To establish a model of *A. subrufescens* growth, the yield, biological efficiency, earliness (expressed as days to first harvest), and precociousness of mushroom cultivation was analyzed.

2. Materials and methods

2.1. Spawn

Strain ABL-99/30 was used and collected from commercial growers in the Piedade region in the Sao Paulo State (Brazil). This strain's fruiting takes place at low temperatures (±23 °C) and mushrooms are medium-sized. The procedures adopted by Zied et al. (2009) were followed for spawn production.

2.2. Compost

Organic compost was prepared using sugar cane bagasse and cost cross straw, as described by Andrade et al. (2008) with a final C/N ratio of 17.98, total N content of 2.24 (%), pH of 6.6 and moisture of 69.20 (%). After conditioning, compost was spawned at a rate of 1.2 g kg^{-1} of fresh weight (f.w.) of compost.

2.3. Casing

The used casing basis was 75% of soil (loamy Oxisol-Alfisol) + 25% of vegetable charcoal, which was treated with 4 different calcium sources (calcitic limestone "CL"; calcitic limestone + gypsum "CL + G"; dolomitic limestone "DL" and dolomitic limestone + gypsum "DL + G") at 2 application times (20 days before casing and at the moment of casing), to increase the levels of pH between 6.0 and 7.0. The soil "in nature" had a pH of 4.3 (Table 1).

2.4. Mushroom cultivation

The experiment was carried out in a plastic greenhouse and lasted 120 days; the first flush was harvested between 24 and 29 days after casing, and primordia formation was observed 22 days after casing. Six flushes were harvested over the cultivation cycle.

2.5. Experimental design

The experiment followed a double factorial design and included 8 treatments (4 sources of calcium × 2 application times) and 4 replicates.

2.6. Evaluated data

Before the breaking of lamellas (their optimal commercial development stage), mushrooms were harvested, counted and

Table 1 Chemical analysis of four calcium sources in two application times in the casing soil (soil + charcoal), and the only the soil analysis "in nature" (without calcium sources and charcoal).

Chemical analysis	pH $CaCl_2$	$H^+ + Al^{3+}$ (mmol dm^{-3})	O.M. (g kg^{-1})	P (mg dm^{-3})	K^+ (mmol dm^{-3})	Ca^{2+}	Mg^{2+}	SB	CEC	V (%)	Fe (ppm)	Mn (ppm)
CL	6.44 AB	16.53 B	11.43 B	5.92 A	1.77 A	13.52 B	0.49 B	15.79 B	32.32 B	48.48 AB	15.55 B	2.32 A
CL + G	6.56 A	16.69 B	11.56 B	7.66 A	2.73 A	22.81 A	1.05 B	26.59 A	43.29 A	59.95 A	15.76 B	1.37 B
DL	6.30 B	19.88 A	11.85 B	4.39 A	1.55 A	6.92 B	3.82 A	12.30 B	32.18 B	37.72 B	18.53 A	1.48 B
DL + G	6.02 C	21.98 A	12.76 A	3.69 A	1.38 A	10.98 B	4.18 A	16.55 AB	38.54 AB	41.64 B	18.75 A	1.37 B
20 days bc*	6.20 b	19.52 a	11.89 a	4.04 b	1.48 a	13.68 a	2.33 a	17.51 a	37.03 a	16.40 b	16.40 b	1.53 a
At casing	6.49 a	18.02 a	11.92 a	6.79 a	2.23 a	13.43 a	2.43 a	18.10 a	36.13 a	49.28 a	17.90 a	1.74 a
Average	6.33	18.78	11.91	5.42	1.86	13.56	2.39	17.81	36.59	46.95	17.15	1.64
Soil	4.31	25.38	7.46	5.21	0.75	3.65	0.82	5.23	30.52	17.08	9.08	0.66

CL: calcitic limestone; CL + G: calcitic limestone + gypsum; DL: dolomitic limestone; DL + G: dolomitic limestone + gypsum.
Capital letters compare different sources of calcium and lower case letters, the time of application; Tukey test (critical value $\leqslant 0.05$).
* Before casing.

weighed daily. The yield was calculated as f.w. of mushroom divided by f.w. of compost multiplied by 100, and expressed as%. The biological efficiency, a practical estimate of the ability of mushrooms to convert substrate into fruiting bodies was calculated as f.w. of mushroom divided by dry weight of compost multiplied by 100, expressed as%. The earliness was calculated as the number of days between the casing and the first harvest as reported by Mamiro and Royse (2008) and Pardo et al. (2003). The precociousness (total harvest time divided by first half of harvest time multiplied by 100, expressed as%) was calculated as reported by Zied et al. (2010). Finally, the chemical properties of casing (pH, $H^+ + Al^{3+}$, organic matter, P, K^+, Ca^{2+}, Mg^{2+}, Fe, Mn, sum of base – SB, cation exchange capacity – CEC and percentage of base saturation – V%) were determined as reported by Teixeira et al. (2008).

2.7. Statistical analysis

The SAS statistical software was used for the variance analysis, and a Tukey test ($P \leqslant 0.05$) was used to separate the means by analyzing all the variables. Finally, multiple linear regression models were done with a forward stepwise regression method of the "Multiple Regression" procedure (Statgraphics). Differences were considered significant for $P < 0.05$.

3. Results and discussion

For years, mushroom growers have been warned about avoiding dolomite in their casings because magnesium delays the onset of fruiting and lowers *A. bisporus* yields (Kurtzman, 1991). Similar results were observed in our data with *A. subrufescens* cultivation.

The application of calcitic limestone (with or without gypsum) increased the pH values more than the other two treatments, which probably anticipated the first flush by 2.2–2.6 days (Table 2). Treatments with DL and DL + G showed higher values of H + AL, organic matter, Mg and Fe than CL and CL + G treatments (Table 1). These treatments showed the later harvest of the first flush (Table 3), which showed a positive correlation with earliness. Despite earliness can be a great advantage, it was negatively correlated with yield and biological efficiency ($r = -0.75$). The highest yield in the first flush was observed in the DL and DL + G treatments (Table 2).

A negative correlation was found for H + Al, organic matter, and Mg content with the yield/biological efficiency values. These results were obtained due to an inadequate application of DL to correct the pH. This effect was even more severe with the application of G, due to a translocation of Al to the compost.

A negative correlation of organic matter with yield/biological efficiency has been found. The increase in the amount of organic matter (Table 2) after adding charcoal was mainly due to the presence of a black powder and should be avoided by consecutive washings of charcoal, before mixing with soil.

The precociousness values indicate that production accumulates in the first 50 days after the first primordial formation (22 days). This is a specific parameter used to monitor the yield at mid-cycle of the crop development. The best values were

Table 2 Data of mushroom production according different source of calcium and time of application.

Treatments	Earliness (days)	Biological efficiency (%)	Total	Yield (%) 1st flush	2nd flush	3rd flush	4th flush	5th flush	6th flush	Precocity (%)
CL	24.72 A	48.37 A	17.07 A	21.18	25.28	16.52	15.68	10.83	10.48	75.40 A
CL + G	25.86 A	43.65 A	15.41 A	27.88	11.47	18.62	12.08	18.02	11.89	63.83 A
DL	26.96 AB	35.90 A	12.67 A	38.37	14.59	18.99	12.64	13.08	2.30	78.36 A
DL + G	28.46 B	34.49 A	12.17 A	29.60	18.40	8.80	11.63	21.46	10.10	67.83 A
20 days before casing	26.29 a	39.48 a	13.93 a	28.14	14.54	19.25	15.52	11.45	11.06	73.76 a
At casing	26.70 a	41.73 a	14.73 a	28.88	20.88	12.89	10.99	19.32	7.01	68.81 a
Average	26.50	40.60	14.33	28.51	17.71	16.07	13.25	15.39	9.04	71.28

CL: calcitic limestone; CL + G: calcitic limestone + gypsum; DL: dolomitic limestone; DL + G: dolomitic limestone + gypsum.
Capital letters compare different sources of calcium and lower case letters, time of application; Tukey test (critical value $\leqslant 0.05$).

Table 3 Correlations between chemical characteristics of treatments and mushroom growth.

Pearson Correlation	Earliness	Precociousness	pH	$H^+ + Al^{3+}$	M.O.	P	K^+	Ca^{2+}	Mg^{2+}	SB	CEC	V (%)	Fe	Mn
Yield	**−0.75**	−0.178	0.538	**−0.709**	**−0.839**	0.210	0.376	0.419	**−0.791**	0.280	−0.037	0.547	−0.373	0.519
BE[a]	**0.032**	0.674	0.169	**0.048**	**0.009**	0.618	0.358	0.302	**0.019**	0.502	0.929	0.161	0.363	0.188
Earliness	–	−0.116	−0.675	**0.868**	**0.884**	−0.096	−0.382	−0.457	**0.882**	−0.283	0.115	−0.539	**0.770**	−0.691
		0.784	0.066	**0.005**	**0.003**	0.819	0.350	0.255	**0.003**	0.497	0.786	0.168	**0.025**	0.057
Precociousness	–	–	−0.269	0.174	−0.081	−0.324	−0.638	**−0.739**	−0.018	**−0.831**	**−0.870**	**−0.712**	−0.041	0.180
			0.519	0.680	0.848	0.433	0.088	**0.036**	0.965	**0.010**	**0.004**	**0.047**	0.922	0.670

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.
[a] BE – Biological efficiency.

obtained with CL and DL treatments; while the CL + G and DL + G treatments showed a reduction of precociousness (63.8% and 67.8%, respectively).

Negative correlations were found between the precociousness and the amount of Ca, SB, CEC and V, showing that the presence of bases (soluble salts) may delay the rate of flushes production.

Table 4 shows the equations obtained with the "step forward" multiple linear regression analysis (Statgraphics), which provided values of $R^2 \geqslant 70\%$ and $P \leqslant 0.01$. These equations analyze the possible relationship between the behavior of mushroom growth and the values of the different chemical components of casing layer that may influence the final mushroom production conditions.

The variability in the *A. subrufescens* yield (R^2 = 70.36% and P = 0.0092) and biological efficiency (R^2 = 70.38% and P = 0.0092) has been explained by the amount of organic matter in the casing soil. In this case, a high amount of organic matter reduces the yield and the biological efficiency values due to the presence of a black powder rich in organic matter, and with other toxic elements, which affect mushroom growth. This equation can be used safely when soil + charcoal is used as cover layer.

The resulting earliness equation depended on the organic matter (R^2 = 78.07%), Fe content (R^2 = 93.91%), Mn content (R^2 = 99.41%) and Mg content (R^2 = 99.80%), with a P value of 0.0002. In this case, a low amount of organic matter, Fe and Mg content and high Mn content, promotes an earlier first flush in the crop. So the main element that influences the results of earliness is the Mg content, followed by Mn and Fe contents and organic matter. Thus, it has been verified that the amount of organic matter in casing soils provides important variations in the performance of agronomic behavior (yield, biological efficiency and earliness).

Finally, precociousness was found to depend on the cation exchange capacity (R^2 = 75.72% and P = 0.0050). In this case, the lower the cation exchange capacity, the higher the precociousness. The treatments with the lower cation exchange capacity (CL and DL) had mushroom harvesting concentrated in the first half of the crop cycle (75.40% and 78.36%).

No statistical differences were found (Tukey test, $P \leqslant 0.05$) in the number and weight of mushrooms obtained by the different treatments; although the addition of soluble salts to the casing layer has been reported to delay fruiting, decrease the number of basidiocarps and yield, and increase the average mushroom size (Flegg, 1960, 1961; Reeve et al., 1960).

The negative influence of H + Al on *A. blazei* yields due to the potential acidity of soil has been reported (Zied, 2008). In addition, a negative correlation between the CEC and yield because of the high influence of H + Al, which represents 58% of the total CEC value has been showed.

The use of calcium carbonate ($CaCO_3$) to correct pH of casings has been suggested, combined with calcium sulfate (gypsum) to improve particle separation (Stamets, 1993). This practice should not be used in the cultivation of *A. subrufescens*, due to the slight changes that occur in the soil structure when it is used, especially compared to organic material based casings (peat, coconut fiber or spent mushroom substrate). The casing layer should contain a low level of easily degradable organic matter in order to maintain the ecological selectivity of the medium unaltered and to avoid the appearance of undesirable microorganisms (Visscher, 1988).

It has been reported that the precociousness values of *A. blazei* yields depend on the type of compost and casing used,

Table 4 Stepwise regression analysis for the mushroom production parameters based on the chemical characteristics of casing soils.

Correlation with variable	Equation	Variables related	R^2 (%)	P	SEE
Yield	YIELD = 59.605 − 3.80361 * OM	OM	70.36	0.0092	1.687,%
Biological efficiency	BE = 168.834 − 10.7732 * OM	OM	70.38	0.0092	4.777,%
		OM	78.07		
Earliness	EARL = 2.87104 − 0.148048 * Mg + 1.65 * OM + 0.353268 * Fe − 1.04326 * Mn	Fe	93.91	0.0002	0.161, days
		Mn	99.41		
		Mg	99.80		
Precociousness	PREC = 118.749 − 1.29747 * CEC	CEC	75.72	0.0050	4.457,%

R^2: determination coefficient; P: probability; SEE: standard error of estimate; BE: biological efficiency (%); EARL: earliness (days); PREC: Precociousness (%); OM: organic matter (g kg^{-1}); Fe: Fe content (ppm); Mn: Mn content (ppm); Mg: Mg content (mmol dm^{-3}); CEC: cation exchange capacity (mmol dm^{-3}).

with the highest values for tifton straw compost and polyacrylamide gel casing (75%) and the lowest values for tifton straw compost and soil/charcoal casing (51%) (Zied et al., 2009). The precociousness values were also affected by soil texture and cultivation environments (Zied et al., 2010). These results can be partly explained by the chemical correlations noted in this work.

Other factors influencing precociousness values are the strains used, the compost formulation, the physical characteristics of the casing, the cultivation technique adopted, the thickness of the casing layer, and the degree of technology used in the cultivation environment (Stoller, 1952; Pardo et al., 2003).

A positive correlation between the concentration of the iron ion and the number of primordia formed *in vitro* has been observed during *A. bisporus* growth (Hayes, 1972). In addition, calcium is an essential nutrient for the development of mycelium in pure growth (Treschow, 1944 cited by Flegg, 1956), and potassium contributes to the yield, although it is not a limiting factor (Stoller, 1952).

4. Conclusions

Different calcium sources influenced differently *A. subrufescens* growth, especially earliness that showed significantly the highest values when dolomitic limestone with gypsum was applied. Negative correlations were observed between yield/biological efficiency and H + AL, organic matter and Mg concentrations. Positive correlations were found between earliness and H + Al, organic matter, Mg and Fe concentrations. On the other hand negative correlations were also noted between earliness and the amount of Mn. Finally, negative correlations were observed between precociousness and amount of Ca, SB, CEC and V%. The models presented in this work are extremely important for predicting the agronomic performance or the production of *A. subrufescens* on the basis of chemical analysis provided by the casing soil.

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