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"INDOOR" METHOD OF COMPOSTING AND GENETIC BREEDING OF THE STRAINS TO IMPROVE YIELD AND QUALITY OF THE ALMOND MUSHROOM *AGARICUS SUBRUFESCENS.*

DIEGO C. ZIED *[1]; A. PARDO-GIMENEZ [2]; J.-M. SAVOIE [3]; J.E. PARDO-GONZALEZ [4], P. CALLAC[3]

[1]Módulo de Cogumelo. Departamento de Produção Vegetal, Universidade Estadual Paulista . Fazenda Lageado, PO box 237, CEP 18603-970, Botucatu, SP, Brazil.
dczied@gmail.com
[2]Centro de Investigación, Experimentación y Servicios del Champiñón (CIES), PO box 63, 16220 Quintanar del Rey, Cuenca, Spain
[3]INRA, UR1264, Mycologie et Sécurité des Aliments, F-33883, Villenave d'Ornon, France.
[4]Escuela Técnica Superior de Ingenieros Agrónomos, Universidad de Castilla-La Mancha, Campus Universitario, s/n, 02071 Albacete, Spain

ABSTRACT

The aim of the present work was to evaluate the potential efficiency of an *indoor* composting method and the genetic breeding of strains on the agronomic performance (yield, number and weight of basidiocarps, precociousness and earliness) and quality of *A. subrufescens* mushrooms. The experiment followed a factorial combination (3 composts types x 4 strains) with five replicates per treatment. One strain was a hybrid between French and Brazilian isolates. Strains and composts affected all variables analyzed (yield, number of basidiocarps, precociousness and earliness), except the weight of basidiocarps harvested. According to agronomic performance, yield was positively correlated with the number of basidiocarps and precociousness but was negatively correlated with earliness. According to chemical characteristics of basidiocarps, moisture was positively correlated with the amount of fat; protein was negatively correlated with the amount of hemicellulose and finally, hemicellulose was negatively correlated with the amount of cellulose present in the mushrooms. Despite the observed differences between composts, the best composting process for the cultivation of *A. subrufescens* is still unknown, requiring further research with management approaches, methods and formulations to be used for the commercial production of a selective substrate. The intercontinental hybrid possessed improved quality characteristics while yielding similar to its better parent. Breeding programs for improving mushroom quality and yield of *A. subrufescens* would be warranted.

Keywords: *Agaricus subrufescens*; compost; genetic breeding; chemical characterization; agronomic performance.

INTRODUCTION

Since the first tests performed in 1980 by Takatoshi Furumoto, agronomist, production of *Agaricus subrufescens* (formerly *A. blazei*, *A. brasiliensis*) was done on basis of cultivation practices adopted for the production of *Agaricus bisporus*. Even today, little has changed especially with

growing practices to improve yield (15-25%), earliness (70% of total yield in the first half of the crop), duration of flushes (4 days of harvest), interval between flushes (3-5 days) and crop cycle (50-60 days).

Strains used for the cultivation of *A. subrufescens* in Brazil are marketed as varieties collected indigenously, that were selected through domestication and adaptation to the cultivation conditions of the farms (type and formulation of the compost and local environmental conditions). The consequences are a great variability in yield, a long growing cycle and a lack of control over the specific growth characteristics of the strains.

Agaricus subrufescens has been characterized as a tropical mushroom with fruiting temperatures used during cultivation usually between 25 and 29°C. However mycologists have collected fruiting bodies in temperate countries such as Belgium and France [1; Guinberteau, pers. com.], showing the species has an extended geographic distribution. Because of this great geographic distribution, the important work of genetic breeding and acquisition of new hybrids can be performed, creating individuals with specific characteristics for production in different conditions worldwide.

In Brazil, the traditional process of composting has been widely practiced by growers, following the steps of: pre-wetting (4-7 days), fermentation (formation of the windrow 2 m wide x 2 m high, with intervals of turning every 2-3 days), pasteurization (58±2°C) and physical, chemical and biological conditioning (47±2°C) [2]. The raw materials commonly used as bulk compost are: sugar cane bagasse (*Saccharum officinarum*), various grasses (*Braquiaría* sp., *Cynodon dalactylon*, *Panicum maximum*, etc), cereal straw (*Triticum aestivum*, *Avena sativa*, *Oryza sativa*, etc.) and manure. Already as concentrated material (nitrogen source or not) soybean, wheat, corn and cotton meal, urea, ammonium sulfate, superphosphate, calcium carbonate and gypsum are used [3].

In 1986, the first method of "indoor" composting used for the production of *A. bisporus* was proposed [4], later called "environmental control" [5] and "accelerated" [6] composting, in order to accelerate the composting process to limit anaerobiosis and bad smells, to decrease the loss of material during the composting process, to reduce the physical space of the operations, and the use of machines [7]; and especially to increase process efficiency and productivity. Productivity is a direct consequence of operating quality practiced during the composting process, both with respect to the design of the theoretical formulation, as well as a civil structure existing and used [8].

As important as the agronomic performance of the species, the final quality of mushrooms (physical, chemical and biological control of harvested mushroom) should also be taken into consideration. It can be defined as physical aspects: size, degree of maturation, absence of pests and diseases, etc.; chemical aspects: the amount of β-glucan, no heavy metal, high presence of proteins and minerals, etc.; and finally biological activities: bactericidal, antitumor and antioxidant activity.

In general, it is difficult to compare the chemical results obtained and cited in the literature by several authors working with the same species, since there are many variables influencing the nutritional composition of mushrooms [9], such as differences between strains, composition of compost, type of casing layer, environmental conditions and methods of cultivation, besides the inherent inaccuracy in methods of analysis and precision of the analyst [10]. New cultivation technologies should be investigated to increase the agronomic performance without changing the physical-chemical characteristics of harvested mushrooms. Thus, the present study focused on evaluation of potential efficiency of *indoor* composting and genetic breeding of strains on yield, number and weight of basidiocarps, precociousness, earliness and quality of *A. subrufescens*.

MATERIALS AND METHODS

Spawn. Four strains were used described as follows:

- 99/30: strain stored in the mycology collection, Mushroom Research Center (FCA/UNESP), isolated in Piedade (1999) from a commercial farm of the Atushi Group, São Paulo State (Brazil).
- CA454: originated from Brazil, corresponding to ATCC 76739, deposited as the original strains of *A. blazei* Murill used for the development of the cultures.
- CA487: wild strain isolated by Jacques Guinberteau in 2006 at Saint-Léon, Gironde, France, on waste of leaves lawn mowing
- CA454 x CA487: hybrid between Brazilian (C454) and French (C487) strain obtained by crossing mycelia from single spore isolates. All the CA strains are from the CGAB collection (INRA, UR MYCSA, France)

Production of spawn followed procedures adopted by Zied et al. [11].

Compost (Phase I and II). Three composts were used, made from different plants of "Indoor" composting, that were produced by different methods.
Compost 1: wheat straw was moistened for 6 days, then the straw was mixed and transferred to the 1st Bunker with chicken manure and concentrated ingredients where they remained for 5 days; afterward the compost was mixed and transferred to the 2nd Bunker where it remained for another 5 days, finally the compost was mixed again and transferred to the 3th Bunker where it remained for an additional two days. Phase II lasted 7 days (8 hours at 60°C and 6 days at 45-50°C).
Compost 2: wheat straw and chicken manure were moistened for 8 days then held 3 days and turned; then the compost was transferred to the 1st Bunker together with the concentrated ingredients where it remained for 2 days; afterward the compost was mixed and transferred to the 2nd Bunker where it remained for 2 days. Finally the compost was mixed again and transferred to the 3th Bunker where it remained for 2 days. Phase II lasted 7 days (13 hours at 57°C and 6 days at 45-50°C).
Compost 3: wheat straw and chicken manure were moistened for 6 days with turning on the 3rd day; then the compost was transferred to the 1st Bunker together with the concentrated ingredients where it remained for 2 days; afterward the compost was mixed and transferred to the 2nd Bunker where it remained for 2 days; then the compost was mixed again and transferred to the 3th Bunker where it remained for 2 days. Finally the compost was transferred to 4th Bunker where it remained for 2 days. Phase II lasted 8 days (8 hours at 58°C and 7 days at 45-50°C).

Table 1 shows the characteristics of each compost type at the end of Phase II of the composting process.

Inoculation and spawn run. The compost was inoculated with 1% spawn in relation to the wet weight of the compost and incubated at 28±2°C with relative humidity at 50±10% for 15 days.

Casing layer. A mixture of casing with black peat + soil (4:1, v/v) added calcium carbonate and formaldehyde in the amount of 50 ml per m^3 of material was used. With fully developed mycelium, the casing was added over the compost at a depth of 3 cm (2.6 liters of material per plastic box containing 6 kg of compost). The boxes with compost and casing were taken to a chamber with air temperature of 26±1°C, compost temperature of 27±1°C, relative humidity of 90±5% and CO_2 content of 2,100 ppm, during 8 days following the methodology presented by Minhoni et al. [12].

Table 1. Physico-chemical characteristics of three compost types (at end of Phase II).

Parameter	Compost 1	Compost 2	Compost 3
pH, 1:5, v/v	7.35	7.24	7.51
Moisture, g kg^{-1}	678	675	668
Nitrogen, g kg^{-1}	23.8	21.7	24.7
Protein, g kg^{-1}	104.2	95.0	108.1
Ash, g kg^{-1}	245.5	294.5	297.1
Organic matter, g kg^{-1}	754.5	704.5	702.9
C/N	18.4	18.8	16.5

Pinning and harvest. The environmental variables were controlled in order to obtain 4 flushes of production over the crop. For this the temperature, relative humidity and aeration were conducted according to methodology presented by Zied [13]. Fig. 1 demonstrates the behavior of environmental variables and reflects the flush of production according to the strain used.

The total production time of the crop was 70 days, and the presence of primordia was observed at 17 days. The mushrooms were collected manually with the largest weight possible before pileus opening and lamella breaking. Then, mushrooms were evaluated for their agronomic performance and chemical characteristics.

Experimental design and data analysis. The experiment was conducted using 4 strains and 3 composts, totaling 12 treatments. Each treatment consisted of five repetitions of boxes with 6 kg of compost. The Sisvar 3.2 statistical program was used to separate treatment means with Tukey's test ($P \leq 0.05$). Linear correlation between agronomic performance and chemical characteristics of *A. subrufescens* was done using the statistical software Sigma Stat 3.5.

Evaluated data. The agronomic performance was evaluated by yield [14], number and weight of basidiocarps [10], precociousness [12] and earliness [15]. The chemical characteristics were evaluated by moisture [16], protein, N-free extract and ash [17], fiber [18], fat [19] and hemicellulose and cellulose [20; 21].

RESULTS AND DISCUSSION

Strain CA487 had the highest yield, followed by CA454 x CA487, 99/30 and CA454, which demonstrates the potential production of the strains from a temperate country and also of the hybrid (Table 2). Regarding the compost used, compost 2 had the highest yield, followed by compost 1 and 3, and shows that compost with a low C/N ratio and high nitrogen content may result in low yield. Another factor that may have influenced the low yield obtained for compost 3 is a lower compost moisture (66%).

Kopytowski-Filho [22] emphasizes that compost obtained with an initial mixture having a high C/N ratio, (40-33/1) tends to show higher yield than compost obtained from an initial C/N ratio around 29-26/1.

The behavior of production flushes of strains according to management of environmental variables controlled is shown in Fig. 1. The strains 99/30, CA454 and CA454 x CA487 showed flushes well distributed during cultivation at 27°C. This distribution responded to the phase of induction by decreasing temperature to 20°C and increasing to 27°C. Strain CA487 was harvested at ± 27°C as the others, but it also produced fruiting bodies during the decrease in temperature to 20°C, as observed on days 43 and 58. More studies are required on the management of environmental variables to obtain the flushes of production for this strain of temperate origin.

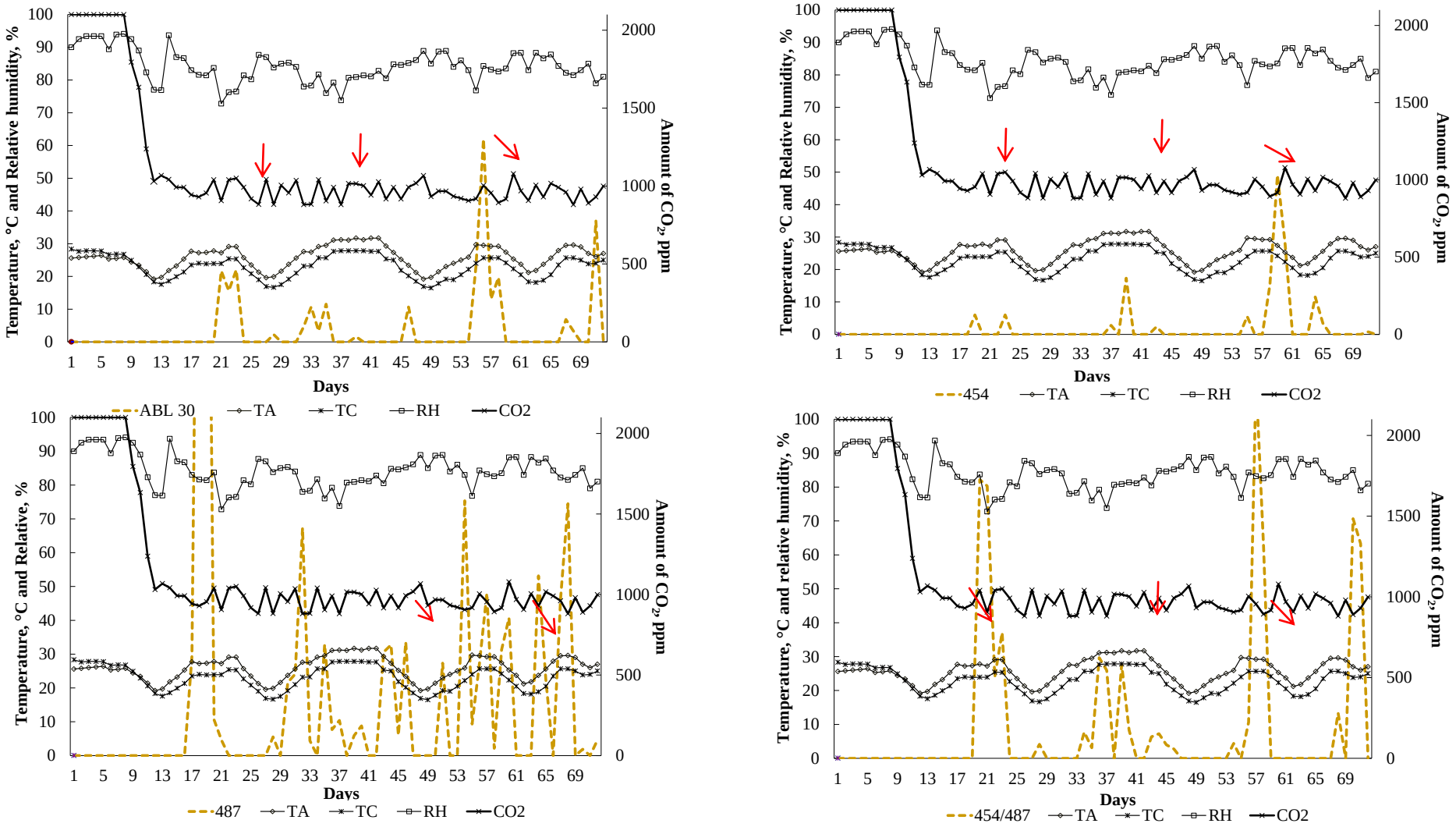

Figure 1. Environmental variables and crop flushes during the 70 days of cultivation, where: ABL 30, 454, 487 and 454/487 indicates the production of the strains (grams) of harvested mushrooms during the growing period; TA, air temperature; TC, compost temperature; RH, relative humidity and CO_2, the amount in environment.

Table 2. Agronomic performance of four strains of *Agaricus subrufescens* produced on three different composts.

Strain	Compost		
	1	2	3
Yield (%)			
99/30	4.7 a CB	3.2 ab C	1.2 b C
454	1.9 a C	2.3 a C	1.1 a C
487	14.4 a A	18.6 a A	13.5 b A
454 x 487	6.1 b B	11.2 a B	8.0 b B
Number of basidiocarps, u			
99/30	21 a B	13,8 abC	5,8 b C
454	3.8 a C	6,8 a C	3,6 a C
487	72.4 a A	74,8 a A	65,6 a A
454 x 487	25.6 b B	41,4 a B	30,8 ab B
Weight of basidiocarps, g			
99/30	27.2 a A	19.4 a A	10.9 a A
454	17.9 a A	15.2 a A	24.5 a A
487	17.0 a A	12.6 a A	15.5 a A
454 x 487	18.7 a A	15.2 a A	17.4 a A
Precociousness, %			
99/30	30.5 a AB	37.8 a A	27.7 a AB
454	12.6 a B	25.2 a A	20.0 a B
487	64.4 a A	59.4 a A	65.1 a A
454 x 487	51.4 a A	52.6 a A	28.1 a AB
Earliness, days			
99/30	28.7 a A	26.8 a AB	38.0 a AB
454	50.0 a B	44.0 a B	52.8 a B
487	18.8 a A	18.2 a A	18.5 a A
454 x 487	29.0 a A	25.4 a AB	25.5 a A

Lowercase letters compare the results on the same line and capital letters compare the results in the same column in the Tukey's test ($P \leq 0.05$).

Another positive factor that should be highlighted is the convenience and ease in working with the CA487 strain that in just 70 days of crop had a yield between 13.5-18.6%, high number of mushrooms (mean of 71 u), precociousness (mean of 63%) and earliness of production (18.5 days). Similar yields were observed with commercial strains by Siqueira et al. [23] and Zied et al. [3] with values of 16.3% and 18.3%, respectively, but with crop time above 110 days of production.

The numbers of mushrooms followed the patterns of yield, that had a positive correlation with precociousness ($r = 0.868$, $P = 0.001$), and a negative correlation with earliness ($r = -0.829$, $P = 0.001$). Thus high yield in the crop is associated with a large number of mushrooms harvested, concentrated in the first half of the crop; but with low yield in the first flush (this trend was clear for the Brazilian strains).

The present work illustrates the interest of intercontinental breeding programs for the development of efficient new varieties, since the first hybrid used responded exactly the goals that has been developed; maintained a good agronomic performance (close to its better parent) and increased the weight of harvested mushroom (although no significant difference at Tukey's test, was observed that the hybrid increased approximately 12.3% of the weight of mushrooms when compared with the CA487 strain).

The compost used did not affect the weight of mushrooms, precociousness and earliness, but the strain affected the earliness and precociousness. The best composting process for the cultivation of *A. subrufescens* is still unknown, requiring further research with management approaches (performance of "traditional" composting or composting in Bunkers with Phase II and

III together), methods (production of substrate composted or sterilized "axenic") and formulations (range in C/N ratio, content N, organic matter and ash) to be used for the commercial cultivation.

According to Table 3, little variation in chemical characteristics of mushroom were observed according to strains and composts used, but some features need to be mentioned. 99/30 and CA454 strains had higher amount of protein when grown in compost 3, on the other hand the CA487 and CA454 x CA487 strains has higher amount of protein when grown in compost 1. The higher levels of fiber were observed in 99/30 and CA454, and the highest levels of fat were observed in CA 487 and CA 454 x 487 strains.

Table 3. Physico-chemical characteristics of mushrooms according to strain and compost type

Strain	99/30			454			487			454 x 487		
Compost	1	2	3	1	2	3	1	2	3	1	2	3
Moisture, %	85.3	85.4	84.1	84.7	85.8	85.0	83.0	87.6	88.3	86.1	86.5	86.0
Protein, %	30.3	32.8	33.3	28.9	30.1	30.5	30.7	26.2	29.0	34.9	33.7	28.6
Ash, %	5.8	7.0	6.1	6.2	7.1	6.8	6.3	7.1	7.2	6.6	6.3	5.9
Fiber, %	6.8	6.8	5.6	8.2	7.8	8.1	5.1	6.7	5.1	5.6	6.7	5.1
Fat, %	0.97	0.86	1.13	0.94	1.04	1.00	0.96	1.71	1.66	1.26	1.18	1.52
N-free extracts, %	56.0	52.3	53.7	55.5	53.8	54.0	56.8	58.1	56.8	51.5	51.8	58.7
Hemicellulose, %	19.1	19.6	17.5	21.5	17.2	19.0	21.0	22.7	21.4	18.8	18.1	20.6
Cellulose, %	6.6	6.8	6.9	6.2	8.9	7.0	5.7	3.8	4.5	7.1	7.7	6.9

Comparing the results of protein, ash, fiber and fat of *A. subrufescens* mushrooms with those obtained by Hernández [9], Andrade et al. [24] and Pardo et al. [10] for *Agaricus bisporus* and *Lentinula edodes* we have, protein: *A. subrufescens* (32.17%), *L. edodes* (20.33%) and *A. bisporus* (23.22%); ash: *A. subrufescens* (6.35%), *L. edodes* (3.10%) and *A. bisporus* (12.62%); fiber: *A. subrufescens* (6.4%), *L. edodes* (8.04%) and *A. bisporus* (20.41%) and finally fat: *A. subrufescens* (0.98%), *L. edodes* (2.00%) and *Agaricus bisporus* (5.2%).

It should be noted that the moisture content had a positive correlation with the amount of fat ($r = 0.780$, $P = 0.002$); the protein had negative correlation with the amount of cellulose ($r = -0.712$, $P = 0.009$) and finally cellulose had a negative correlation with the amount of hemicellulose ($r = 0.623$, $P = 0.030$) present in the mushrooms.

Polysaccharides are the main chemical compounds found in fungal cell walls. Glucose – usually as glucans, β(1→4) cellulose, α(1→4) and α(1→6) glycogen, β(1→3) and β(1→6) yeast glucan – constitutes from 80 to 90% of the cell wall material of many species, and glucosamine (in chitin) constitutes from 1 to 58% (range of values), usually 5 to 20% [25].

Park et al. [26] compared the amount of β-glucan in mushrooms produced in Brazil (greenhouse and field) and in Japan (greenhouse), concluded that *A. blazei* cultivated in greenhouses have a lower amount of β-glucan (7.6 ± 2.8g $100g^{-1}$ mushroom produced in Japan and 8.4 ± 0.9g $100g^{-1}$ mushroom produced in Brazil) than those cultivated in the field (10.1 ± 2.1g $100g^{-1}$ mushroom). Zied [27] evaluated different cropping practices that influenced the amount of β-glucan in *A. subrufescens* mushrooms and found a variability of 35.8% of the value of β-glucan influenced by strain and 9.9% of the value of β-glucan influenced by the compound used.

According to the review carried out by Manning [28] on the chemical composition and nutritional value of cultivated mushrooms, carbohydrates are the main component of mushrooms apart from water, and account for an average of 4.2% of the fresh weight. Among them, glycogen and hemicellulose are the main polysaccharides found in mushrooms; contents of 8.18% (dry weight) of crude hemicellulose have been recorded in *A. campestris*, markedly lower than those obtained in this work with *A. subrufescens* (between 17.5 and 22.7%).

CONCLUSIONS

Despite the observed differences between composts, the best composting process for the cultivation of *A. subrufescens* is still unknown, requiring further research with management approaches, methods and formulations to be used for the commercial production of a selective substrate. The intercontinental hybrid resulted in an increase in the weight of basidiocarps, while still maintaining high yield close to its better parent. Breeding programs for improving mushroom quality and yield of *A. subrufescens* are worth being developed.

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