USE OF PHASE II MUSHROOM COMPOST IN *AGARICUS SUBRUFESCENS* PRODUCTION

ARTURO PARDO-GIMÉNEZ[1]*, JOSÉ EMILIO PARDO[2], JAIME CARRASCO[1], MANUEL ÁLVAREZ-ORTÍ[2] AND DIEGO C ZIED[3]

[1]Centro de Investigación, Experimentación y Serviciosdel Champiñón (CIES), 16220 Quintanardel Rey, Cuenca, Spain
[2]Escuela Técnica Superior de IngenierosAgrónomos y de Montes, Universidad de Castilla-La Mancha (UCLM) 02071 Albacete, Spain
[3]Universidade Estadual Paulista, UNESP - Campus de Dracena, 17900-000 Dracena, SP, Brazil
apardo.cies@dipucuenca.es

ABSTRACT

In the last years, the cultivation of the medicinal mushroom *Agaricus subrufescens* Peck has evoked great interest worldwide. During the crop development, techniques and methods previously established for *A. bisporus* production have been adopted, trying to take advantage of existing buildings and technologies. This work assesses the production parameters and composition of the harvested fruiting bodies of *A. subrufescens* that have been produced from three commercial compost specifically prepared for the production of *A. bisporus*.Two loading densities of compost (60 to 70 kg m^{-2}) were used. A growth cycle of *A. subrufescens* under controlled conditions has been carried out.There were no significant differences among treatments for any of the considered parameters. Biological efficiency values obtained were between 46.10 and 58.52 kg dt^{-1}, which is higher than those registered in the references consulted.The fact that significant differences in behavior between different compost used have not been observed, along with the good agronomic performance of them, allows confirming its suitability for the production of *A. subrufescens* in the conditions used. As regards the loading density, the range between 60 and 70 kg m^{-2} could be applied to commercial scale. The proximate analysis of the harvested carpophores did not provide significant differences between treatments or factors. With regard to the content of other cultivated species of edible fungi, *A. subrufescens* is remarkable in general for its low content of water, crude fat, crude fiber and ash, and high content in protein and total carbohydrates.

Keywords: *Agaricus subrufescens*, *Agaricus blazei*, compost, production, proximate analysis

INTRODUCTION

The cultivation of the medicinal mushroom *A. subrufescens* Peck has generated a notable interest worldwide in the last few years, becoming increasingly popular and as a result expanding through many countries. This is mainly due to its high international market price, which is related not only to the remarkable medicinal properties contributed by high content of bioactive compounds but also to the culinary value added by its slightly nutty pleasant aroma [1]. Several studies recently reviewed by Wisitrassameewong *et al*. [2] highlight the importance of *A. subrufescens* medicinal properties. It was traditionally used to treat many common diseases like atherosclerosis, hepatitis, hyperlipidemia, diabetes, dermatitis and cancer [3]. Among the beneficial properties from *A. subrufescens* that have been published are tumor growth reduction, immune modulatory activities, immune stimulatory effects, antimicrobial and antiviral activities and anti-allergy effects [2].

Besides its medicinal interest, *A. subrufescens* is a food of high nutritional value, rich in protein, fiber and minerals (mainly potassium, phosphorus and calcium), with low lipid content [1]. Nowadays consumers concern to look for healthier food, find on the consumption of mushroom an alternative with two major advantages, in some varieties, the therapeutic properties and an important source of high value proteins [4]. Considering the special requirements of the crop, based on the high temperature needed, the production of *A. subrufescens* could be a good choice by the edible mushroom growers of different countries, particularly during the summer months. This crop allows the use of poor technology facilities with low energy costs. The well known *Agaricus bisporus* (Lange) Imbach production technology can mostly be used for *A. subrufescens*, so the growers should not have any problem in adjusting to the new species. Furthermore, the marketing conditions of this mushroom, mainly dried, facilitate to deal with the short life of edible cultivated mushroom allowing a flexible commercialization of the product throughout the year.

Along the crop development, those processes and techniques established for the production of *A. bisporus* have been considered, although the specific technology for the crop studied is still under review. To increase the yield and to get a proper adaptation of each country, it is necessary to evaluate different crop parameters. The cultivation conditions in the different stages of the crop cycle (temperature, relative humidity, carbon dioxide concentration, light cycle) as well as the aspects concerning the casing materials should be thoroughly analyzed. In this context, the aim of this work was to achieve the production of *A. subrufescens* while using substrates specifically prepared for *A. bisporus* cultivation by a traditional composting system of two phases, and making use of the existing facilities and technologies for this purpose.

MATERIALS AND METHODS

To determine the physical, chemical and biological characteristics of composts, the following measurements were taken: water content, pH, total N content, organic matter and ash, C/N ratio, crude fibre, crude fat, nitrogen-free extracts, cellulose, hemicellulose, lignin and neutral detergent-solubles, pathogenic nematodes, mites and competitor moulds. The methodology previously described by Pardo-Giménez *et al.* [5] was used. Composts characteristics are presented in Table 1.

The experimental design used was a 3×2 equilibrated factorial plan with six replicates (randomised blocks with two factorial factors). Factor 1, with three levels, corresponded to three phase II commercial composts (obtained from three different plants of composting) prepared for the production of *A. bisporus*. Factor 2, with two levels, corresponded to the loading density of compost (60 and 70 kg m^{-2}). A total of 36 trays (1450 cm^2 each one) were employed, which were placed at two levels on both sides of the growth chamber. Strain ABL99/30 (Mycotec of the Moìdulo deCogumelos, FCA-UNESP, Brazil) was selected for the experiment. This strain, collected in Piedade (São Paulo, Brazil) in 1999, is characterized by its medium to small size fruit bodies, strong texture, high yield and precociousness, reduced time to first harvest (±38 days), and slightly low fructification temperature (±25 °C).

A. subrufescens trial was carried out in a 20 m^3 experimental growth chamber equipped with a humidification system, a heating/cooling system, and internal air circulation/external ventilation. This allowed the automatic control of temperature, relative humidity and carbon dioxide. Composts were inoculated with grain spawn at a rate of 12 g kg^{-1} of fresh weight of compost. The mushroom growth cycle was carried out according to the fast cooling method: incubation with a compost temperature of 28 ºC, drop to 20 ºC for fruiting and compost temperature of 26 ºC and 700 ppm CO_2 during the cultivation. Temperatures and plan for primordial induction is presented in Fig. 1. A peat based casing was used.The total growth cycle lasted 110 days; five flushes of mushrooms were harvested.

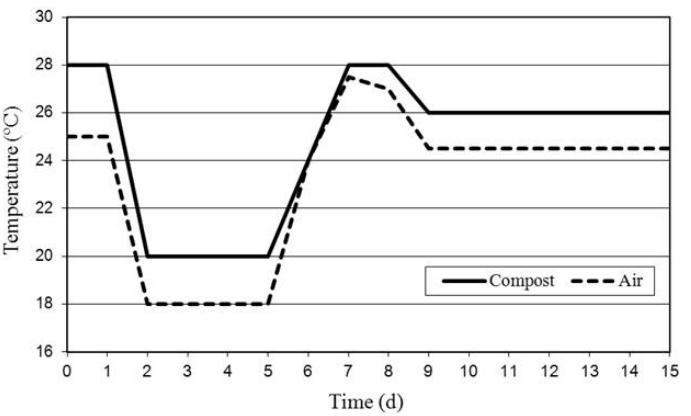

Figure. 1. Temperatures of induction for *A. subrufescens* fruiting in fast cooling process

The main production parameters (earliness, number of mushrooms, yield per unit of area, biological efficiency, unitary weight and dry matter content) were evaluated, and a proximate analysis of harvested mushrooms was carried out. Mushrooms were harvested every day at their optimal commercial development stage, with the highest possible weight before the opening of the pileus.To assess the production parameters, weight before stipe trimming and the total number of mushrooms picked from each tray (1450 cm^2) were recorded daily. Yield is expressed as kg per cultivated area; biological efficiency, an estimation of mushrooms' ability to convert substrate into fruiting bodies, was calculated by dividing the total fresh weight of mushrooms harvested from one crop (several flushes) by the total substrate dry weight, expressing the fraction as kg dt^{-1} compost.The size of the mushrooms, expressed as unit weight in g, was calculated from the yield and number of mushrooms harvested. Earliness, or days to the first harvest, was expressed as the number of days between casing and the beginning of the first flush harvest. On the day when most mushrooms were picked during the first flush, the

dry matter and proximate analysis were determined for those mushrooms of uniform size and at the same development stage.

Dry matter and water contents were determined by measuring weight loss after oven drying at 105°C for 72 h [6]. Mushrooms protein content was calculated by multiplying the total nitrogen content, obtained by the Kjeldahl method [7], by a conversion factor of 4.38. Crude fat was estimated gravimetrically by filter bag technique after petroleum ether extraction of the dried sample in an extraction system Ankom XT10 [8]. To determine the content of crude fiber, Weende technique adapted to the filter bag technique was applied. This method determines the organic residue remaining after digestion with solutions of sulfuric acid and sodium hydroxide, using an Ankom 220 Fiber Analyzer [9]. Total carbohydrate content was calculated by subtracting the sum of the crude protein, total fat, water and ash from the total weight of the mushrooms [10]. Nitrogen-free carbohydrates content is calculated by subtracting the crude fiber from the total carbohydrate content [6]. To determine ash content, mushrooms were ashed at 540°C for at least 6 h. The energy value of the mushrooms was estimated as per Lau [6]. ANOVA was used to analyze the data and the Tukey-HSD test employed to establish significant differencesbetween means (P = 0.05). All calculations were performed using Statgraphics Plus v. 4.1 software (Statistical Graphics Corp., Princeton, NJ, USA).

Table 1. Chemical characteristics of different composts used

Characteristics	Compost 1	Compost 2	Compost 3
pH (1:5, w/v)	7.64	7.61	7.40
Water (g kg^{-1})	695.0	672.3	689.3
Total nitrogen (g kg^{-1})	20.7	23.0	22.7
Protein (g kg^{-1})	129.4	143.8	142.1
Ash (g kg^{-1})	224.8	258.8	281.7
Organic matter (g kg^{-1})	775.2	741.2	718.3
C/N	21.7	18.8	18.3
Crude fiber (g kg^{-1})	333.2	334.2	310.6
Crude fat (g kg^{-1})	3.9	1.8	3.6
N-free extract (g kg^{-1})	308.7	261.6	262.0
Hemicellulose (g kg^{-1})	134.4	125.2	151.7
Cellulose (g kg^{-1})	235.9	201.5	202.1
Lignin (g kg^{-1})	185.7	214.3	166.9
Neutral-det. sol. (g kg^{-1})	219.2	200.3	197.6

RESULTS AND DISCUSSION

No significant differences were registered among the treatments for none of the considered parameters (Table 2). The biological efficiency (BE) values ranged between 46.10 and 58.52 kg dt^{-1}, with yield per unit of area between 9.06 and 11.23 kg m^{-2}, being generally higher than the obtained by the literature reviewed.

Kopytowski-Filho and Minhoni [11] evaluated the behaviour of the variety ABL 99/30, used in this work, over three different kinds of compost, registering BE values between 21.1 and 34.9 kg dt^{-1} by using a mixture of mineral soil and vegetal carbon. In Taiwan, Liang *et al*. [12] obtained a highest yield of 1.93 kg/m^2 through substrates basedon two kinds of sawdust [12]. In Slovenia, Gregori *et al*. [13] employed *A. bisporus* commercial compost achieving 700 g of mushrooms from 3 kg of compost. Also in 2008, Cavalcante *et al*. evaluated in Brazil several casing materials based on local mineral soils and sand with different additives, obtaining BE values between 17.73 and 25.97 kg dt^{-1} [14]. Siqueira *et al*. [15] reached substantial BE values, by studying compost from crushed sugarcane bagasse, Coast cross hay and corn husk

Table 2. Mean values of production parameters assessed in the mushrooms originating from the various treatments

	Number of mushrooms m^{-2}	**Yield (kg m^{-2})**	**Unitary weight (g)**	**Biological efficiency (kg dt^{-1})**	**Earliness (days from casing)**	**Dry matter (%)**
Compost 160 kg/m^2	535 a	10.61 a	19.90 a	57.99 a	34.5 a	10.70 a
Compost 170 kg/m^2	575 a	11.04 a	19.38 a	51.71 a	33.3 a	10.76 a
Compost 260 kg/m^2	534 a	9.06 a	16.97 a	46.10 a	34.1 a	10.98 a
Compost 270 kg/m^2	618 a	11.23 a	18.71 a	48.92 a	33.3 a	11.00 a
Compost 360 kg/m^2	572 a	10.91 a	19.13 a	58.52 a	34.5 a	11.61 a
Compost 370 kg/m^2	553 a	10.61 a	19.64 a	48.74 a	33.6 a	12.12 a
MEAN	565	10.58	18.96	52.00	33.9	11.20

Values followed by a different letter within a column are significantly different at 5% level according to Tukey's HSD test.

amended with wheat bran, lime, gypsum, super phosphate and urea [15]. Similarly, Zied *et al*. [16], with two kinds of compost prepared with Tifton grass and oat straw, respectively achieved BE values between 23.25 and 36.25 kg dt^{-1}. Colauto *et al*. (2010) got BE values of 27.3 kg dt^{-1} from compost based on different local materials and a Brazilian pasteurized peat casing layer [17], and 36.1 kg dt^{-1} by using pasteurized lime schist as casing layer [18]. In repeat trial with lime schist as casing material, the same group obtained BE of 46.8 kg dt^{-1} for a crop cycle of 66 days [19] and 60.4 kg dt^{-1} for crop cycle of 90 days [20]. González Matute *et al*. reported BE values between 13.1 and 34.3 kg dt^{-1} on the uncomposted substrates with a mixture of peat and calcium carbonate as casing layer [21]. Zied *et al*. studied different varieties of *A. subrufescens* using compost based on wheat straw and chicken manure; a highest yield of 18.6%, equal to a BE of 57.2 kg dt^{-1} was obtained [22]. Recently, Pardo-Giménez *et al*. analyzed the effect of different casing layers over several strains of *A. subrufescens*, using compost based on wheat straw and chicken manure; a highest BE of 27.57 kg dt^{-1} was achieved for the strain ABL99/30 with a peat-based casing material [23].

The fact of non-significant differences among the compost kinds used (Table 3), together with the good agronomic behavior of them, allows to confirm that the use of the compost studied is suitable for the production of *A. subrufescens* in the conditions employed. Concerning the loading density, though significant differences were not recorded (Table 3), it is noteworthy that with70 kg m^{-2} a higher yield was obtained per unit of area than with 60 kg m^{-2} (10.96 against 10.19 kg m^{-2}), despite the biological efficiency registered (49.81 kg dt^{-1}) was lower than that obtained with the lowest density (54.19 kg dt^{-1}). This range between 60 and 70 kg/m^2 could be applied at commercial scale.

The proximate analysis of the harvested carpophores (Tables 4 and 5) did not show any significant differences between treatments or factors, with the exception of the crude fat content between composts. The harvested sporophores were found to contain (dry wt. basis) protein 291.1 g kg^{-1}; crude fat 20.4 g kg^{-1}; total carbohydrates 615.6 g kg^{-1}; available carbohydrates 540.9 g kg^{-1}; crude fiber 74.7 g kg^{-1}; ash 73.0 g kg^{-1}; energy value 353 kcal per 100g. In general, the gross composition of mushrooms is water (90%), protein (2–40%), fat (2–8%), carbohydrates (1–55%), fiber (3–32%) and ash (8–10%) [3]. With regard to the composition of other edible species of fungi, *A. subrufescens* is, in general, low in water, crude fat, crude fiber and ash, high in protein, total carbohydrate and mean values of available carbohydrates and energy value [24].

The protein contents, between 268.8 and 307.9 g kg^{-1}, are similar to those recently reported by Siqueira *et al.* (283.3-293.5 g kg^{-1}) [25] and Zied *et al*. (262-349 g kg^{-1}) [22]. According to data collected by Eira [26] on sporophores of *A. subrufescens* grown in Brazil, the protein contents (289.4-391.8 g kg^{-1}) is higher than those recorded for *A. bisporus* (263 g kg^{-1}), *Lentinula edodes* (175 g kg^{-1}), *Pleurotus florida* (189 g kg^{-1}), *Volvariella diplasia* (285 g kg^{-1}) and *Pleurotus ostreatus* (187 g kg^{-1}) [26]. Chang and Miles (2004) describe it as one of the species with higher protein

Table 3. Mean values of production parameters assessed in mushrooms for the different factors considered in the experimental design

	Number of mushrooms m^{-2}	**Yield (kg m^{-2})**	**Unitary weight (g)**	**Biological efficiency (kg dt^{-1})**	**Earliness (days from casing)**	**Dry matter (%)**
Compost 1	555 a	10.83 a	19.64 a	54.85 a	33.9 a	10.73 a
Compost 2	576 a	10.15 a	17.84 a	47.53 a	33.7 a	10.99 a
Compost 3	562 a	10.76 a	19.38 a	53.63 a	34.1 a	11.87 a
Density 60 kg/m^2	547 a	10.19 a	18.67 a	54.19 a	34.3 a	11.10 a
Density 70 kg/m^2	582 a	10.96 a	19.24 a	49.81 a	33.4 a	11.29 a
Mean	565	10.58	18.96	52.00	33.9	11.20

For each factor within a column, values followed by a different letter are significantly different at 5% level according to Tukey's HSD test.

Table 4. Proximate analysis of mushrooms originating from the various treatments

	Water (g kg^{-1})	**Crude Protein (Nx4.38)**	**Crude Fat (g kg^{-1} d.m.)**	**Total Carboh. (g kg^{-1} d.m.)**	**Nitrogen-Free Extract**	**Crude Fiber (g kg^{-1} d.m.)**	**Ash (g kg^{-1} d.m.)**	**Energy Value (kcal/100g d.m.)**
Compost 160 kg/m^2	893.0 a	290.2 a	19.1 a	613.6 a	537.7 a	75.9 a	77.1 a	350 a
Compost 170 kg/m^2	892.5 a	291.5 a	21.6 a	611.4 a	534.4 a	77.0 a	75.6 a	351 a
Compost 260 kg/m^2	890.2 a	297.8 a	24.2 a	604.5 a	525.9 a	78.6 a	73.6 a	352 a
Compost 270 kg/m^2	890.1 a	307.9 a	23.9 a	594.6 a	516.6 a	78.0 a	73.6 a	352 a
Compost 360 kg/m^2	883.9 a	290.1 a	18.2 a	620.6 a	549.0 a	71.6 a	71.1 a	354 a
Compost 370 kg/m^2	878.8 a	268.8 a	15.3 a	648.9 a	581.9 a	67.0 a	67.0 a	358 a
Mean	888.0	291.1	20.4	615.6	540.9	74.7	73.0	353

Values followed by a different letter within a column are significantly different at 5% level according to Tukey's HSD test.
d.m.= dry matter

Table 5. Proximate analysis of mushrooms for the different factors considered in the experimental design

	Water (g kg^{-1})	**Crude Protein (Nx4.38)**	**Crude Fat (g kg^{-1} d.m.)**	**Total Carboh. (g kg^{-1} d.m.)**	**Nitrogen-Free Extract**	**Crude Fiber (g kg^{-1} d.m.)**	**Ash (g kg^{-1} d.m.)**	**Energy Value (kcal/100g d.m.)**
Compost 1	892.7 a	290.8 a	20.3 ab	612.5 a	536.1 a	76.5 a	76.3 a	351 a
Compost 2	890.1 a	302.8 a	24.07 a	599.5 a	521.3 a	78.3 a	73.6 a	352 a
Compost 3	881.3 a	279.5 a	16.8 b	634.7 a	565.4 a	69.3 a	69.0 a	356 a
Density 60 kg/m^2	889.0 a	292.7 a	20.5 a	612.9 a	537.5 a	75.3 a	73.9 a	352 a
Density 70 kg/m^2	887.1 a	289.4 a	20.2 a	618.3 a	544.3 a	74.0 a	72.1 a	354 a
Mean	880.0	291.1	20.4	615.6	540.9	74.7	73.0	353

For each factor within a column, values followed by a different letter are significantly different at 5% level according to Tukey's HSD test.d.m.= dry matter

content among all the cultivated edible mushrooms, over two times higher than *L. edodes* [24]. Fat content reported in this paper (15.3-24.2 g kg^{-1}) is, in general, of the same order as those reported in the literature. According to Chang and Miles [24], the fat content of different species of mushrooms varies between 11 and 83 g kg^{-1}on dry weight basis, with a mean content of 40 g kg^{-1} [24]. Recently, Pardo-Giménez *et al*. recorded mean values of 15.9 g kg^{-1} in carpophores of *A. subrufescens*cultivated in Spain [27].

The harvested carpophores had an average of 74.7 g kg^{-1} of crude fiber content. The data collected by Eira [26] in sporophores of *A. subrufescens* cultivated in Brazil set out fiber contents (55.6-118.1 g kg^{-1}) in the same range as *A. bisporus* (104 g kg^{-1}), *Lentinula edodes* (80 g kg^{-1}) and *Pleurotus florida* (115 g kg^{-1}), although lower than those of *Volvariella diplasia* (174 g kg^{-1}) and *Pleurotus ostreatus* (156 g kg^{-1}) [26]. The mineral content ranged between 55.6-118.1 g kg^{-1}, with an average of 73.0 g kg^{-1}, as reviewed by Pardo-Giménez *et al*. [27]. These values are, in general, lower than the found for other cultivated mushrooms [24, 26]. Finally, the energy value, with an average of 353 kcal per 100g of dry matteris low, though similar to those values found within the reviewed literature (344 and 362 g kg^{-1}) [27].

CONCLUSION

After the investigation and pursuant there to, compost prepared for *A. bisporus* cultivation, based on cereals straw (wheat, barley) and chicken manure, and produced by a classical composting two-phase system, are valid for the commercial growing of *A. subrufescens*. This opens the possibility to take advantage of existing technologies and infrastructures to facilitate the diversification of the edible fungi production. As regards the loading density, the range between 60 and 70 kg m^{-2} could be applied to commercial scale. The carpophores gathered had a composition similar to that recorded by other authors under different conditions.

ACKNOWLEDGEMENTS

We would like to thank the "Diputación Provincial de Cuenca" and the "Consejería de Agricultura de Castilla-La Mancha" (Spain) for the financial support.

REFERENCES

[1] Largeteau ML *et al*. (2011).The medicinal *Agaricus* mushroom cultivated in Brazil: biology, cultivation and non-medicinal valorisation. *Appl. Microbiol. Biotechnol.* 92: 897-907.

[2] Wisitrassameewong K. *et al*. (2012). *Agaricus subrufescens*: A review. *Saudi J. Biol. Sci.*19: 131-146.

[3] Firenzouli F *et al*. (2008). The medicinal mushroom *Agaricus blazei* Murrill: review of literature and pharmaco-toxicological problems. *Evid. Based Complement. Alternat. Med.* 5(1): 3-15.

[4] Henriques GS *et al*. (2008). Avaliação *in vivo* da qualidadeprotéica do champignon do Brasil (*Agaricus brasiliensis* Wasser *et al.*). *Rev. Nutr.* 21: 535-543.

[5] Pardo-Giménez A *et al*. (2012). Effect of supplementing compost with grapeseed meal on *Agaricus bisporus* production. *J. Sci. Food Agric.* 92: 1665-1671.

[6] Lau O. (1982). Methods of chemical analysis of mushrooms. In: Tropical Mushrooms. Biological Nature and Cultivation Methods. Chang ST and Quimio TH Eds. The Chinese University Press, Hong Kong. 87-116.

[7] FOSS. (2003). The determination of nitrogen according to Kjeldahl using block digestion and steam distillation. Foss Application Note AN 300. FOSS Tecator AB Höganäs, Sweden.

[8] ANKOM. (2009). Rapid Determination of Oil/Fat Utilizing High Temperature Solvent Extraction. ANKOM Technology Method 2, AOCS Official Procedure Am 5-04. ANKOM Technology. Macedon (NY), USA.

[9] ANKOM. (2008). Crude Fiber Analysis in Feeds By Filter Bag Technique. ANKOM Technology Method 7, AOCS Approved Procedure Ba 6a-05. ANKOM Technology. Macedon (NY), USA.

[10] Sullivan DM. (1993). Proximate and mineral analysis. In: Methods of Analysis for Nutrition Labeling. Sullivan DM and Carpenter DE Eds. AOAC International. Arlington (VA), USA. pp. 105-109.

[11] Kopytowsky-Filho and Minhoni MTA. (2007). Produtividade e eficiênciabiológica da linhagemABL 99/30 de *Agaricus blazei* emtrêstipos de compostos e emdoisambientes de cultivo. *Energ. Agric. (Botucatu)*. 22: 65-78.

[12] Liang ZC *et al*. (2008). Heavy metal contents of the cultivated culinary-medicinal Royal Sun Agaricus (the himematsutake mushroom) *Agaricus brasiliensis* S. Wasser *et al*. (agaricomycetideae) using different casing materials. *Int. J. Med. Mushrooms* 10: 181-188.

[13] Gregori A *et al*. (2008). Influence of carbon dioxide, inoculum rate, amount and mixing of casing soil on *Agaricus blazei* fruiting bodies yield. *Acta Agriculturae Slovenica*. 91: 371-378.

[14] Cavalcante JLR *et al*. (2008). Cultivation of *Agaricus blazei* in the environmental protection area of the Baturité region under three types of casing soils. *Acta. Sci. Agron.* 30: 513-517.

[15] Siqueira FG *et al*. (2009). Cultivation of *Agaricus blazei* ss. Heinemann using different soils as source of casing materials. *Sci. Agric.(Piracicaba, Braz.)*. 66: 827-830.

[16] Zied DC *et al*. (2009). Produção de *Agaricus blazei* ss. Heinemann (*A. brasiliensis*) emfunção de diferentescamadas de cobertura e substratos de cultivo. *Interciencia*. 34: 437-442.

[17] Colauto NB *et al*. (2010). Pasteurização de turfabrasileirapara o cultivo de *Agaricus brasiliensis*. *Semina: Ciências Agrárias, Londrina* 31(Supl. 1): 1331-1336.

[18] Colauto NB *et al*. (2010).Tratamentostérmicos do calxistoparausocomocamada de cobertura no cultivo de *Agaricus brasiliensis*. *Ciência Rural*. 40: 1660-1663.

[19] Colauto NB *et al.* (2010). Alternative to peat for *Agaricus brasiliensis* yield. *Bioresource Technol.* 101: 712-716.

[20] Colauto NB *et al*. (2011). Production flush of *Agaricus blazei* on Brazilian casing layers. *Braz. J. Microbiol.* 42: 616-623.

[21] González Matute R *et al*. (2011). *Agaricus blazei* production on non-composted substrate based on sunflower seed hulls and spent oyster mushroom substrate. *World J. Microbiol. Biotechnol.* 27: 1331-1339.

[22] Zied DC *et al*. (2011).”Indoor” method of composting and genetic breeding of the strains to improve yield and quality of the almond mushroom *Agaricus subrufescens*. In: *Mush. Biol. Mush. Prod.* Savoie JM, Foulongne-Oriol M., Largeteau M., and Barroso G. Eds. 7: 424-432.

[23] Pardo-Giménez A *et al*. (2014). Adaptabilidad de cepasbrasileñas de *Agaricus subrufescens* Peck a la fructificaciónsobrediferentescapas de cobertura en cultivocomercial. *Rev. Iberoam. Micol.* 31: 125-130.

[24] Chang ST and Miles PG. (2004).Mushrooms. Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact (2nd ed.). CRC Press. Boca Raton (FL), USA.

[25] Siqueira FG *et al*. (2011). Biological efficiency of *Agaricus brasiliensis* cultivated in compost with nitrogen concentrations. *Hortic.Bras.* 29: 157-161.

[26] Eira AF. (2003). Cultivo do Cogumelo Medicinal *Agaricus blazei* (Murrill) ss. Heinemann o *Agaricus brasiliensis* (Wasser *et al*.). Aprenda Fácil Editora.Viçosa (MG) Brasil.

[27] Pardo-Giménez A *et al*. (2013). Análisis proximal de carpóforos de *Agaricus subrufescens* Peck producidos sobre diferentes capas de cobertura. *ITEA-Inf. Tec. Econ. Agrar.* 109: 290- 302.