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Full Length Research Paper

Efficient and cost-effective mushroom production using coffee husk substrate: Challenges and opportunities

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Coffee husk is a residue generated in large amount in Brazil and which contains caffeine and tannins, among other compounds, resulting in disposal problem. Recently, studies have shown that coffee husk can be used as substrate for mushroom cultivation. However, the main problem seems to be a low mushroom yield. Moreover, sterilization was always performed, making difficult the mushroom cultivation dissemination in deprived regions. Here, the viability of alternative methods was evaluated using lime immersion and boiling, to produce *Pleurotus ostreatus* mushrooms in coffee husk of two varieties. These alternative methods produce mushrooms with higher or similar yield as compared to sterilization method. Therefore, immersion of the substrate in water for removing toxic compounds, careful control of moisture in the substrate, and selection of strains adapted to growth in coffee husk seems to be efficient approaches to improve mushroom yield.

Key words: Coffee husk, *Pleurotus ostreatus,* cultivation, sterilization, mushroom yield.

INTRODUCTION

Brazil is the largest producer of coffee in the world (FAOSTAT, 2010). The industrial processing of coffee cherries generated a residue called coffee husk. The coffee husk is rich in nutrients (Rodriguez and Gordillo, 2011). However, it also contains compounds such as caffeine, tannins and polyphenols (Fan et al., 2000a, 2003), which can be toxic and limit the uses of this residue (Pandey et al., 2000). Therefore, most coffee husk is discarded or burned leading to environmental pollution.

Aiming to give a destination to this agro-industrial residue, studies have been made to use coffee husk as substrate for mushroom production (Fan et al., 2003; Dias et al., 2003; da Silva et al., 2012). Furthermore,

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coffee husk can be easily found in Brazil throughout the year, a very important characteristic to use any residue as substrate for mushroom production. The production of mushroom is an interesting strategy, because it provides food that is a good source of protein, fiber, vitamins and minerals for humans (Barros et al., 2008). In addition, the mushrooms can be enriched with minerals like selenium, copper and zinc,that increase their antioxidant activity (Carrasco-Gonzalez et al., 2017; Poniedziałek et al., 2017).

However, biological efficiency in coffee husks is lower than 30%, or were not able to produce mushrooms (Beaux and Soccol, 1996; Dias et al., 2003; de Assunção et al., 2012; Nunes et al., 2012).

All previous studies used sterilized coffee husk for mushroom production. Sterilization is an expensive method that requires great investment, which makes difficult its implementation in deprived regions and countries. Utilization of alternative simple and cheap methods is essential to disseminate the mushroom production, as has been shown in Mexico (Léon-Monzón et al., 2004).

Herein, the technical viability of alkaline immersion method (AIM), the quick and cheap method for mushroom production in coffee husk, was evaluated. The effect of these methods on the *Pleurotus ostreatus* mushroom yield, flush cycle and composition was tested, using coffee husk of two varieties. Appearance of contaminant fungi during mycelial run was also evaluated. Moreover, the main factors that influence the mushroom yield was assessed. This will offer useful information to produce mushroom in coffee husk, independently of the method used to treat the coffee husk.

MATERIALS AND METHODS

Microorganism and spawn production

The fungus used was *P. ostreatus*, strain PLO 06 (collection of the Laboratório de Associações Micorrízicas/Departamento de Microbiologia/BIOAGRO/UFV). This strain was grown in a Petri dish containing potato dextrose agar (PDA) culture medium (Fluka) at pH 5.8 and incubated at 25°C. After seven days, four disks of PDA containing mycelia were used for spawn production in 75 g of rice grains that were previously boiled and autoclaved at 121°C for 90 min.

Substrate, methods of treatment and inoculation

The substrates used was coffee husk of two species: Arabica coffee (*Coffea arabica*) and Conilon coffee (*Coffea canephora*). The former was obtained from the Incofex Coffee Corporation, in Viçosa, Minas Gerais State, and the last from a small farmer from Espírito Santo states, Brazil. Both substrates were mixture in a proportion of 4:1 with wheat bran (w/w, coffee husk /wheat bran).

The substrates were treated as follow: (i) boiled it in water containing 0.5% of commercial lime (CL) during 15 min, (ii) boiled it in water containing 0.5% of CL during 30 min or (iii) submerging it in water containing 2% of CL during 4 h. After treated, the substrates were centrifuged at 1800 rpm for 5 min to remove excess of water. An additional treatment was sterilization, where the substrates were moistened with water at 70% of the retention capacity and 300 g of each substrate was placed in polypropylene bags. The bags containing the substrates were autoclaved at 121°C for 2 h. Each bag containing 300 g of treated substrate was inoculated with 15 g of spawn. The bags were closed and incubated at 25°C for 20 to 30 days. After incubation period, the packages were transferred to a fruiting room with controlled temperature and humidity of 20°C and 80%, respectively. Six packages were performed for each substrate and method.

Mushroom production

Mushrooms were harvested three times, from the 15^{th} to the 65^{th} days after inoculation. The fresh weight of mushrooms was recorded to determine the biological efficiency:

BE = (Fresh weight of mushrooms/Dry weight of substrate) x100

Subsequently, the mushrooms were dried in an oven at 60°C, grounded using a knife mill and passed through a 2-mm sieve, to determine the content of minerals and the nutritional value.

Contaminations

The contaminant fungi growth was evaluated, daily, through visual observations of the fungal colonies growth along the substrate, using a scale (Figure 1).

Chemical composition

The chemical composition (moisture, protein, fat, carbohydrates and ash) was determined using AOAC (2005). The crude protein content (N × 4.38) was estimated by the macroKjeldahl method; the crude fat was determined by extracting a known weight of powdered mushroom sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15°C. Total carbohydrates were calculated by the difference. Total energy was calculated according to the following equation (AOAC, 2005):

Energy (kcal) = 4 x (g protein + g carbohydrate) + 9 x (g lipid).

Mineral content

Two hundred milligrams of dried mushrooms were digested with a 10 mL mixture of nitric acid and perchloric acid (3:1, v:v) and 500 µL H_2O_2 at 200°C. (Tedesco et al., 1995). At last, the mixture was cooled, and dilute with ultra-pure water (Milli-Q system, Millipore, USA) to a final volume of 25 mL. The solutions were filtered through Whatman No. 42 filter paper (Kent, U.K). Resulting solutions were used for direct spectrophotometric analysis. Three blank digest was carried out in the same way.

The concentrations of iron (Fe), zinc (Zn), potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg) were determined in an atomic absorption spectrometry (Optima 3300 DV; Perkin Elmer, Waltham, MA), using specific standards and procedures for each mineral as recommended by the manufacturer.

Caffeine analysis

Caffeine content was analyzed by high performance liquid

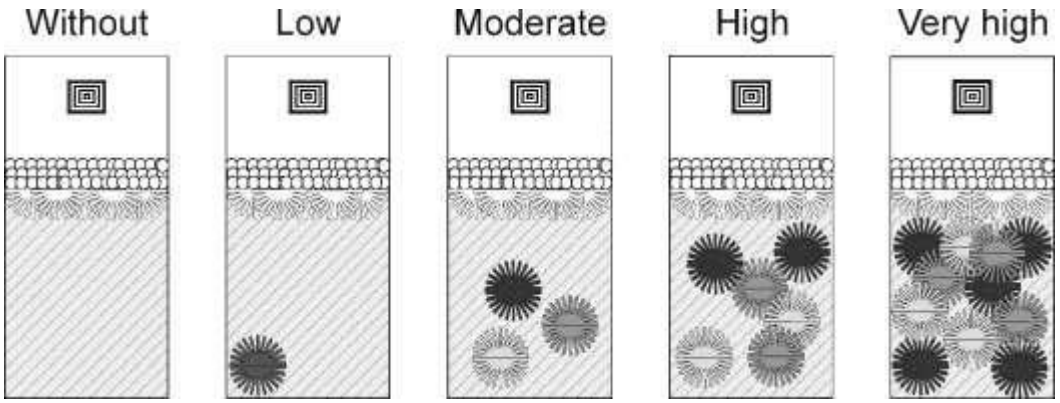

Figure 1. Qualitative parameters used to evaluate the growth of fungal contaminants in *P. ostreatus* using different substrate treatments.

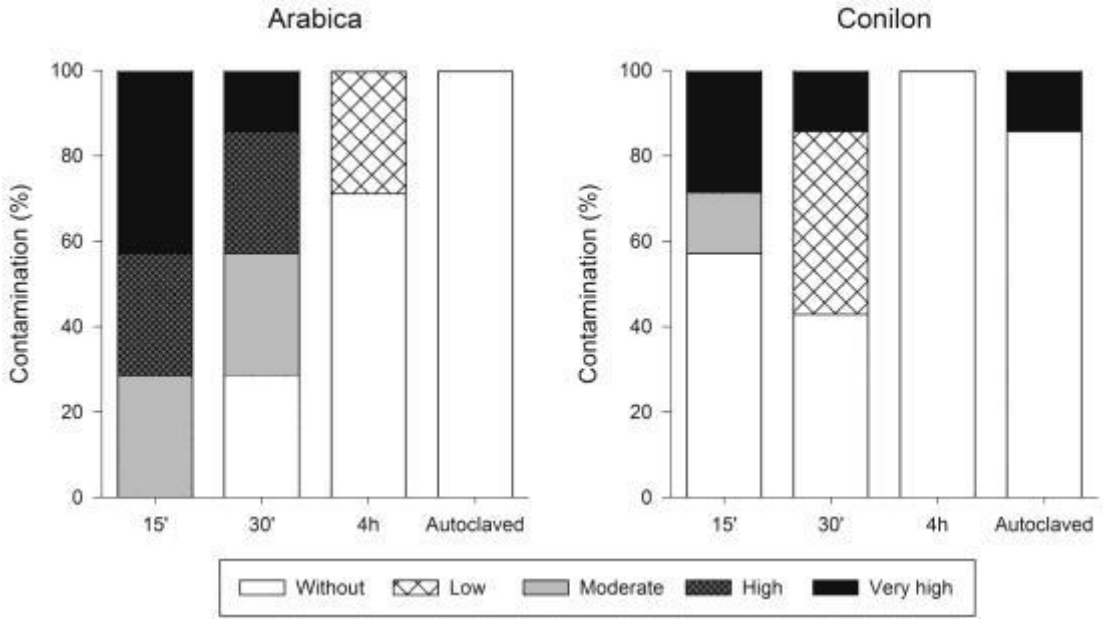

Figure 2. Percentage of *P. ostreatus* packages contaminated by other fungi when grown in substrate treated by different alkaline methods, as compared to the autoclaved one.

chromatography (HPLC) as described by Casal et al. (2000). Forty grams of the each substrate, before inoculation, were dried in oven at 75°C over-night. Then, 2 g of each substrate were boiled in 50 mL of water during 15 min. This mixture was filtered through 0.45 µm membrane. The 20 µL of the permeate was injected into HPLC (Shimadzu, C18 reverse phase and UV detection at 273 nm) and eluted with flow isocratic of 1 mL/min of a solution 50% of methanol and water (v/v). The column C18 (Ultracarb 5 ODS20, Phenomenex, Torrance, CA) had a 5 pm particle size. These analyses were made in triplicate.

Caffeine (Sigma, St. Louis, MO) was dissolved in purified water to form standard solutions. These solutions were prepared in duplicate and serially diluted to prepare the standard curve. The eluted caffeine was detected at 273 nm, after 7 min. The standard curve had a linear correlation of 0.999. Caffeine reference material (Sigma) was used to determine analytical precision. The certified caffeine was recovered totally, showing an acceptable analytical precision.

Statistical analysis

The experiment used a randomized design. The results were expressed as mean values and standard deviation. The data, except the qualitative data and the estimative of cost, were subjected to analysis of variance (ANOVA), and the averages were compared by Tukey's test ($p < 0.05$).

Pearson's correlation coefficients were used to analyze the relationship between relative contamination growth (RCG) and mushroom yield ($p < 0.05$). The degree of contaminant growth was numerically classified as follows: 0 = without; 1 = low; 2 = moderate; 3 = high; 4 = very high. Then, these values and the percentage of contaminated packages (Figure 2) were used to calculate the RCG (RGC = 0 * without% + 1 * low% + 2 * moderate% + 3 * high% + 4 * very high%).

Table 1. Biological efficiency, number of flushes and unviable packages of Arabica and Conilon coffee husk treated by four different methods (mean ± SD).

Substrate	Disinfestation method *	Biological efficiency (%)				Number of flushes	Unviable packages** (%)		
		1º	2º	3º	Total		1º	2º	3º
Arabica	15 min	09.25 ± 6.84^{b}	03.86 ± 1.77 ^{b}	-	12.46 ± 08.62^{b}	2	0.00	0.00	100.00
	30 min	11.58 ± 5.73^{b}	06.79 ± 7.67 ^{b}	-	15.65 ± 08.37^{b}	2	0.00	50.00	100.00
	4 h	24.01 ± 8.37^{a}	16.29 ± 5.93 ^{a}	-	40.30 ± 13.15^{a}	2	0.00	0.00	100.00
	Autoclaved	05.00 ± 1.94^{b}	0.00 ± 0.00 ^{c}	-	05.50 ± 02.9^{c}	1	0.00	100.00	100.00
Conilon	15 min	15.28 ± 5.70^{a}	16.18 ± 4.85 ^{a}	7.40 ± 5.03^{a}	37.37 ± 07.35^{a}	3	0.00	16.66	33.33
	30 min	20.03 ± 2.59^{a}	15.94 ± 4.32 ^{a}	8.04 ± 4.49^{a}	42.68 ± 04.06^{a}	3	0.00	0.00	16.66
	4 h	13.07 ± 7.65^{a}	06.66 ± 3.07 ^{b}	0.00 ± 0.00^{b}	17.51 ± 10.57^{b}	2	0.00	16.66	100.00
	Autoclaved	0.00 ± 0.00^{b}	0.00 ± 0.00 ^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0	100.00	100.00	100.00

*15 min: boiled for 15 min in 0.5% lime solution; 30 min: boiled for 30 min in 0.5% lime solution; 4 h: immersion for 4 h in 2.0% lime solution; Autoclave: Sterilization for 60 min in 121°C. ** Percentage of package that produced no mushroom. Means with different letters within a column are significantly different (P< 0.05) according to Tukey's test.

Table 2. Harvest time required for mushroom formation of Arabica and Conilon coffee husk treated with four different methods (mean ± SD).

Substrate	Disinfestation method *	Harvest time (d)		
		1º Flush	2º Flush	3º Flush
Arabica	15 min	40.43 ± 6.60^{a}	57.40 ± 6.50^{a}	-
	30 min	43.00 ± 7.77^{a}	56.67 ± 8.74^{a}	-
	4 h	31.50 ± 2.51^{b}	51.17 ± 6.01^{a}	-
	Autoclaved	46.60 ± 0.54^{a}	-	-
Conilon	15 min	34.00 ± 1.22^{a}	46.33 ± 1.63^{a}	57.17 ± 2.86^{a}
	30 min	31.67 ± 4.41^{a}	46.50 ± 6.09^{a}	57.00 ± 1.41^{a}
	4 h	34.80 ± 3.90^{a}	53.00 ± 5.57^{a}	-
	Autoclaved	Inhibited	Inhibited	Inhibited

*15 min: boiled for 15 min in 0.5% lime solution; 30 min: boiled for 30 min in 0.5% lime solution; 4 h: immersion for 4 h in 2.0% lime solution; Autoclave: Sterilization for 60 min in 121°C. Means with different letters within a column, for each substrate, are significantly different (P < 0.05) according to Tukey's test.

RESULTS

Mushroom yield

Independent of substrate, the mushroom yield was higher in AIM than sterilization process (Table 1). However, the biological efficiency was different among the substrates and treatments (Table 1; $p < 0.05$). The highest mushroom yield for Arabica substrate was observed when AIM for 4 hour, without boiling was used. On the other hand, for Conilon substrate, the boiling methods were more efficient (Table 1). Furthermore, the percentage of unviable packages (no mushroom) was higher in sterilized substrate (Table 1).

Harvest time required for mushroom formation

No difference in the harvest time was required for mushroom formation among the treatments for both coffee varieties (Table 2; $p > 0.05$). The AIM for 4 h was able to produce mushrooms faster ($p < 0.05$) than other treatments in the first flush for Arabica substrate; however, this difference was compensated for in the second flush ($p > 0.05$).

Contamination

The highest fungi contamination was in the boiled substrates (Figure 2). However, no correlation between contamination and mushroom yield for Arabica ($r = -0.272$, $p = 0.728$) and Conilon ($r = 0.264$, $p = 0.736$) was observed.

Chemical composition and mineral content in the mushroom

Protein and lipid content decreased when coffee husk

Table 3. Nutritional value of mushrooms on a fresh weight basis (mean ± SD).

Substrate	Disinfestation method*	Moisture (g/100)	Protein (g/100)	Fat (g/100)	Ash (g/100)	Carbohydrate (g/100)	Energy (Kcal)
Arabica	15 min	92.40 ± 0.07 ^{a}	2.41 ± 0.16 ^{ab}	0.10 ± 0.02 ^{b}	0.69± 0.01 ^{a}	4.37 ± 0.25 ^{a}	27.03 ± 0.14 ^{a}
	30 min	92.70 ± 0.38 ^{a}	2.30 ± 0.12 ^{b}	0.10 ± 0.02 ^{b}	0.71± 0.01 ^{a}	4.17 ± 0.31 ^{a}	25.84 ± 1.42 ^{a}
	4 h	92.38 ± 0.49 ^{a}	2.66 ± 0.03 ^{a}	0.15 ± 0.03 ^{ab}	0.68± 0.02 ^{a}	4.12 ± 0.45 ^{a}	27.48 ± 1.93 ^{a}
	Autoclaved	93.08 ± 0.09 ^{a}	2.68 ± 0.05 ^{a}	0.19 ± 0.01 ^{a}	0.63± 0.01 ^{b}	3.49 ± 0.15 ^{a}	25.27 ± 0.34 ^{a}
Conilon	15 min	92.56 ± 0.49 ^{a}	2.32 ± 0.11 ^{b}	0.10 ± 0.01 ^{b}	0.58± 0.01 ^{b}	4.49 ± 0.45 ^{a}	26.80 ± 1.91 ^{a}
	30 min	92.55 ± 0.11 ^{a}	2.01 ± 0.12 ^{c}	0.08 ± 0.01 ^{b}	0.59± 0.02 ^{b}	4.88 ± 0.23 ^{a}	26.59 ± 0.33 ^{a}
	4 h	92.16 ± 0.44 ^{a}	2.69 ± 0.16 ^{a}	0.13 ± 0.01 ^{a}	0.66± 0.01 ^{a}	4.26 ± 0.25 ^{a}	27.80 ± 0.71 ^{a}

*15 min: boiled for 15 min in 0.5% lime solution; 30 min: boiled for 30 min in 0.5% lime solution; 4 h: immersion for 4 h in 2.0% lime solution; Autoclave: Sterilization for 60 min in 121°C. Means with different letters within a column, for each substrate, are significantly different (P< 0.05) according to Tukey's test.

Table 4. Mineral content in mushrooms (mean ± SD; mg kg^{-1} of dry mass).

Substrate	Disinfestation method *	Fe	K	Na	Mg	Ca	Zn
Arabica	15 min	119.4±03.8^{b}	24754.0±866.0^{a}	75.0±25.0^{a}	1229.2±17.7^{ab}	738.0±236.0^{a}	83.5±01.7^{a}
	30 min	105.2±04.0^{b}	25492.0±070.7^{a}	145.7±31.7^{a}	1166.7±35.4^{b}	825.0±035.4^{a}	73.2±01.8^{a}
	4 h	170.2±23.8^{a}	23679.0±778.0^{a}	150.0±43.3^{a}	1291.7±28.9^{a}	1150.0±238.0^{a}	56.1±15.5^{ab}
	Autoclaved	83.3±04.2^{b}	21183.0±437,0^{b}	151.8±66.6^{a}	1000.0±14.4^{c}	225.0±110.9^{b}	47.4±08.6^{b}
Conilon	15 min	237.5±75.0^{a}	24006.0±907.0^{a}	253.3±112.5^{a}	2050.0±158.1^{a}	1531.0±518.0^{a}	105.2±14.6^{a}
	30 min	237.5±59.5^{a}	25019.0±646.0^{a}	185.1±066.6^{a}	1912.5±116.4^{a}	2650.0±926.0^{a}	98.0±23.6^{a}
	4 h	162.5±17.7^{a}	24625.0±106.0^{a}	331.3±027.6^{a}	2012.5±088.4^{a}	1175.0±177.0^{a}	102.2±23.9^{a}

* 15 min: boiled for 15 min in 0.5% lime solution; 30 min: boiled for 30 min in 0.5% lime solution; 4 h: immersion for 4 h in 2.0% lime solution; Autoclave: Sterilization for 60 min in 121°C. Means with different letters within a column, for each substrate, are significantly different (P< 0.05) according to Tukey's test.

substrate was boiled and ash content when the substrate was sterilized (Table 3; $p < 0.05$). The mineral content in the mushrooms was not influenced by substrate treatments in Conilon coffee (Table 4; $p > 0.05$). On the other hand, in general, there was an increase ($p < 0.05$) in the mineral content in the mushrooms produced in alternatives AIM for 4 h in Arabica substrate (Table 4).

Caffeine

The AIM methods were efficient in decreasing the caffeine content in the coffee husks (Table 5; $P > 0.05$).

DISCUSSION

One problem of the coffee industry is the management of the coffee husk generated during the coffee processing. Usually, this residue is discarded without any processing step, leading to environmental problems. The use of this residue as substrate for mushroom cultivation seems to be an interesting strategy to add economic value to this residue and prevent environmental pollution. Many studies successfully reported production of mushrooms in sterilized coffee husk

Table 5. Caffeine in coffee husk after disinfestation treatments.

Substrate	Disinfestation method*	Caffeine (g kg^{-1})
Arabica	15 min	1.19 ± 0.06^{b}
	30 min	0.92 ± 0.04^{c}
	4 h	1.11 ± 0.07^{b}
	Autoclaved	3.85 ± 0.09^{a}
Conilon	15 min	5.63 ± 0.67^{b}
	30 min	3.98 ± 0.45^{c}
	4 h	4.86 ± 0.22^{bc}
	Autoclaved	7.68 ± 0.93^{a}

*15 min: boiled for 15 min in 0.5% lime solution; 30 min: boiled for 30 min in 0.5% lime solution; 4h: immersion for 4 h in 2.0% lime solution; Autoclave: Sterilization for 60 min in 121°C. Means with different letters within a column, for each substrate, are significantly different ($P < 0.05$).

(Fan et al., 2001, 2003; Dias et al., 2003; da Silva et al., 2012). However, in South America, mushrooms are an expensive food, especially when compared with meat, making its commercialization difficult, thus justifying investigations of cheap methods to cultivate mushrooms in coffee husk. This will improve the competitiveness of this commodity; besides making cheaper the infrastructure implementation required for mushroom cultivation. So mushrooms, such as *P. ostreatus,* could be excellent food for malnutrition problem (Kane et al., 2017).

In this study, it has been showed that AIM can be used to produce mushroom in coffee husk. The mushroom yield was similar to the production obtained in sterilized coffee husk (Dias et al. 2003; de Assunção et al., 2012; Nunes et al., 2012). Furthermore, the time required to produce the mushrooms by AIM was not influenced (Table 2). The AIM had effect on the nutritional value and the mineral content of the mushrooms, decreasing protein and lipid levels when the substrate was boiled (Table 3) and increasing K, Mg, Zn and Ca levels when AIM was used, showing clearly a bioaccumulator of Ca in the mushrooms (Table 4). However, the protein, lipid and minerals levels observed here were similar or higher than those reported in others studies (Vetter, 1994; Çağlarırmak, 2007; Gupta et al., 2013), showing that mushrooms growing in AIM present an adequate profile of macro and micronutrients (Tables 3 and 4).

Relevant differences in mushroom yield, between disinfection methods depending on the substrates, were observed (Table 1), suggesting that characteristics of the coffee varieties are influencing the mushroom production. Some authors have also observed a change in the nutritional composition according to the substrate, for both *Pleurotus pulmonarius* and *P. ostreatus* (Garuba et al., 2017).

One of these characteristics seems to be presence of tannins and caffeine in the coffee husk, compounds that can exert toxic effect on *P. ostreatus* and *Lentinula edodes* (Beaux and Soccol, 1996; Pandey et al., 2000; Fan et al., 2003) and can significantly affect the fungal growth. Immersion in water or boiling can remove those compounds from the substrate (Table 5), leading to levels low enough to enable adequate mycelial growth and consequently, to an increase in mushroom yield, as observed in this study (Table 1). Indeed, prior studies reported boiling the coffee husk before sterilization of this residue for mushroom production (da Silva et al., 2012; de Assunção et al., 2012). Therefore, immersion in water seems to be a simple and efficiency alternative to improve the mushroom cultivation in coffee husk.

Other characteristic is the low moisture holding capacity of the coffee husk. The moisture content of dry coffee husks is naturally low, with values lower than 20% (Brand et al., 2000; Duku et al., 2011). Its low moisture holding capacity led to no homogeneous water distribution, allowing some sites of the substrate to be with high moisture level, which decrease the substrate porosity and prevents oxygen transfer (Pandey, 1992). On the other hand, low moisture level leads to poor accessibility of nutrients, resulting in poor growth (Pandey, 1992). In fact, prior researches showed inhibition or decrease of fungal growth due to high moisture level in coffee husk (Fan et al., 2000b; Dias et al., 2003). To avoid or minimize this problem, previous studies reported adjusting the moisture of the substrate 4 to 5 h before the autoclaving (Fan et al., 2001, 2003; Fan and Soccol, 2001). Moreover, supplementation with agroindustrial residues with high moisture holding capacity (e.g. sawdust and sugar cane bagasse) and decrease of coffee husk granulometry can be interesting strategies to improve the moisture holding capacity of the substrate.

So, the use of approaches to efficiently select fungi strains more adapted to grow in coffee husk is essential to achieve high mushroom production. The studies of mushroom cultivation in coffee husk carried out so far with high mushroom yields performed a prior screening of fungi strains in PDA medium containing extract of coffee husk (Fan et al., 2001, 2003; Fan and Soccol, 2001). This leads to selection of fungi strains more adapted to degrade tannins and caffeine, as well as to metabolize other carbon resource found in the coffee husk, which will result in a higher energy availability to support the mycelial growth and fructification process.

Here, it is shown that AIM are simple and cheap methods that can be used to produce mushroom in coffee husk. However, immersion of the substrate in water for removal of the toxic compounds, besides controlling the moisture in the substrate and selection of strains, adapt to growth in coffee husk which seems to be efficient approaches to improve mushroom yield.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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