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Alkalinization and moist heat treatments of substrates for cultivation of edible mushrooms in pupunha and cocoa residues

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In search of a healthier diet, the consumption of edible mushrooms has been expanding, as well as the use of agro-industrial residues for cultivation and the use of less costly techniques. In this work, residues of pupunha palm (*Bactris gasipaes* Kunth.) and cocoa (*Theobroma cacao* L.) were tested as a substrate to produce *Pleurotus pulmonarius* CCB19, as well as two forms of disinfection: Moist heat (autoclaving) and alkalinization with calcium hydroxide solution. Substrate compositions based on the pupunha palm residue (P) mixed with the cocoa testa (T) were tested in three different proportions (100% P; 90% P + 10% T; and 80% P + 20%T). The production time, biological efficiency (BE), production rate (PR), and diameter of the mushroom caps were analyzed. These parameters did differ neither with substrates formulations, nor with disinfection methods. The results showed the potential of P and T to be used as substrate in mushroom cultivation and alkalinization technique is as effective as autoclaving for substrate disinfection. From the study, it is concluded that alkalinization technique is a good alternative for the production of edible mushrooms by the small rural producer, since it is less expensive and easier to handle.

Key words: *Bactris gasipaes* Kunth, *Theobroma cacao*, *Pleurotus pulmonarius*, biological efficiency, agro-industrial residues.

INTRODUCTION

Within the agricultural scenario, the cultivation of edible mushrooms becomes an interesting income option for

rural producers, especially when agricultural waste can be used as substrates for the cultivation of the mushrooms. Edible mushrooms are foods which provide benefits to human health by having high protein content, essential amino acids, carbohydrates (mainly fibers) as well as low content of lipids (Ragunathan and Swaminathan, 2003; Okwulehie et al., 2014; Bach et al., 2017). They may also be a source of minerals such as calcium, potassium, iron, and zinc (Mallikarjuna et al., 2013) as well as vitamins such as riboflavin, niacin and folates (Mattila et al., 2001). Also, they have bioactive substances that give them medicinal properties, making them functional foods (Roncero-Ramos and Delgado-Andrade, 2017).

They were initially cultivated in China, around the sixth century (Chang and Miles, 2004). In 2015, mushroom production was recorded in more than 100 countries (Singh, 2015). The species most produced in the world are *Lentinula edodes* and species of the genera *Pleurotus*, *Auricularia*, *Agaricus* and *Flammulina* (Royse et al., 2017). *Pleurotus* mushrooms are fast, easy to grow and have the ability to colonize many agricultural residues (Dias, 2010). In the production of *Pleurotus*, substrates such as coffee shells, cocoa shells, coconut fibers, sawdust from coconut husks supplemented with rice bran, cupuaçu exocarp, corn cobs and sugarcane bagasse have been tested (Bermúdez et al., 2001; Marino et al., 2008; Fonseca et al., 2015; Hoa and Wang, 2015). In order to decrease the initial microbial load of the substrate, different forms of disinfection have also been studied, including autoclaving, which is considered a standard method on a large scale for mushroom production, and alternative methods, such as alkalinization (Contreras et al., 2004; León-Monzón et al., 2004; Nunes et al., 2017) along with pasteurization (Moda et al., 2005). Due to the cost, energy consumption and necessary training, autoclaving substrates are difficult to adopt for small and medium rural producers. Considering the costs, alkalinization can be an alternative for disinfesting substrates. This technique consists in raising the pH of the substrate to alkaline levels, inhibiting the growth of contaminating fungi and bacteria without substantially affecting the development of *Pleurotus* sp. (Contreras et al., 2004; León-Monzón et al., 2004; Bernabé-González and Cayetano-Catarino, 2009; Nunes et al., 2017).

Another way to reduce the cost of production is the use of local agro-industrial residues as substrate for mushroom cultivation. In southern Bahia, the production activities of peach palm (*Bactris gasipaes* Kunth.) for obtaining industrialized palm hearts, and cacao (*Theobroma cacao* L.) for obtaining cocoa beans, in 2019, was 5,200 and 41,637 tons, respectively (IBGE, 2019). In these productive chains, a large amount of waste is produced. Approximately 13 kg of waste is generated for every 400 g of palm heart (Fermino et al., 2010) and to obtain one ton of cocoa almond, 80-120 kg of cocoa seed shell is generated after processing (Silva et al., 2015). Therefore, considering the availability of these agro-industrial residues, the objective of this study was to evaluate their use in the production of *Pleurotus pulmonarius* CCB19 and the effectiveness of alkalinization in calcium hydroxide solution as a disinfection method.

MATERIALS AND METHODS

Microorganism

Culture of *P. pulmonarius* CCB19 was obtained from the culture collection of the State University of Maringá, Paraná, Brazil, and maintained at 4°C in potato dextrose agar (PDA). Reactivation was performed in Petri dishes containing PDA medium and incubated at 25 ± 2°C in the absence of light until the PDA medium was completely covered by the fungal mycelium (Oliveira et al., 2007).

Preparation of the inoculum or spawn

The spawn was prepared using wheat grains in according to Bononi et al. (1999) with some modifications. The grains were previously cooked in boiling water for 15 min. After, draining the excess water, calcium carbonate ($CaCO_3$) and plaster (1:4, w:w) were added in the proportion of 3% of the dry mass of the grain. Portions of 300 g were placed in polypropylene bags and sterilized for 1 h at 121°C. After cooling the grains, plugs (7 mm) of the *P. pulmonarius* mycelium grown in PDA (of one-third of the cultivated mycelium) were inoculated on the surface of the autoclaved wheat grains. The inoculated bags were kept at 25°C, in the absence of light, until the fungus mycelium completely colonized the grains.

Residues, substrate preparation and mycelium inoculation

The discarded pupunha leaf sheath residue from the processing of palm heart was used as the substrate. This residue was subjected to drying in a forced air greenhouse at 50°C for 2 days and then crushed in a knife mill, using a 12-mm sieve. The dried and crushed cocoa testa (cocoa shell) was also used along with the pupunha residues. For the preparation of the substrates, residues were mixed in three different proportions: 100:0; 90:10 and 80:20 of pupunha leaf sheath: cocoa testa, respectively. Afterwards, they were subjected to two different types of disinfection, heat treatment (autoclaving) and immersion in alkaline solution ($CaOH_2$), composing six different types of tested substrates (S1 to S6), as shown in Table 1.

The autoclaving and alkalinization treatments were developed based on the studies of Nunes et al. (2017). The water holding capacity was adjusted to between 66 to 67%, thereafter 500 g of substrates (S1-S3) were distributed in polypropylene bags and

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Table 1. Proportions of residues of pupunha leaf sheath and cocoa testa, and disinfection treatments (autoclaving and alkalinization) of the substrate for *Pleurotus pulmonarius* CCB19 cultivation.

Substrate	Substrate formulation (%)		Disinfection treatments
	Pupunha	Cocoa	
S1	100	0	
S2	90	10	Autoclaving
S3	80	20	
S4	100	0	
S5	90	10	Alkalinization
S6	80	20	

autoclaved at 121°C and 1 atm for 2 h. For the alkalinization treatment, the residues were subjected to immersion in a 2% $CaOH_2$ for 18 h and centrifuged at 363 g for 6 min to remove the liquid excess. Further, 500 g of each substrate (S4-S6) were packed in plastic bags (capacity of 1 kg).

For pH analysis, 5 g of sample was diluted in 50 ml of distilled water and stirred for 30 min. For moisture content analysis, 5 g of the previously treated substrate sample was taken to the greenhouse (50°C) until a constant mass was obtained. The residues were also submitted to analysis of carbon and nitrogen content in according to Brazilian Ministry of Agriculture (MAPA, 2006). For the analysis of the carbon content, 5 g of the substrate previously dried in a greenhouse was submitted to a muffle incineration at 550°C for 1 h to determine the organic matter content and then, the carbon content was calculated. The nitrogen analysis was performed by the Kjeldahl method by distillation (Kjeldahl, 1883).

Cultivation of *P. pulmonarius* CCB19

After autoclaving or $CaOH_2$ solution immersion, the spawn (10% w/w) were inoculated on the substrate surface. The bags with substrates and spawn were incubated at 25°C, moisture of 60 - 70% (monitored by a hygrometer), and absence of light until the mycelium occupies all the substrate (Oliveira et al., 2007). The time (days) required for colonization of the substrate by the fungus (mycelial run) was monitored. After complete colonization, the mycelium was subjected to thermal shock at 4°C for 24 h. For fruiting, the colonized substrates were transferred to a room with a temperature of 20°C, moisture of 80 - 90% and the presence of light until initial formation of the mushrooms (Nunes et al., 2012). Thereafter, the bags were opened to expose the substrate surface to air until harvesting of the basidiocarps. After the first harvest (1st flush), the substrates were retransferred to the incubation room (25°C, moisture of 60 - 70%, absence of light), to allow new mycelial growth aiming at the second harvest (2nd flush).

Determination of biological efficiency (BE), productivity rate (PR) and morphological analysis (cap size)

From each cultivation bag, the mass of the harvested mushrooms was determined and with the dry mass of the substrate, the biological efficiency (BE) was determined using the formula:

BE (%) = (Fresh mushroom mass / Initial dry mass of the substrate) × 100

In addition, the productivity rate (PR) was calculated by dividing the BE by the time in days required for harvesting (Oliveira et al., 2007). After harvesting, the size of each mushroom's cap was measured with a millimeter ruler (Liasu et al., 2015).

Statistics

The experimental design was completely randomized in a 1 × 3 × 2 × 4 factorial scheme, corresponding to 1 fungal strain, 3 substrate formulations, 2 substrate disinfestation modes and 4 repetitions for each treatment, totaling 24 portions represented by each culture bag. The data were submitted to analysis of variance (three-way ANOVA) and compared by Tukey's test at 5% probability. The statistical procedures used in this study were performed in the R program (R Core Team 2019). 'ExpDes' package: ANOVA, Shapiro-Wilk test, and Tukey's Test (Ferreira et al., 2019); 'lattice' package: graphic elements (Sarkar, 2008).

RESULTS

Physico-chemical evaluation of the cultivation substrate and cultivation time of *P. pulmonarius* CCB19

The values of the carbon and nitrogen ratio (C/N), moisture, pH, mycelial colonization time (TMC), and mushroom harvest time (MH) of the two fruiting cycles are presented in Table 2. The pH values of the initial substrates were 4.6 in the autoclaved substrates (S1, S2 and S3) and between 10 and 11 in the alkalized substrates (S4, S5 and S6). Moisture values ranged from 66 to 67% for autoclaved substrates, and from 71 to 73% for alkalized substrates. The carbon-nitrogen ratio ranged from 33.6 to 37.7 for autoclaved substrates and 41.7 to 44.1 for alkalized substrates. The time (days) of colonization and harvest were recorded during the first and second flushes. In the first flush, the colonization time of the alkalized substrates (26 days) was significantly shorter ($p<0.05$) than the colonization time of the autoclaved substrates (37.3 to 41.3 days). In this same first flush, the harvest time of alkalized substrates was also significantly shorter (30.5 to 31.3 days) than for autoclaved substrates (43.3 to 46.8 days). In the second flush, the colonization time was significantly longer for

Table 2. Physico-chemical evaluation (pH, moisture and C/N), Time of mycelial colonization (TMC) and mushroom harvest (MH), in two production flushesof *Pleurotus pulmonarius* CCB19 in pupunha leaf sheath and cocoa testa residues in different proportions and submitted to autoclaving or alkaline treatment.

Substrate*	pH	Moisture (%)	C/N	Flush 1 (day)		Flush 2 (day)	
				TMC	MH	TMC	MH
S1	4.6	67	37.7	41.3[a]	46.8[a]	69.8 [a]	76.8 [a]
S2	4.6	66	35.7	37.3[b]	43.3[a]	59.3 [ab]	64.3 [ab]
S3	4.6	66	33.6	39.8[ab]	45.8[a]	64.5 [ab]	71.3 [ab]
S4	10	72	44.1	26.0 [c]	31.3[b]	53.0 [b]	59.5 [b]
S5	11	73	42.0	26.0 [c]	30.8[b]	53.3 [b]	60.0 [ab]
S6	10	71	41.7	26.0 [c]	30.5[b]	53.0 [b]	58.8 [b]

*S1: 100% pupunha residue (autoclaved); S2: 90% pupunha residue + 10% cocoa testa (autoclaved); S3: 80% pupunha residue + 20% cocoa testa (autoclaved); S4: 100% pupunha residue (alkalinized); S5: 90% pupunha residue + 10% cocoa testa (alkalinized); S6: 80% pupunha residue + 20% cocoa testa (alkalinized). Means followed by different superscripts in the same column show a significant difference (p<0,05) according to the Tukey test.

Table 3. Results of biological efficiency (BE) of the two flows, total BE and productivity rate (PR) in the different substrates for the cultivation of *Pleurotus pulmonarius* CCB19 in residues with different proportions of pupunha leaf sheath and cocoa testa residues submitted to autoclaving and alkaline treatment.

Substrate*	BE(%)		BETotal(%) (Flushes1+2)	PR
	Flush1	Flush2		
S1	55.4[a]	11.1[b]	66.5[a]	0.9[a]
S2	42.0[ab]	13.4[b]	55.3[a]	0.9[a]
S3	32.6[ab]	16.4[ab]	49.0[a]	0.7[a]
S4	26.9[b]	37.0[a]	63.9[a]	1.1[a]
S5	31.5[b]	22.6[ab]	54.1[a]	0.9[a]
S6	32.8[ab]	38.5[a]	71.2[a]	1.2[a]

*S1: 100% pupunha residue (autoclaved); S2: 90% pupunha residue + 10% cocoa testa (autoclaved); S3: 80% pupunha residue + 20% cocoa testa (autoclaved); S4: 100% pupunha residue (alkalinized); S5: 90% pupunha residue + 10% cocoa testa (alkalinized); S6: 80% pupunha residue + 20% cocoa testa (alkalinized). Means followed by different superscripts in the same column show a significant difference (p<0.05) by Tukey test.

autoclaved substrate (S1) when compared to the alkalized substrates, and the harvest time was significantly longer also for S1, but only when compared to two of the alkalized substrates (S4 and S6). In general, only the first flush showed a significant difference in colonization time and harvest time between alkalized and autoclaved substrates. Considering the same treatment of disinfection, autoclaving (S1, S2, S3) or alkalinization (S4, S5, S6), in these two flushes, different proportions of pupunha leaf sheath and cocoa testa substrates did not show significant difference for the mycelial colonization time and harvest time.

Biological efficiency (BE), productivity rate (PR) and morphological analysis (cap size)

Table 3 shows the biological efficiency results for each flush and total. In the first flush, the BE values between the different substrates (autoclaved or alkalized, with or without cocoa testa) did not show significant difference, except for the value of S1 which was higher than S4 and S5. In the second flush, in general, a difference in BE was observed between autoclaved and alkalized treatments ($p < 0.05$), and the alkalized substrates presenting a higher EB value. The results of total BE (first flush+ second flush) varied from 49 to 71.2% while PR was between 0.7 and 1.2. BE and PR showed no significant difference between treatments. The presence of contaminants was observed in only two samples of the alkalized substrates, which means 11% of all samples (data not shown). The results of the cap size of the mushrooms collected during the two flushes are presented in Table 4. In flush 1, the cap size varied from 2.0 to 3.2 cm and only the mushrooms collected in substrate S1 had a significantly smaller cap size when

Table 4. Cap diameter of the *Pleurotus pulmonarius* CCB19 mushrooms grown in residues of pupunha leaf sheath and cocoa testa residues, in different proportions, subjected to autoclaving and alkaline treatment

Substrate*	Cap diameter (cm)	
	Flush 1	Flush 2
S1	2.0[b]	2.6[b]
S2	3.1[a]	3.0[ab]
S3	3.2[a]	2.8[ab]
S4	2.8[a]	3.6[a]
S5	2.8[a]	3.3[ab]
S6	2.7[a]	3.6[a]

*S1: Mushrooms grown in 100% pupunha (autoclaved); S2: mushrooms grown in 90% of pupunha residue + 10% cocoa testa (autoclaved); S3: mushrooms grown in 80% of pupunha + 20% cocoa testa (autoclaved); S4: mushrooms grown in 100% pupunha residue (alkalinized); S5: mushrooms grown in 90% of pupunha residue + 10% cocoa testa (alkalinized); S6: mushrooms grown in 80% of pupunha residue + 20% cocoa testa (alkalinized). The different superscripts in the same column show a significant difference ($p<0.05$) according to the Tukey test for an average of four repetitions per substrate.

compared to the other mushrooms. In flush 2, the cap size varied from 2.6 to 3.6 cm and S1 continued to have the smallest cap, but it differed significantly only from S4 and S6.

DISCUSSION

Physico-chemical evaluation of the cultivation substrate and cultivation time of *P. pulmonarius* CCB19

Values of pH, moisture and the carbon and nitrogen ratio (C/N) are variables that impact on the time of substrate colonization by the fungal mycelium and consequently, the basidiocarp harvest time (Bellettini et al., 2016). The difference between the pH values in autoclaved and sterilized substrates was due to the solution composed of $CaOH_2$ used only in the alkalinization methodology. According to Chang and Miles (2004), the pH range for mycelial growth of *Pleurotus* spp. is about 5.5 to 6.5. However, some *Pleurotus* spp. have good growth over a wider pH range, from 5.5 to 7.5 (Yadav, 2001). Sardar et al. (2015) showed that a substrate with a pH of lower than those recommended can reduce the mycelial growth rate.

The initial pH of the autoclaved substrates (S1, S2, S3) was less than the range recommended in the literature (Table 2). This may have negatively influenced the mycelial growth rate of *P. pulmonarius* with an increase in the mycelial colonization time. Meanwhile, in alkaline substrates (S4, S5, S6) with high pH, the mycelial colonization time and harvest time were shorter than those times for autoclaved substrates. In general, in the second flush there was no difference between autoclaved and alkalized substrates for the times of mycelial colonization and harvest time. Similar results were found by Nunes et al. (2017) when testing the feasibility of alternative methods for producing *Pleurotus ostreatus* in coffee husks. These authors reported that the method of immersion in alkaline solution for 4 h produced mushroom faster than the autoclaved treatments in the first flush. However, in the second flush, no mushrooms were harvested from the autoclaved substrates (Nunes et al., 2017). Another factor to consider when growing mushrooms is the substrate moisture content (Rajarathnam and Bano, 1988; Dias et al., 2003). On the other hand, the lower concentration of water reduces mycelial growth, since water is essential for the assimilation and transport of nutrients (Stamets and Chilton, 1983). The recommended substrate moisture content for basidiomycete growth is between 50 and 75% (Chang and Miles, 2004). However, Oliveira et al. (2007), managed to grow *P. pulmonarius* on a substrate formed by a mixture of wheat bran, corn straw, peanut husks with 80% of humidity. In this work, the alkalized substrate had higher moisture content than the autoclaved substrates (Table 2). This difference was probably due to the different wetting processes between the two disinfection treatments (autoclaving and alkalinization). The autoclaved substrates were moistened with the controlled addition of water. In alkalinization, the substrate was immersed in an aqueous solution for 18 h, a difference that probably did not negatively affect the development of the mycelium.

The substrate carbon and nitrogen (C/N) ratio is an important factor for influencing mycelial growth and mushroom production (Nunes et al., 2012). Therefore, the C/N ratio needs to be adjusted to promote higher mushroom productivity, avoiding excess or deficiency of nitrogen, as the high nitrogen content can inhibit the development of the mycelium and the lack of nitrogen can compromise the mushroom quality, biological efficiency, and productivity (Yang et al., 2013). In the present work, the C/N ratio (Table 2) according to Kurt and Buyukalaca (2010); Bernardi et al. (2013) and Cueva et al. (2017), indicating that the substrates used (peach palm leaf sheath alone or supplemented with cocoa testa) provide the C/N ratio necessary for *P. pulmonarius* CCB19 for the development of its mycelium and mushroom growth.

Different supplementation rates impact on C/N ratios, and consequently in the productivity (Zanetti and Ranal, 1997; Yang et al., 2013; Cueva et al., 2017). Therefore, in this work, different proportions of cocoa skin were tested to increase the nitrogen content in the substrate to evaluate the impact of the difference in the C/N ratio on the growth of *P. pulmonarius*. The low variation of the C/N ratio between treatments was probably not enough to differentiate the fungus growth time, biological

efficiency and productivity rate in the different treatments (Tables 2 and 3).

Biological efficiency (BE), productivity rate (PR) and morphological analysis (cap size)

From the point of view of the producer, the biological efficiency, productivity and morphology of mushrooms show how much a given technique is viable. In this study, the results of alkalinization technique did not significantly differ from the moist sterilization technique, showing that the alkalinization of the substrates tested is as viable as autoclaving in the cultivation of *P. pulmonaryius*. In addition, alkalinization has the advantages of greater power savings and easier handling.

So far, few scientific works have studied the potential of peach palm residue as a substrate for the cultivation of edible mushrooms. In addition, there is no work in the literature so far that reports on this residue being treated with alkalinization. Sales-Campos et al. (2010) used sterilized substrate prepared with peach palm stem for the cultivation of *P. ostreatus*, and obtained BE values ranging from 123.13 to 128.66%.

Regarding the cultivation of *P. pulmonarius* in several agro-industrial residues, the literature shows different values of BE. To evaluate the spawn production with different substrates and using the same lineage of *P. pulmonarius* CCB19 of this study, BE ranging from 25 to 43% was reported (Oliveira et al., 2007). When another lineage of *P. pulmonarius* was grown in different types of wood (*Milicia excelsa*, *Gmelina arborea*, *Afzelia africana* and *Khaya senegalensis*), BE ranging from 25.56 (*A. africana*) to 36.13% (*G. arborea*) was observed (Adewoyin and Ayandele, 2018) and when grown in brachiaria straw treated with different volumes of $CaCO_3$ solution, BE ranged from 69.87 to 135.50% (Iossi et al., 2018). Another experiment involved using dried banana leaves or dried leaves of *Chrysalidocarpus lutescens*, a palm tree, treated with immersion in alkaline solution or in hot water at 80°C for 1 h. The highest BE was 120.1% for mushrooms grown on banana leaves immersed in alkaline solution, followed by BE of 81.24% for the same substrate treated in hot water. On the other hand, the BE of dry palm leaves were 41.4 and 44.9% in the treatments with alkaline solution and hot water, respectively (Bernabé-González and Cayetano-Catarino, 2009). When using corn stalk supplemented with different concentrations of wheat bran or corn flour, a BE of 113 and 132% was found, respectively (Mkhize et al., 2016). These authors indicated that the addition of supplements had a positive influence on the production of *P. pulmonarius*, thus showing the importance of supplementation for some residues. In this study, the cocoa seed testa was used as a potential supplement for the pupunha residue. The different concentrations of the studied substrates (0, 10 and 20% of cocoa seed testa) did not result in a difference ($p > 0.05$) regarding BE and PR with the tested mushroom (Table 3). Therefore, it turns out that the pupunha residue with or without cocoa seed testa addition is a substrate with potential to produce *P. pulmonarius* CCB19 mushrooms. This is also reinforced by PR between disinfection treatments since there was no statistical difference between them (Table 3).

Regarding the morphological analysis, mushrooms grown on different substrates, in general, did not show difference in the size of the cap between the different disinfection methods (Table 4). Cap size varied from 2.0 to 3.2 cm in the first flush, and from 2.6 to 3.6 cm in the second flush. It could be an advantage for the producer, in terms of cap size, in continuing the production of the mushroom in the second flush. Oliveira et al. (2007), working with the same isolate used in this study (*P. pulmonarius* CCB19), obtained mushrooms with a cap size between 5 and 10 cm, when the inoculum was prepared with corncob residue. Cap sizes similar to that found in this work (less than 5 cm) were obtained when the inoculum was prepared with rice husk. Other studies on cultivating *P. pulmonarius* on different substrates, found cap sizes ranging from 3.20 to 5.50 cm (Mkhize et al., 2016) and from 2.9 to 13.5 cm (Liasu et al., 2015). The size of the cap of the mushrooms is affected by environmental conditions, such as aeration, characteristics of each species and by a specific response of each species to environmental conditions. In addition, substrate conditions, such as type, presence or absence of supplementation, and technique for treating the substrate can influence the size of the cap (Chang and Miles, 2004; Ng'etich et al., 2013; Yang et al., 2013). In this work, the use of different disinfection method (autoclaving and alkalinization), as well as different concentrations of pupunha and cocoa testa residues did not demonstrate an effect on the cap diameter size of the *P. pulmonarius* CCB19.

Conclusion

Pupunha residues and pupunha residues plus cocoa are equally efficient to produce *P. pulmonarius* CCB19 mushrooms. The use of the calcium hydroxide solution as a chemical agent for disinfesting the substrate has biological efficiency similar to the cultivation in autoclaved substrates. Based on the production of mushrooms by small farmers, the method of treating the substrate by alkalization is a better alternative, considering the BE, PR of the mushrooms, as well as the ease of access to the technique, low cost and easy handling.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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