African Journal of Biotechnology Vol. 11(53), pp. 11630-11635, 3 July, 2012
Available online at http://www.academicjournals.org/AJB
DOI:10.5897//AJB12.451
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Cultivation of *Pleurotus* mushrooms in substrates obtained by short composting and steam pasteurization

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Accepted 10 May, 2012

This paper presents results of two experiments for cultivation of *Pleurotus ostreatus*, *Pleurotus pulmonarius* and *Pleurotus eryngii* grown with different formulations of grass and straw mixtures derived from agro-industrial residues. Cultivation was prepared through a number of approaches, such as short composting/pasteurization and axenic culture. In the first experiment, *P. pulmonarius* was grown on two formulations of different grasses, with no significant differences observed for either productivity or biological efficiency, with values close to 20 and 60%, respectively. The second experiment revealed similar productivity and biological efficiency between *P. pulmonarius* and *P. ostreatus* for both forms of substrate treatment (short composting/pasteurization vs. axenic culture), with similar values to those observed in the first experiment. *P. eryngii* did not produce mushrooms in the composting treatment and showed lower productivity (17.5%) than the other two species (20.5 and 20.8%, respectively) when the substrates were autoclaved (axenic culture). The preparation for short composting and steam pasteurization was described in illustrative figures in order to provide expertise to small producers who wish to initiate economic and sustainable mushroom cultivation making use of regional lignocellulosic residues.

Key words: Steam pasteurization, lignocellulosic biomass, straw mixtures, mushrooms.

INTRODUCTION

The cultivation of *Pleurotus* species occupies third place in terms of global mushroom production, with versatility in terms of growth substrates and cultivation conditions. As such, it represents an alternative source of income for small holder farmers (Mansur et al., 1992; Sánchez and Royse, 2001; Valencia and López, 2005; Urben, 2004). Many lignocellulosic materials, including agricultural and industrials residues, such as sugarcane bagasse, straws (wheat, bean, rice, corn, etc), sawdust, coffee pulp and cotton textile industry residues, amongst others, can be used as substrates for *Pleurotus* spp. cultivation (Oei, 2003; Peksen and Küçükomuzlu, 2004). This versatility allows for cultivation in lower cost substrates and different climatic conditions.

Appropriate treatment to obviate the risk of substrate contamination can, however, constitute a limiting factor for small producers in tropical countries, given the limited investment in appropriate infrastructures (Dias, 2010). According to Laborde and Delmas (1974) and Valencia and López (2005), a number of different methods for substrate pasteurization or sterilization have been proposed: (a) autoclaving (axenic), (b) axenic and inoculation with thermophilic microorganisms, (c) rapid substrate steam treatment between 80 and 100°C for several hours, (d) NADASI system: pasteurization at 72°C for four or five days, and (e) pasteurization by substrate steam treatment for several days (60°C) in a tunnel.

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Table 1. Formulations of the substrates used in short fermentation with steam pasteurization for cultivation of *Pleurotus pulmonarius* (Experiment 1).

Formulation 1			Formulation 2		
Substrate	**Amount (kg)**	**Dry matter (kg)**	**Substrate**	**Amount (kg)**	**Dry matter (kg)**
Coast-cross hay*	20	19.1	Corn stover*	40	36.2
Soybean straw*	30	26.6	Bean straw*	40	35.9
Corn stover*	20	19.1	Bahia grass*	20	18.0
Sugarcane bagasse*	30	26.8	-	-	-
Total	100	91.6	Total	100	90.1

*Raw material: *Cynodon dactylon* (L.) Pers (coast-cross hay); *Glycine max* (L.) Merrill (soybean); *Zea mays* L (corn); *Saccharum officinarum L.* (sugarcane); *Phaseolus vulgaris* L. (bean); *Paspalum notatum* Flueggé (bahia grass).

Table 2. Total nitrogen content of raw materials used as substrates after short fermentation with steam pasteurization for cultivation of *Pleurotus pulmonarius* (Experiment 1).

Formulation 1				Formulation 2			
Substrate	**N-Total (%)‡**	**Dry matter (kg)**	**N-Total (kg)**	**Substrate**	**N-Total (%)‡**	**Dry matter (kg)**	**N-Total (kg)**
Coast-cross hay*	1.00 ± 0.05	19.1	0.191	Corn stover*	0.94 ± 0.05	36.2	0.34
Soybean straw*	0.64 ± 0.03	26.6	0.170	Bean straw*	1.60 ± 0.08	35.9	0.57
Corn stover*	0.94 ± 0.04	19.1	0.179	Bahia grass*	1.39 ± 0.07	18.0	0.25
Sugarcane bagasse*	0.40 ± 0.02	26.8	0.107	-	-	-	-
Total		91.6	0.647	Total		90.1	1.164
			%NT1 = 0.71%				%NT2 = 1.29%

‡Total nitrogen was estimated by the Kjeldahl method (five replicates). *Raw material: *Cynodon dactylon* (L.) Pers (coast-cross hay); *Glycine max* (L.) Merrill (soybean); *Zea mays* L (corn); *Saccharum officinarum L.* (sugarcane); *Phaseolus vulgaris* L. (bean); *Paspalum notatum* Flueggé (bahia grass).

In Brazil, most research on evaluation of substrates for *Pleurotus* cultivation has been performed using the axenic cultivation system, which is accessible only to research institutions or companies with investment capacity (Dias, 2010). Nevertheless, reports of contamination problems with sterilized substrates are common, as maintenance of growing conditions free of contaminant microorganisms is arduous. Steam pasteurization and composting are traditional procedures which are more appropriate for small scale mushroom growers, as these substrates are more stable and less susceptible to contamination (Chang and Miles, 2004). The aim of this study was to evaluate the applicability of Brazilian agricultural residues or grasses as substrates for the production of *Pleurotus* species using short composting followed by steam pasteurization. Determination of the technical adequacy of this simple, low cost procedure for growth substrate preparation and culture conditions will promote acceptance by small producers, ultimately increasing the number of *Pleurotus* producers in Brazil.

MATERIALS AND METHODS

Spawn production

Pleurotus ostreatus, *Pleurotus pulmonarius and Pleurotus eryngii* were grown in potato dextrose agar (PDA) medium for maintenance and for spawn production. The mushroom spawn was prepared in rice husk substrate enriched with wheat bran, limestone and gypsum, as described by Siqueira et al. (2011).

Biological efficiency of *P. pulmonarius* in two substrate formulations (types) obtained by composting

P. *pulmonarius* was evaluated by a short composting technique followed by steam pasteurization of two formulations produced with different nitrogen concentrations (Tables 1 and 2). Total nitrogen was estimated by the Kjeldahl method (Table 2). The basic steps of the process are shown in Figure 1. Residues were prepared in alternating layers up to a weight of 100 kg and arranged into 1.2 x 1.2 × 1.2 m windrows (Figure 1A). Water was added to each of the residue layers throughout the process to ensure sufficient wetting of the substrate. Composting was conducted for seven days and the compost was turned every two days during each process (Figure 1B). During the overturning, compost humidity was checked and adjusted to approximately 65%. After seven days of composting, the substrate was transferred to a 500 kg capacity pasteurization tunnel (Figure 1C) for 12 h of continuous steaming, with temperatures maintained between 60 and 70°C. After 12 h, the compost was overturned to ensure homogeneity and cooled to a temperature below 30°C. The compost was then packed in bags (10 kg/bag) (Figure 1D), and thoroughly inoculated with 200 g of spawn (Figure 1E). After inoculation, the compost was incubated at room temperature, oscillating between 25 and 30°C, for a period of 30 days. Following incubation, bags were completely opened and humidity maintained above 80% using nebulizers (Figure 1F).

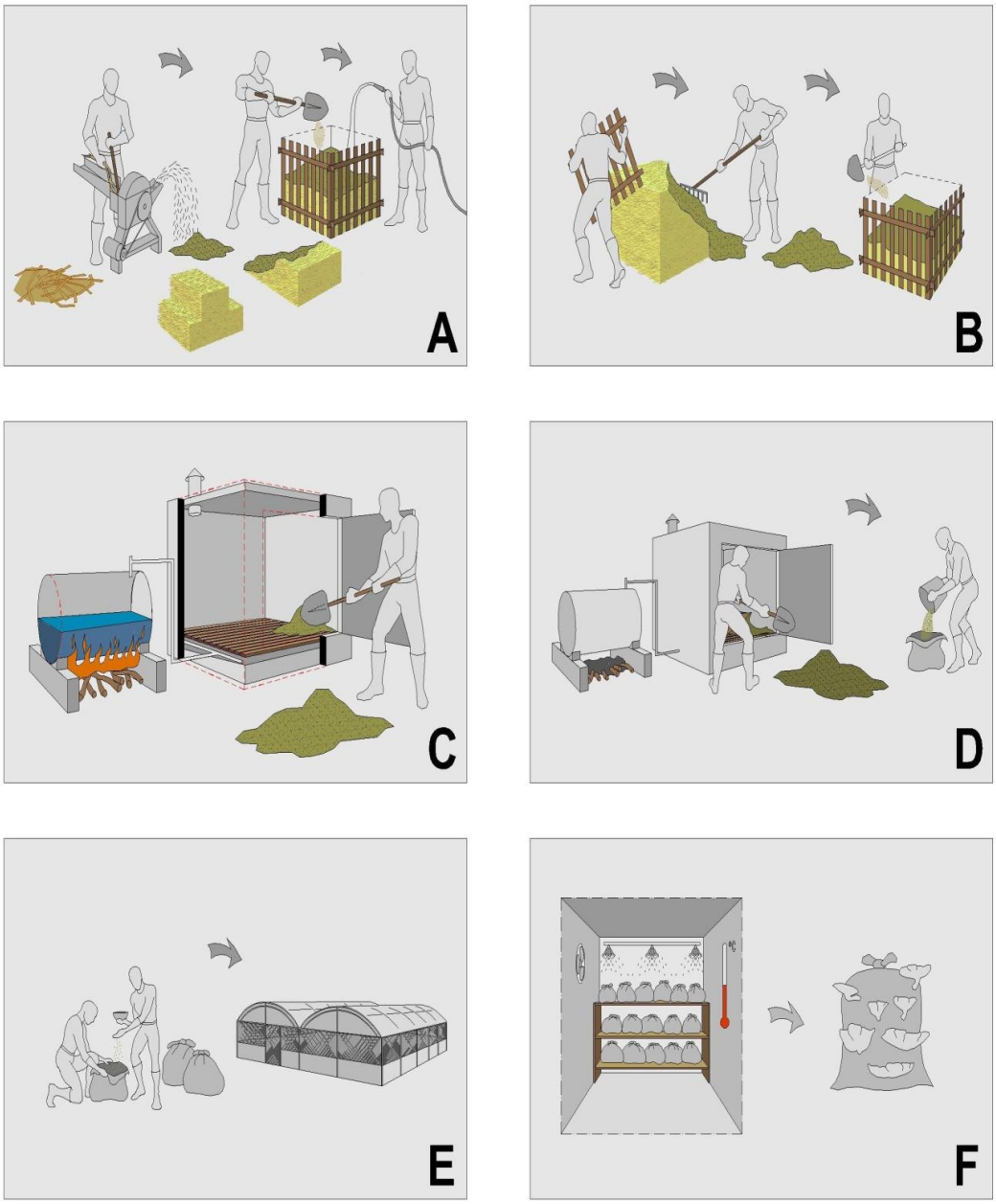

Figure 1. Scheme for the preparation of substrates for composting and steam pasteurization for cultivation of *Pleurotus* spp. (A) Preparation of raw ingredients in long rectangular piles (known as ricks or windrows), approximately 1.2 m high. (B) Periodical turning, watering and remounting. This phase is essentially a microbiological process resulting in the release of energy and heat. (C) Steam pasteurization of the material after composting. (D) Packaging substrate and pasteurization in plastic bags. (E) Compost inoculation. (F) Fruiting induction and cultivation.

Comparison of biological efficiency of *Pleurotus* species cultivated in bean straw prepared by axenic sterilization and composting

Axenic cultivation

Substrate was packed in polypropylene bags with a gas exchange filter (1.5 kg/bag) and sealed using a sealing machine. Bags were autoclaved twice at 24 h intervals, at 121°C/2 h. One bag was separated for analysis of humidity and dry matter content by drying at 65°C for 48 h for subsequent estimation of biological efficiency. Substrate inoculation was conducted in a horizontal laminar flow chamber by adding 5 g of the spawn to each bag. Only 5 g of spawn was applied as it was added as inoculum at the substrate surface, to allow monitoring of mycelial growth in each treatment. After inoculation, bags were sealed and incubated at room temperature, between 25 and 30°C. After complete colonization, the bags colonized with *P. pulmonarius* were transferred to

Table 3. Productivity and biological efficiency of *Pleurotus pulmonarius* in substrates processed by short fermentation with steam pasteurization (Experiment 1).

Substrate formulation (Tables 1 and 2)	Colonization time (days)	Productivity (%)*	Biological efficiency (BE) (%)**
Formulation 1	30	19.1[a]	54.5[a]
Formulation 2	25	21.2[a]	60.5[a]

Means followed by same letter do not differ statistically in the Scott-Knott test at the 5% probability level.*,Productivity (%): fresh harvest weight/ substrate wet weight) × 100; **BE (%): fresh harvest weight/ substrate dry weight) × 100.

mushroom-house cultivation at room temperature and relative air humidity maintained above 80%. In the case of bags colonized with *P. ostreatus* and *P. eryngii*, mushroom-house cultivation maintained a temperature at 18 ± 2°C, again with relative air humidity above 80%. All bags were completely opened to allow mushroom fruiting during a two month period.

Cultivation in substrates produced by short composting

For composting prepared using bean straw, 100 kg of dry straw were prepared according to the procedures described in Figure 1. Compost was packed in polypropylene bags with a gas exchange filter (1.5 kg/bag), inoculated with 5 g of spawn and sealed, as described previously for axenic cultivation. All incubation conditions for compost colonization, fruiting induction and cultivation were as described in the previous section for axenic cultivation, according to each *Pleurotus* species.

Statistical analysis, yield and biological efficiency (BE)

Plots for both axenic and composting were arranged together in a completely randomized design (CRD), with 10 replications per treatment. Productivity [(fresh harvest weight/substrate wet weight) x 100], biological efficiency (BE) [(fresh harvest weight/substrate dry weight) x 100], and mycelial growth were analyzed using the SISVAR®-UFLA software (Lavras, Minas Gerais, Brazil) (Ferreira, 2000). Treatment means were compared for statistical significance using the Scott-Knott test at the 5% probability level.

RESULTS AND DISCUSSION

In the first experiment, two composting formulations were tested for *P. pulmonarius* cultivation. Both were composed of three or more ingredients, which resulted in formulations with different final nitrogen concentrations (Tables 1 and 2). On the fourth day after fruiting induction, the formation of the first mushrooms appeared. *P. pulmonarius* showed a biological efficiency of 54.5 and 60.5% for formulations 1 and 2, respectively, with no significant differences between them (p> 0.05) (Table 3). These biological efficiency values corresponded to a productivity of 19.1 and 20.2%, respectively, which can be considered highly satisfactory in terms of commercial production scale. These results demonstrate that, the short composting system and steam pasteurization are very suitable for mushroom substrate cultivation, with the advantage of contamination avoidance throughout the whole cultivation period.

It is interesting to note that the difference in initial nitrogen concentration between the two formulations did not influence productivity and biological efficiency of mushroom production. It shows the system's versatility, which allows the use of locally available residues or grasses, without the requirement for bran supplementation. However, it is important to consider that substrates with lower final nitrogen concentration may result in mushrooms poorer in protein (Silva et al., 2007). The combination of different agro-industrial residues or grasses in the substrate presupposes that formulations composed of different raw materials may provide better conditions for composting, promoting a greater balance between the nutrients. The same reasoning can be applied to the axenic culture system in which the combination of different ingredients can improve the balance in available nutrients for mushroom species cultivation.

The composting process is probably the most appropriate system for *Pleurotus* cultivation in small farms, in projects for product development with high aggregate value (Dias, 2010). However, simplification of formulations can also be very important for commercial mushroom cultivation, as the availability of residues can vary considerably from region to region. Therefore, adaptation of the composting system for the simplest formulations, preferably with only one agro-industrial residue, is also important for simple and economically viable *Pleurotus* cultivation. Bernardi et al. (2007) provided evidence that a single grass (elephant grass) could be used successfully for the cultivation of different species of oyster mushrooms (*Pleurotus* spp), but the authors reported the use of pasteurization of the raw material rather than composting.

Therefore, for the second experiment in this study, bean straw was chosen as the only substrate for both the axenic and for the composting system/steam pasteurization for *P. eryngii*, *P. ostreatus* and *P. pulmonarius* cultivation. Although composting was performed with only one ingredient, the process was successful, as evidenced by the rapid increase in temperature, which reached 74°C during the second day of the process. A rise in temperature is a useful indicator for microbial activity during composting, typically with mesophilic microorganisms metabolizing soluble sugars, followed by thermophilic microorganisms, which become

Table 4. Productivity and biological efficiency of mushrooms in bean straw according to two systems: axenic and composting/steam pasteurization (Experiment 2).

Mushroom species cultivation on bean straw	System: axenic		System: composting	
	P*	BE*	P*	BE*
P. eryngii	17.5^{B}	53.7^{B}	-	-
P. ostreatus	20.5^{Aa}	59.52^{Aa}	21.08^{Aa}	61.75^{Aa}
P. pulmonarius	20.89^{Aa}	62.7^{Aa}	24.32^{Aa}	68.8^{Aa}
Coefficient of variation (CV)	18.39	18.77	18.39	18.77

Capital letters compare the means of productivity and biological efficiency across species, while lower case letters compare means between the two methodologies for each species. Means followed by the same letter do not differ statistically in the Scott-Knott test at the 5% probability level. -, Absence of mushroom production. *P, Productivity; BE, biological efficiency.

dominant in the compost. This establishment of thermophilic microorganisms provides selectivity and contamination avoidance during cell cultivation. This system is therefore an interesting option for small holder farmer conditions. Steam pasteurization is typically employed after the composting to ensure elimination of any pests and microbial contaminants that may survive the composting process.

Of the three species of *Pleurotus* used in this study, only *P. eryngii* showed poor growth on the substrate obtained through composting and, hence, no productivity results were obtained. For this species, colonization was successful only on autoclaved bean straw, with a biological efficiency and productivity of 53.7 and 17.5%, respectively. By contrast, for *P. ostreatus* and *P. pulmonarius*, the substrate obtained by composting showed excellent results for biological efficiency and productivity, as seen in Table 4, which were numerically superior to those obtained in the axenic system, although with differences not significant ($p > 0.05$). These results indicate versatility in the mushroom cultivation systems, since they indicate the possibility of simplifying the compost formulation, depending on the availability and cost of production or transportation of necessary raw materials.

A number of studies have demonstrated the need to supplement nitrogen-poor substrates with wheat or soybean bran or to combine different straws or grasses for *Pleurotus* cultivation (Basak et al., 1996; Zanetti and Ranal, 1996; Hernández et al., 2003; Moda et al., 2005; Siqueira et al. 2011; Zied et al., 2011). Curvetto et al. (2002) also showed that enrichment of sunflower hulls with manganese and nitrogen resulted in an increase of 50% or more in *P. ostreatus* production. However, other studies showed that *Pleurotus sajor-caju* can be cultivated on simple substrates, using only one ingredient (Ragunathan et al., 1996; Ragunathan and Swaminathan, 2003; Bernardi et al., 2007). Furthermore, Silva et al. (2007) observed that the addition of 2 or 3% urea to the substrate for *P. sajor-caju* cultivation completely inhibited substrate colonization. The authors noted that the use of sugarcane bagasse and coast-cross hay only in equal proportions provide the same values of biological efficiency in substrates enriched with wheat bran (10%) and urea (1%). According to the authors, the enrichment of the substrate with nitrogen is important to increase the protein content of mushrooms and not productivity. The increase in nitrogen content of 0.65 to 1.3% led to an increase in protein content from 17.1 to 28%, but productivity was unaltered. However, when concentrations of 1.75 and 2.2% nitrogen were used, there was no colonization of the substrate (Silva et al., 2007). Therefore, despite being an important strategy for enabling utilization of certain locally available residues in the cultivation, enrichment of the substrate can result in negative effects, in addition to the increase in production costs.

Consequently, it is important to determine the nitrogen content of each raw material used in the formulation of the substrate for mushroom cultivation and then decide on appropriate supplementation or combinations with other ingredients. The results obtained in this study indicate the suitability of bean straw as a unique substrate for *P. ostreatus* and *P. pulmonarius* cultivation, using the composting process for substrate preparation for mushroom cultivation. High productivity and biological efficiency, associated with the absence of contamination indicate this process as an ideal system for mushroom cultivation by small holder farmers without resources for investment in specialized facilities. In the case of *P. eryngii*, alternative agricultural residues or grasses are required, as bean straw did not present encouraging results in this work. Factors such as initial concentration of nitrogen or the presence of ammoniac nitrogen may also have affected substrate colonization. Further studies are required to evaluate alternative residues or grasses for cultivation according to this system.

ACKNOWLEDGEMENTS

The authors wish to thank the Brazilian agencies Fundação de Amparo a Pesquisa de Minas Gerais – FAPEMIG (Research Support Foundation of Minas

Gerais) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES (Coordination of Improvement of Higher Education Personnel) for financial support.

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