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AXENIC CULTIVATION OF *Pleurotus ostreatus* var. Florida IN SUPPLEMENTED SUGARCANE BAGASSE BRIQUETTES

Anderson C. Magalhães^{1*}, Bruno R. de A. Moreira², Diego C. Zied²

^{1*}Corresponding author. College of Agricultural and Technological Sciences, São Paulo State University (Unesp)/ Dracena – SP, Brazil. E-mail: ac.magalhaes@unesp.br

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ABSTRACT

Articles on the applicability of briquetting to the production of mushrooms are rare. Therefore, this research provides the scientific community and, mainly, producers, unpublished technical information regarding the productive yield of oyster mushroom cultivated in sugarcane bagasse briquettes supplemented with bioproducts of cereals. In 30 days of axenic cultivation, 6 tons-force briquettes with 80% humidity resulted in higher productivity (30.4%), and generated the lowest physical volume of exhausted compound (44.8%), thus, overcoming the technical performance of the other briquetting matrices tested, and even of methods traditionally used for the preparation of substrate for *P. ostreatus* cultivation on a commercial scale. The conclusion is, therefore, that the axenic cultivation of *P. ostreatus* var. Florida in sugarcane bagasse briquettes, supplemented with bioproducts of cereals, has technically qualified as an original and efficient method for the production of high biological value protein by the recycling of agricultural residues and can therefore guarantee multiple economic benefits to the agribusiness of the oyster mushroom, in addition to allowing reduction of environmental impacts by the restricted generation of exhausted compound in the post-harvest stage.

INTRODUCTION

In Brazil, an international reference in technology for sugar and bioethanol industrialization, a considerable physical volume of sugarcane bagasse is annually generated as a co-product by the sugar-energy sector; if it is disposed incorrectly to the environment, this generates irreparable adverse effects on native fauna and flora. Researches emphasize that this agricultural residue enables sustainable applications such as soil conditioner and cultivation substrate for edible mushrooms (Shu et al., 2015; Wang et al., 2017).

Known as oyster mushroom, shimeji or hiratake, *P. ostreatus* qualifies as an excellent source of high biological value proteins, essential amino acids, vitamin D, macro and micronutrients, and carbohydrates, in addition to possessing low cholesterol. As for the cultivation and commercialization aspects, this does not require expensive investments in the installation and maintenance of production infrastructure; it adapts well to lignocellulosic substrates and has a guaranteed demand for vegan and vegetarian cultures. Therefore, the oyster mushroom agribusiness is considered profitable and ideal for non-

capitalized producers (Bach et al., 2017; Khan et al., 2017).

In *P. ostreatus* commercial cultivation, the substrate selection and preparation are crucial steps to productivity and product quality. If possible, we should opt for a substrate of excellent nutritional quality, easy to acquire, whose supply is regular and the source of production is located as close as possible to the place of cultivation. Although the enzymatic complex of *P. ostreatus* is an excellent degrader of complex carbohydrates such as cellulose, hemicellulose and lignin, it is possible that there is unproductivity in lignocellulosic substrates, if these are nutritionally inappropriate. For this reason, experts recommend supplementing them with rice, wheat and corn bran, bean leaves, or any other additional source capable of balancing the carbon/nitrogen ratio (C/N) (Naraian et al., 2016, Vieira & Andrade, 2016; Higgins et al., 2017).

Added to the nutritional quality, the sanitary aspect and physical properties of substrate also considerably influence the productive yield of *P. ostreatus*. Traditionally, the substrate sterilization is promoted by thermal treatments such as pasteurization, composting and autoclaving, making it axenic, that is, free of harmful

² São Paulo State University (Unesp)/ Dracena - SP, Brazil.

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microbial agents. Although this substrate category offers advantages to producers, such as little or no contamination propensity and accelerated cultivation cycle, the preparation of the substrate requires considerable volume of raw material, making it difficult the management of waste in the post-harvest stage. Scientific researches highlight the fight against excessive generation of mushroom drawn compound in post-harvest stage as one of the main challenges regarding the cultivation of *P. ostreatus* (Atila, 2016; Higgins et al., 2017). Therefore, it was assumed that the briquetting could be useful to the sustainability of the oyster mushroom production chain by the production of reduced volume substrate.

Historically, the briquetting technology is applied to the industrialization of solid biofuels. Hence, it is less popular in relation to food production. In this technique, the density of the raw material is significantly reduced by compaction at constant pressure and temperature, with or without the presence of natural or synthetic binder substances. Due to its physicochemical properties and moderate nutritional quality, the sugarcane bagasse is simultaneously viable to the briquetting and production of culture substrate for oyster mushroom (Khorasgani et al., 2017).

Therefore, it was verified the unavailability of articles that relate oyster mushroom production to briquetting, this research was conducted on axenic cultivation of *P. ostreatus* var. Florida in sugarcane bagasse briquettes, supplemented with cereal bioproducts, with the intention of disseminating unpublished information on viability of briquetting to the oyster mushroom crop and benefits that such technology could offer to fungus farmers, especially those with limited physical space for storage, preparation, cultivation and management of drawn compound.

MATERIAL AND METHODS

The research was conducted in the laboratories of Machinery and Agricultural Mechanization/Applied Physics and Agricultural Microbiology/Center for Mushroom Studies (CECOG), São Paulo State University Júlio de Mesquita Filho, College of Agricultural and Technological Sciences (FCAT/Campus of Dracena), located in the municipality of Dracena, São Paulo, Brazil.

The Raw materials, defibered sugarcane bagasse, maize and wheat bran, and calcite were collected from the beef sector and CECOG, respectively. In relation to the species of cultivated oyster mushroom, *P. ostreatus* var. Florida, it belongs to the CECOG mycology collection and is available to those who may be interested in studying it.

For the preparation of the primary matrix, healthy fruiting bodies were selected. Prior to cloning, the laboratory apparatus was sterilized in a laminar flow chamber (Filterflux, class II-A1 biological safety cabin), exposing them to ultraviolet radiation for 1 hour. Fruiting bodies were collected by collecting and depositing internal active tissue fragments in Petri dishes containing PDA culture medium, duly sterilized in a vertical autoclave

(Primatec, line CS-A), at 121°C and 1 atm, for 30 minutes. Finally, the Petri dishes were conditioned in BOD incubator (Filterflux, photoperiod SL-224), maintaining them at $23.5 \pm 2.7^\circ\text{C}$, for seven days (Tarko & Sirna, 2018).

To the spawn production, 2 kg of sorghum grains were used. After cooking, promoted at 50°C for 1 hour, the grains were cooled in running water, then dried in the sun. To the grains, 20 g of agricultural gypsum were added in order to supplement them and, consequently, to provide nutrients to the mycelial development. The grains were distributed in polyethylene bags, to which a cotton filter was added, so that the water vapor was allowed during the autoclaving process. After sterilization, at 121°C and 1 atm, for 2 hours, the grains were inoculated with primary matrix. The bags were sealed in a plastic sealer (Proels MOD-P 601), finally destined to the incubator, where they remained at $23.0 \pm 2.5^\circ\text{C}$, for twenty days (Mihai & Vamanu, 2015; Tarko & Sirna, 2018).

For the production of briquettes, the experimental design was completely randomized, with eight replicates per treatment, in a 4x3 factorial scheme, corresponding to: briquetting pressure (2, 4, 6 and 8 ton-force) and substrate humidity (40, 60 and 80%), respectively. The scientific research states that the substrate humidity for cultivation of edible mushrooms should range from 60 to 75%, otherwise there is unproductivity due to inappropriate supply of water to microbial metabolic activity (Zied et al., 2011). Therefore, in this study, values not included in the optimum range recommended by the aforementioned authors were selected, aiming precisely to evaluate the effect of water stress on the productive yield of oyster mushroom cultivated in briquettes, since there is no literary information available to respect to such phenomena associated with briquetting.

Prior to the briquetting, the raw materials were pre-treated by grinding and sifting them in a Willey Mill (Marconi 340-A) and 60 mesh mill respectively; particle size equivalent to 1.4 mm were selected. The raw materials were then bagged and then subjected to desiccation in a forced air circulation oven, operating at $65.0 \pm 2.5^\circ\text{C}$ for 96 hours, to dehydrate them completely. After the conditioning phase, 14.4 kg of sugarcane bagasse were homogenized; 1.2 kg of corn meal; 1.2 kg of wheat bran and 0.5 kg of calcite in a wooden crate. The amounts of supplementary materials were defined, according to the technical recommendations of Rossi et al. (2003). The 180 g aliquots were placed in polyethylene bags resistant to autoclaving in order to prevent briquettes from losing humidity by compaction. After adding water to the treatments, with a test tube graduated in milliliters, respecting the pre-established humidities, the polyethylene bags in mold, were designed in rectangular format. For the compacting briquettes, a hydraulic press was used (Bovenau P - 15000), regulated to exert constant pressure for 1 minute. The briquette characteristics are described in Table 1.

TABLE 1. Characterization of briquettes used as axenic substrates in the cultivation of oyster mushroom.

| Dimensional | | Raw materials | |
|-------------|-------------------------------|-------------------|---------|
| Length | 0.2 m | Sugarcane bagasse | 130.0 g |
| Width | 0.1 m | Corn bran | 12.5 g |
| Thickness* | 0.05 - 0.075 m | Wheat bran | 12.5 g |
| Area | 0.02 m ² | Calcium carbonate | 5.0 g |
| Volume* | 0.001 - 0.0015 m ³ | Standard Mass | 180.0 g |

* Variable as a function of applied briquetting pressure.

Immediately after the pressing, the briquettes were sealed in order to avoid loss of humidity by evaporation. A cotton filter was added in the upper right corner of the autoclaving bag, aiming at gas exchanges between substrate and atmosphere, since *P. ostreatus* is an aerobic microorganism, it needs a continuous flow of oxygen to fully develop. Subsequently, the briquettes were sterilized by autoclaving, at 121°C and 1 atm, for 4 hours. After reaching the thermal equilibrium, the briquettes were inoculated with 4.5 g of spawn in a laminar flow chamber. Finally, they were sealed, destined to the incubation infrastructure, in which the run of mycelium was promoted at the temperature and relative humidity of the air equivalent to $24.0 \pm 2.5^\circ\text{C}$ and $65.0 \pm 3.5\%$, respectively. The briquettes were transferred to the growing

environment, as the beginnings of fruiting bodies emerged (Hoa et al., 2015; Mihai & Vamanu, 2015; Tarko & Sirna, 2018).

Prior to transferring the briquettes to the culture chamber, this was sterilized by simultaneous application of 10% formaldehyde solution and virgin lime on shelves and floor. The environment was kept closed for five days, so that there was satisfactory control of pre-existing contaminants. The cultivation was conducted for 30 days, at the temperature and relative humidity of $29.0 \pm 3.5^\circ\text{C}$ and $89.3 \pm 6.7\%$, respectively, monitored by digital sensor (Highmed HM-02). The main steps taken to produce axenic briquettes for oyster mushroom cultivation are presented in Figure 1.



FIGURE 1. Main stages of production of sugarcane bagasse briquettes, supplemented with cereal bioproducts, for axenic cultivation of oyster mushroom: (a) obtaining a primary matrix; (b) production of spawn in sorghum grains; (c) homogenization of raw materials; (d) packaging of raw materials in polyethylene bags resistant to autoclaving; (e) briquetting in hydraulic press; (f) axenic briquettes; (g) addition of cotton filter for gas exchange; (h) substrate sterilization by autoclaving; (i) spawn inoculation; (j) incubation or mycelium run; (k) culture environment conditioning; (l) visual aspect of fruiting of *P. ostreatus* var. Florida.

As for the technical evaluation, immediately after the briquetting, the following physical characteristics of briquette were measured:

Apparent density: mass and dimensions of briquette were determined using an analytical balance, with an accuracy equivalent to 0.01 g, and digital caliper, respectively; this parameter was calculated by the quotient of wet mass per briquette volume.

Mechanical resistance to penetration: arranged horizontally on a carbon steel plate, the briquettes were tested for their ability to withstand the vertical incision of the penetrometer (penetroLOG Falker), an electronic soil compaction meter.

In relation to the performance of the oyster mushroom, in the incubation stage, the time necessary to complete colonization of the substrate was analyzed as indicative of vegetative vigor, observing the briquette coating by the mycelium of *P. ostreatus* var. Florida. In addition, during the growing season, the following indicators of productive yield were evaluated daily:

Number of bunches (NB) and fruiting bodies (NFb): measured by visual counting.

Fresh bunch mass (FBM) and fruiting body (FFBM): measured by weighing in analytical balance.

Productivity (Pr): calculated by the relation between total fresh mass (TFM) of fruiting bodies and wet mass of briquette (m), adopting [eq. (1)], recommended by Tarko & Sirna (2018) as a way to evaluate the bioconversion of organic matter to tradable structures.

$$Pr (\%) = \left[\frac{TFM (kg)}{m (kg)} \right] \times 100 \tag{1}$$

In the post-harvest stage, the briquettes (M) were weighed for the quantification of the drawn mushroom compound (SMS), performed by [eq. (2)]:

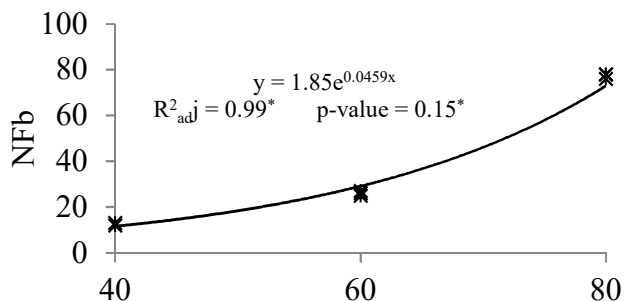
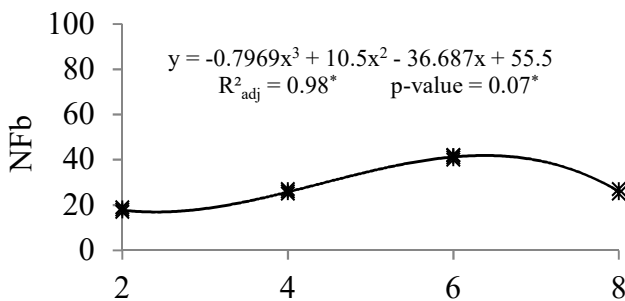
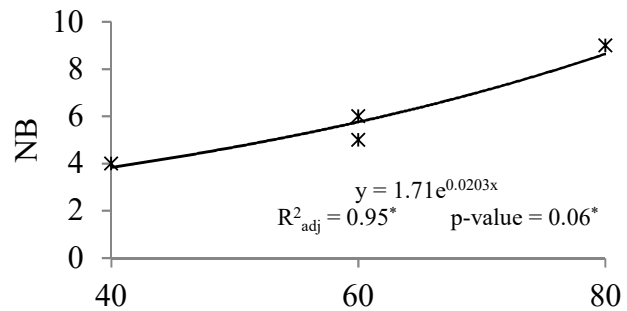
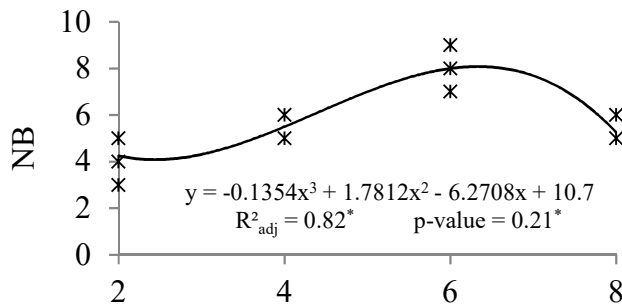
$$SMS (\%) = \left[\frac{M (kg)}{m (kg)} \right] \times 100 \tag{2}$$

For the statistical analysis of the data set, the Shapiro-Wilk, Fisher, Tukey and regression tests were applied using software R version 3.3.1.

RESULTS AND DISCUSSION

Figure 2 shows the isolated effects of briquetting pressure and substrate humidity in the productive performance measures of *P. ostreatus* var. Florida cultivated in sugarcane bagasse briquettes supplemented with cereal bioproducts. Particularly, third order mathematical models were better adjusted to the briquetting pressure effect, while exponential and quadratic regressions made possible significant adjustments to the humidity effect. Therefore, it is reasonable to infer that oyster mushroom growth in briquettes derived from agricultural residues is a complex biological phenomenon that only the causes of variation adopted in this study were not sufficient to fully explain it.

The best averages of NB, NFb, FBM, FFBM and Pr occurred in 6 tons-force briquettes, indicating that, from this pressure level, the efficiency of *P. ostreatus* var. Florida on bioconversion of organic matter into effective fruiting bodies was significantly compromised. It was presumed, therefore, that briquetting pressures higher than 6 tons-force were able to alter the total porosity of the substrate, restricting the oxygen supply.



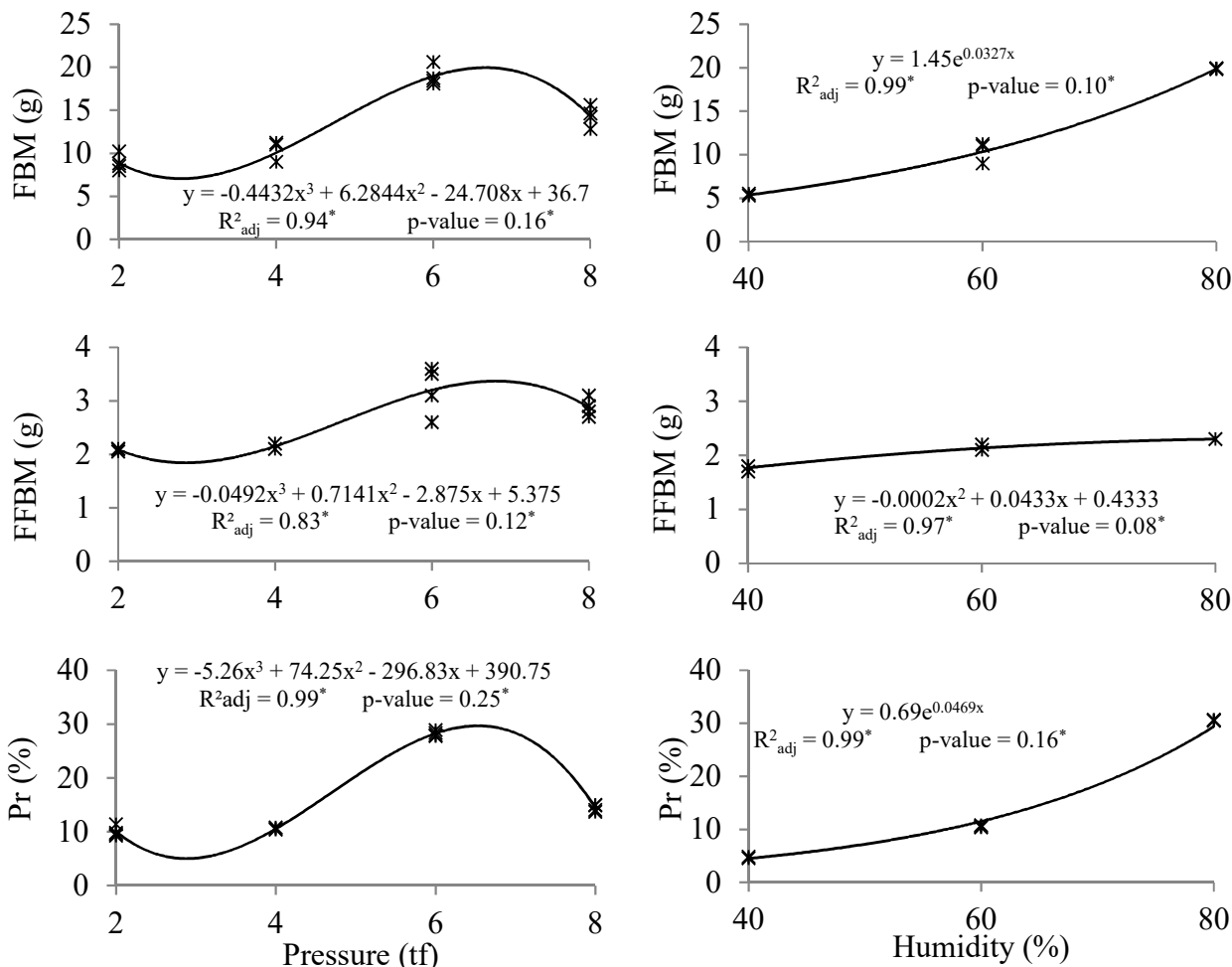


FIGURE 2. The isolated effects of briquetting pressure and substrate humidity in the productive performance of *P. ostreatus* var. Florida cultivated in sugarcane bagasse briquettes supplemented with cereal bioproducts; * significant ($p < 0.05$) by the Shapiro-Wilk and Fisher tests.

Therefore, it was attributed the lower availability of fruiting structures in 8 tons-force briquettes to the possible formation of anaerobic zones, a limiting factor to the development and productive yield of oyster mushroom. In addition, larger amounts of marketable structures were harvested in 80% humidity equivalent briquettes. According to Zied et al. (2011), substrates with humidity greater than 75% are limiting to the development of the

majority of edible mushroom species produced on a commercial scale; this means that the briquetting technique alters the dynamism of water stress in axenic cultivation of oyster mushroom.

In Table 2, the apparent density and mechanical resistance to penetration in sugarcane bagasse briquettes are shown.

TABLE 2. Isolated effects of briquetting pressure and substrate humidity on apparent density and mechanical resistance to penetration of sugarcane bagasse briquettes supplemented with cereal bioproducts, using as axenic substrates in the cultivation of *P. ostreatus* var. Florida.

| Humidity (%) | Pressure (tons-force) | | | | Isolated Effect |
|---|-----------------------|---------------------|---------------------|---------------------|--------------------|
| | 2 | 4 | 6 | 8 | |
| Apparently density (kg m^{-3}) | | | | | |
| 40 | 235.8 ^{aA} | 248.8 ^{aA} | 256.4 ^{aA} | 262.2 ^{aA} | 250.7 ^a |
| 60 | 235.3 ^{aA} | 249.0 ^{aA} | 256.4 ^{aA} | 262.0 ^{aA} | 250.6 ^a |
| 80 | 235.5 ^{aA} | 248.7 ^{aA} | 256.3 ^{aA} | 261.9 ^{aA} | 250.6 ^a |
| Isolated Effect | 235.5 ^D | 248.9 ^C | 256.3 ^B | 261.9 ^A | |
| Average = 250.7 P-value = 0.3* CV (%) = 0.1 | | | | | |
| Mechanical resistance to penetration (kg m^{-2}) | | | | | |
| 40 | 5.4 ^{aA} | 5.7 ^{aA} | 6.2 ^{aA} | 7.6 ^{aA} | 6.2 ^a |
| 60 | 5.4 ^{aA} | 5.6 ^{aA} | 6.3 ^{aA} | 7.6 ^{aA} | 6.3 ^a |
| 80 | 5.5 ^{aA} | 5.7 ^{aA} | 6.3 ^{aA} | 7.6 ^{aA} | 6.2 ^a |
| Isolated Effect | 5.5 ^D | 5.7 ^C | 6.3 ^B | 7.5 ^A | |
| Average = 6.2 P-value = 0.2* CV (%) = 2.2 | | | | | |

Averages followed by the same uppercase letters in the row and lower case letters in the column do not differ by the Tukey test ($p < 0.05$); * significant ($p < 0.05$) by the Shapiro-Wilk test.

There was a direct relation between briquetting pressure and apparent density. Such behavior was predicted because the substrate volume is proportionally reduced as the compaction intensity rises, resulting in greater mass availability per cubic meter of raw material. This is the principle of briquetting technique. By studying the biological behavior of *A. bisporus* in briquette matrices composed of wheat straw, bovine manure, agricultural gypsum, limestone, superphosphate and common bean pulp, exclusively differentiated by apparent density, Geng et al. (2016) observed that denser briquettes were associated to the best productivity averages, converging, therefore, to the results of this research for the productive performance of *P. ostreatus* var. Florida in 2, 4 and 6 tons-force briquettes.

After suffering induced stress, the mushrooms fruited, as a strategy to perpetuate themselves, it is possible that the substrate densification by briquetting acts to a certain extent as an inducing agent of fruiting in *Agaricus spp* and *Pleurotus spp*. Therefore, considering the results of this research for the isolated effect of briquetting pressure on productive yield measurements of oyster mushroom, it is believed that 6 tons-force corresponds to the ideal pressure to induce fruiting in

P. ostreatus var. Florida grown in sugarcane bagasse briquettes supplemented with cereal bioproducts, and which levels higher than this are so stressful that they reflect unproductiveness.

In relation to the mechanical resistance to penetration, this also established a direct relation with briquetting pressure. Although there is no scientific study linking productive yield of oyster mushroom to mechanical resistance of briquette to penetration, it is believed that such parameter can be used as a criterion and evaluation of physical quality of substrate, since it can interfere, directly or indirectly, in mycelial development, hindering the access to fundamental elements to the microbial metabolism.

In Table 3, the interactive effect of briquetting pressure and humidity in the productive performance measures of *P. ostreatus* var. Florida is shown. The 2 tons-force briquettes, with humidity equivalent to 40%, delayed in more than one week the time of colonization of substrate by the mycelium of *P. ostreatus* var. Florida, compared to the other briquetting matrices. This result confirms that humidity values lower than 60% are, indeed, detrimental to the vigor of oyster mushroom at the beginning of vegetative development.

TABLE 3. Interactive effect of briquetting pressure and humidity in the productive performance measures of *P. ostreatus* var. Florida cultivated in sugarcane bagasse briquettes supplemented with bioproducts of cereals.

| Humidity (%) | Pressure (tons-force) | | | |
|---|-----------------------|---------------------|--------------------|--------------------|
| | 2 | 4 | 6 | 8 |
| Substrate colonization time (days) | | | | |
| 40 | 28.0 ^{bB} | 20.8 ^{aA} | 20.8 ^{aA} | 20.8 ^{aA} |
| 60 | 20.8 ^{aA} | 20.8 ^{aA} | 20.8 ^{aA} | 20.8 ^{aA} |
| 80 | 20.8 ^{aA} | 20.8 ^{aA} | 20.8 ^{aA} | 20.8 ^{aA} |
| Average = 21.3 P-value = 0.1* CV (%) = 2.5 | | | | |
| Number of bunches | | | | |
| 40 | 5.0 ^{bAB} | 4.0 ^{bB} | 4.0 ^{bB} | 5.5 ^{aA} |
| 60 | 4.2 ^{bB} | 5.5 ^{aB} | 8.0 ^{aA} | 5.2 ^{aB} |
| 80 | 6.2 ^{aB} | 3.2 ^{bC} | 9.0 ^{aA} | 5.2 ^{aB} |
| Average = 5.4 P-value = 0.2* CV (%) = 12.2 | | | | |
| Number of fruiting bodies | | | | |
| 40 | 29.5 ^{bA} | 27.8 ^{aA} | 12.8 ^{cB} | 24.8 ^{bA} |
| 60 | 17.8 ^{cC} | 25.8 ^{aBC} | 42.0 ^{bA} | 26.0 ^{bB} |
| 80 | 43.8 ^{aC} | 14.0 ^{bD} | 76.0 ^{aA} | 63.0 ^{aB} |
| Average = 33.6 P-value = 0.1* CV (%) = 12.6 | | | | |
| Fresh mass of bunches (g) | | | | |
| 40 | 9.8 ^{aA} | 5.4 ^{cB} | 5.6 ^{bB} | 9.7 ^{cA} |
| 60 | 12.6 ^{aBC} | 10.1 ^{bC} | 18.4 ^{aA} | 14.3 ^{bB} |
| 80 | 10.7 ^{aC} | 24.0 ^{aA} | 19.7 ^{aB} | 24.2 ^{aA} |
| Average = 13.7 P-value = 0.5* CV (%) = 13.2 | | | | |
| Fresh fruit body mass (g) | | | | |
| 40 | 1.6 ^{bA} | 0.8 ^{cB} | 1.8 ^{bA} | 2.2 ^{bA} |
| 60 | 2.9 ^{aB} | 2.2 ^{bC} | 3.7 ^{aA} | 2.8 ^{aB} |
| 80 | 1.5 ^{bC} | 5.6 ^{aA} | 2.3 ^{bB} | 1.9 ^{bBC} |
| Average = 2.4 P-value = 0.4* CV (%) = 14.5 | | | | |
| Productivity (%) | | | | |
| 40 | 10.7 ^{abA} | 4.8 ^{cB} | 4.9 ^{cB} | 11.8 ^{cA} |
| 60 | 10.0 ^{bC} | 10.6 ^{bC} | 28.3 ^{bA} | 14.4 ^{bB} |
| 80 | 11.3 ^{aD} | 13.2 ^{aC} | 30.4 ^{aA} | 21.3 ^{aB} |
| Average = 14.3 P-value = 0.3* CV (%) = 4.3 | | | | |

Averages followed by the same uppercase letters in the row and lower case letters in the column do not differ by the Tukey test ($p < 0.05$); * significant ($p < 0.05$) by the Shapiro-Wilk test.

Scientific researches emphasize that substrates that are restrictive to colonization are useless to the sustainable cultivation of oyster mushrooms, since, besides presenting a higher contamination rate, they make it impossible to produce healthy fruiting bodies of high nutritional quality (Paz et al., 2013). Therefore, 2 tons-force briquettes, with 40% humidity, are negligible to oyster mushroom production. In addition, 6 tons-force briquettes, with 60 and 80% humidity, were associated to the higher averages of NB, NFb, FBM, FFBM and Pr. This behavior was predicted, therefore, at the level of isolated effect, yields of *P. ostreatus* var. Florida in substrate of greater humidity, briqueted to the proportional pressure to 6 tons-force; compared to the general average yield, these briquettes intensified at approximately twice the oyster mushroom efficiency in organic matter bioconversion.

When cultivating *P. ostreatus* on substrates derived from lignocellulosic residues, Tarko & Sirna (2018) found that the time required to complete colonization of these residues corresponded to 13.0 ± 1.0 to 19.3 ± 1.6 days. Similarly, Paul & Ngozika (2017), studying the behavior and productive yield of oyster mushroom, cite that it takes approximately 13.2 days to colonize lignocellulosic substrates sterilized by pasteurization. In contrast, Iqbal et al. (2016), evaluating growth, production and nutritional quality of *P. florida* Cetto, report that this species is able to colonize substrates formulated with sugarcane bagasse, rice, sorghum and corn in 26 to 40 days. Dahmardeh et al. (2010) report that the time required for the complete colonization of substrates based on corn and barley bran by *P. ostreatus* is equivalent to 42.3 and 50 days, respectively, meaning that the nutritional quality of the raw material affects the mycelial vigor of mushroom oyster. In addition to nutritional quality, quantity and spawn quality, fungal species, cultivation conditions and physicochemical properties of substrate, mainly carbon/nitrogen (C/N) ratio, are interfering factors, in relation to mycelial colonization.

Additionally, Mondal et al. (2010) cultivated *P. ostreatus* on substrates derived from lignocellulosic agricultural residues and verified complete colonization in 23.3 days, a result lower to that obtained by Pokhrel et al. (2013), that is, 29.7 days. Jahangir et al. (2015) evaluated the effect of sawdust substrate on growth and yield of oyster mushroom cultivated on substrates of cotton and sorghum straw residues and showed that it required from 32.4 to 36.0 days to fully colonize them. Therefore, these literary results extolled the ability of sugarcane bagasse briquettes supplemented with cereal bioproducts to significantly reduce the time required for complete colonization of culture substrate by the mycelium of *P. ostreatus* var. Florida, compared to techniques adopted for substrate preparation for the commercial scale of oyster mushroom production. Iqbal et al. (2016) argue that the substrate physical structure is crucial to mycelial development.

Tripathy et al. (2011), producing straw mushroom (*Volvariella* spp) on lignocellulosic substrates, clarify that the porous materials allow better mycelial development. In

this sense, Paul & Ngozika (2017) mention that sugarcane bagasse is capable of promoting good colonization rate, gas exchange and easy access to carbohydrates, as well as to provide macro and micronutrients necessary for the full functioning of microbial metabolism. Therefore, it is believed that the briquetting pressure and substrate humidity can considerably alter the porosity, nutritional quality and productive performance of sugarcane bagasse briquettes used as axenic substrates in the cultivation of *P. ostreatus* var. Florida, since there was a significant variation in colonization time.

Cultivating *P. ostreatus* in a substrate formulated with cereal bioproducts and cotton stem, Ashraf et al. (2013) produced, on average, 3.9 fruiting bodies, while Owaid et al. (2015), studying the substrate of wheat biomass supplemented with sawdust, obtained 15.5 fruiting bodies. Compared to the average of these values, the briquettes of 6 and 8 tons-force, with humidity proportional to 80%, were about eight and six and a half times more productive, respectively. Complementarily, in relation to Hoa et al. (2015), which obtained 8.1 fruiting bodies, cultivating *P. ostreatus* on no supplemented sugarcane bagasse substrate, the above-mentioned briquettes were around nine and eight times more productive, reiterating the importance of lignocellulosic residues supplementation with high C/N ratio to productive yield of oyster mushroom (Marino et al., 2008; Pardo-Giménez et al., 2016).

According to Sharma et al. (2013), the cultivation of oyster mushroom in sugarcane bagasse sterilized by pasteurization allowed the harvesting of fruiting bodies of 6.5 g. Results similar to this were reported by Pokhrel et al. (2013), which evaluated the yield of *P. sajor-caju* on substrates based on maize biomass and banana leaves and verified the occurrence of fruiting bodies of 5.0 and 6.3 g, respectively, exceeding the verified average in this research. Therefore, the sugarcane bagasse briquettes supplemented with cereal bioproducts diluted the fresh mass of fruiting body, due to the intense production of this reproductive structure. However, the fruiting bodies of *P. ostreatus* var. Florida exhibited visual appearance and mass suitable for commercialization.

Mihai & Vamanu (2015), cultivating *P. ostreatus* in a substrate formulated with lignocellulosic residues, observed productivity proportional to 30%, corroborating the result obtained by the briquette of 6 tons-force, with humidity equivalent to 80%. On the other hand, Bernardi et al. (2013), selecting lignocellulosic raw materials for the production of substrate for oyster mushroom cultivation, found that sugarcane bagasse resulted in productivity of 1.8%, significantly lower than the averages of this research. Due to the higher productivity, an indicator that best represents the technical feasibility of substrate, briquettes of 6 and 8 tons-force, with humidity equivalent to 80%, have qualified as preferable to the axenic cultivation of oyster mushroom.

In Table 4, the percentages of compound generation drawn by sugarcane bagasse briquettes supplemented with cereal bioproducts are shown.

TABLE 4. Percentage of drawn mushroom compost generated by sugarcane bagasse briquettes supplemented with cereal bioproducts, used as axenic substrates in the cultivation of *P. ostreatus* var. Florida.

| Humidity (%) | Pressure (tons-force) | | | |
|--|-----------------------|--------------------|--------------------|--------------------|
| | 2 | 4 | 6 | 8 |
| 40 | 75.3 ^{cC} | 55.6 ^{aA} | 55.6 ^{cA} | 73.3 ^{cB} |
| 60 | 74.6 ^{bD} | 73.7 ^{cC} | 46.7 ^{bA} | 63.5 ^{bB} |
| 80 | 74.0 ^{aD} | 73.1 ^{bC} | 44.8 ^{aA} | 50.4 ^{aB} |
| Average = 63.4 P-valor = 0.8* CV (%) = 0.2 | | | | |

Averages followed by the same uppercase letters in the row and lower case letters in the column do not differ by the Tukey test ($p < 0.05$); * significant ($p < 0.05$) by the Shapiro-Wilk test.

The 6 tons-force briquettes, with 80% humidity, generated the least amount of drawn mushroom compound; such performance was justified by its higher efficiency in terms of organic matter bioconversion in fresh oyster mushroom mass. Researches show that, in China and Brazil, mushroom producers annually produce about 60% drawn compost per crop cycle (Diao & Mao, 2012; Vieira & Andrade, 2016). Therefore, considering the expectation of this study, the briquetting technique was effectively able to reduce the volume of the mushroom drawn compound generated in the post-harvest stage. This technological advance is very interesting to the national fungus production, since, according to Vieira & Andrade (2016), producers in the municipality of Mogi das Cruzes, a national pole of edible mushroom production, have faced serious difficulties in correctly managing waste production.

CONCLUSIONS

From the results obtained, the conclusion is that the axenic cultivation of *P. ostreatus* var. Florida in sugarcane bagasse briquettes supplemented with cereals bioproducts has been technically qualified as an innovative and efficient method for the production of high biological value protein by the recycling of agricultural residues and is therefore capable of conferring multiple socioeconomic benefits and environmental factors to the oyster mushroom production chain, mainly to small and medium producers who have limited space for storage and preparation of substrate, as well as to those who find it difficult to manage the waste generation in the post-harvest stage.

Regarding recommendations for future research: (a) characterization of energetic efficiency of drawn briquette mushroom cultivation could confer it dual economic purpose and, mainly, to make possible the obtainment of extra income by fungus producers who, instead of negligently discarding it to the environment, would market it as value-added solid biofuel; (b) technical-economical feasibility analysis of briquetting for the production of substrate for cultivation of *Agaricus spp* and *Lentinula spp* on a commercial scale would be interesting, both to the popularization of this technology, and to the agribusiness of these edible mushrooms; (c) pressure-induced fruiting efficiency; this would prevent the mushroom industry from using water as a stressing agent, preserving it as a natural resource; (d) development of a machine capable of performing all the operations necessary to produce axenic briquette for mushroom cultivation, that is: drying, grinding, sifting and homogenisation of raw materials; bagging and correction of substrate moisture; compaction; addition of filter and fence, could be useful to save time, space and labor.

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