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Bioconversion of bananiculture waste for Amazon edible mushroom production

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The objective of this study was to evaluate the use of banana stalk and pseudostem residues from two banana cultivars as cultivation substrate for the Amazonian *Pleurotus ostreatus* NATB. The residues of the two cultivars (silver-dwarf and thap-maeo) were used to cultivate *P. ostreatus* NATB from the Mushroom Cultivation Laboratory Micoteca, CTI, INPA, Brazil, reactivated in medium Potato Dextrose Agar, and subsequently incubated in medium composed of banana juice infusion broth. The tertiary matrix or spawn consisted of solid residue without supplementation that was properly autoclaved for secondary matrix fragment incubation. The treatments were performed in HDPE bags with 1 kg of autoclaved residues, and were humidified at 75% with 5% of tertiary culture. Each treatment consisted of 20 repetitions incubated in a chamber at 25ºC for 45 days. Biological efficiency, yield, and loss of organic matter were evaluated. Mushrooms grown on dwarf silver pseudostem substrate had the highest average percentages in efficiency, biological and yield, but the loss of organic matter was not directly related to these productivity parameters or behavior that can be attributed to other factors such as CO_2 and water loss during the process.

Key words: *Pleurotus ostreatus*, agribusiness, biodegradation.

INTRODUCTION

Banana is the second most consumed fruit in the world. It is grown in over 120 countries, and it has a great socioeconomic significance. In addition to being an important human food, it also contributes to the trade balance of many countries as an important export product, generating profit and income for economies. In

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2014, Brazil was the fourth largest producer with 6,953.747 million tons, behind only India, China and the Philippines according to data from FAOSTAT (2014).

As a consequence of large scale banana production, there is abundant waste generation. An estimated 30% of post-harvest value is lost, and this does not consider the waste generated from pseudostem, stalk, straw, and leaves (Carvalho et al., 2011). These residues are considered the most important in terms of volume generated and fibrous potential, but they are usually used as fertilizer in banana plants (Soffner, 2001).

The utilization of banana agroindustry residues can be applied in several value-added products including basketry, cardboard, fibers, and handicraft products because of the resistant fibers. However, they are even more widely applicable, and applications in biotechnological processes seek to discover new products, reduce environmental impacts, and add value to generated products.

Several bioprocesses have been developed using these materials as substrate to produce various high added value molecules like microbial proteins, organic acids, ethanol, enzymes, and biologically active secondary metabolites (Peralta et al., 2008).

Another important bioprocess is the production of edible mushrooms from the reused agro-industry residues. This includes the production of edible mushrooms in banana straw (Bonatti at al., 2003; Bonatti et al., 2004), in cotton waste (Holtz et al., 2009; Gonçalves et al., 2010), peach palm waste (Duprat et al., 2015), palm oil agribusiness waste (Morais et al., 2017), corn silage (Oliveira et al., 2018), and cupuaçu bark (Fonseca et al., 2015) among others.

Among the diversity of edible mushrooms, fungi of the genus *Pleurotus* can grow on a wide variety of agricultural residues. In addition to their protein quality and the presence of essential amino acids, the mushroom *Pleurotus ostreatus* is an important source of carbohydrates, fiber, minerals and vitamins, making it a nutritionally rich food (Rampinelli et al*.,* 2010).

Given the above, this study evaluated the use of different banana cultivar residues as substrate for cultivating the Amazonian edible mushroom *P. ostreatus* NATB.

MATERIALS AND METHODS

Fungal lineage

The strain of the fungus *P. ostreatus* NATB was accessed from the collection of the Edible Fungi Laboratory of the National Institute of Amazonian Research - INPA. Small fragments were aseptically inoculated in Petri dishes in Potato Dextrose Agar (PDA) medium for colony activation.

Waste treatment

The two banana cultivars (silver-dwarf and thap-maeo) and their respective residues (pseudostem and stalk) were collected from small farmers in the municipality of Parintins-Am (Brazil). The primary processing consisted of subjecting the pseudostem and the “in natura” banana stalk to the mechanical action of a trap 200 organic shredder. The residues were deemed shredded after this step. This material was dried outdoors and packed in plastic bags. Crushed pseudostem and stain residues were autoclaved at 121°C for 60 min for asepsis. After cooling, it was used to formulate an alternative culture medium.

Secondary matrix

P. ostreatus NATB inoculum (9 mm diameter discs) previously micellated in BDA medium were transferred to Petri dishes containing alternative culture medium prepared from infusion of banana residues (pseudostem and stalk) to obtain the secondary matrix, which served as a source of inoculum for the seed in culture medium made from the residue of the thap-maeo cultivar (PSTM), dwarf silver-pseudo-stem (PSPA), thap-maeo cultivar (ENTM) and the stalk of the silver dwarf cultivar (ENPA).

Culture media were prepared by infusing 100 g of substrate in 1 L of boiling water for 30 min, filtering on cotton and making up to 1.5 L. It was necessary to add 2% $CaCO_3$ to adjust the pH (6.5) in 98% residue. After filtration, 12 g of dextrose and 15 g of agar were added to each medium. The different media were autoclaved at 121°C for 60 min and then in a fully stereo environment were poured into Petri dishes, incubated at 25ºC in a BOD chamber.

Tertiary Matrix "Spawn"

The matrix was elaborated from the adapted methodology of Eira and Minhonhi (1997) and Sales-Campos (2008). The substrates were homogenized and humidified at 75% and then deposited in 500 ml glass vials in the amount of 500 g which were drilled in the central portion for secondary matrix inoculum packaging, closed and autoclaved at 121°C for 60 min. After cooling under sterile conditions, mycelium fragments of the secondary matrix were inoculated in the glass vials according to the prepared substrates (PSPA, PSTM, ENPA, ENTM). The flasks were closed and kept in BOD at 25ºC until the complete substrate colonization by the fungus. This matrix served as inoculation source for the cultivation substrates for *P. ostreatus* mushroom production of the present study.

P. ostreatus NATB cultivation

The substrates for fungal growth were prepared using dry and crushed residues (pseudostem and stalk) and four treatments (PSPA, PSTM, ENPA, ENTM) with twenty repetitions for each treatment. The humidity was set to 75%. $CaCO_3$ was not added because the mixture pH did not require correction. The substrates were inserted into HDPE (high density polyethylene) bags, drilled into the central portion for tertiary matrix inoculation, and autoclaved for 60 min. After cooling of the bags, portions removed from the tertiary matrix were inoculated in a laminar flow chamber in the substrate bags. The bags received a synthetic sponge breather to facilitate gas exchange, and they were randomly placed in a chamber with controlled temperature and humidity at 80% humidity and 25ºC (Table 1). The treatments were incubated in the dark for myceliation.

After complete myceliation, the bags were transferred to a production chamber with 90% humidity at 22°C with a 12 h photoperiod to inducing mushroom primordia and basidioma production.

Table 1. Cultivation conditions of *P. ostreatus* NATB in banana substrates.

Cultivate	Substrate	Myceliation humidity (%)	Cultivation humidity (%)	Miceliation temperature (ºC)	Cultivation temperature (ºC)
Prata-anã	PSPA	80	90	25	22
	ENPA	80	90	25	22
Thap-maeo	PSTM	80	90	25	22
	ENTM	80	90	25	22

PSPA: Dwarf silver pseudostem; ENPA: Dwarf silver stalk; PSTM: Thap-maeo pseudostem; ENTM: Thap-maeo stalk.

Table 2. Parameters analyzed during *P. ostreatus* NATB production in substrates of banana cultivars.

Cultivate	Prata-anã		Thap-maeo	
Substrates	**PSPA**	**ENPA**	**PSTM**	**ENTM**
Micellalization (days)	20	27	21	28
Formation of primodia (days)	22	29	23	30
Total culture time (days)	45	45	45	45

PSPA: Dwarf silver pseudostem; ENPA: dwarf silver stalk; PSTM: thap-maeo pseudostem; ENTM: thap-maeo stalk.

Mushroom yield was expressed by calculating biological efficiency during cultivation. Biological efficiency (EB) represents the percentage conversion of substrate to fungal biomass (mushrooms).

$$\text{BE}\ (\%) = \frac{\text{Fresh pasta with mushrooms (g)}}{\text{Dry substrate mass (g)}} \times 100$$

Organic matter loss (OML) is the index that evaluates the decomposition of the substrate by the fungus. This index is based on the loss of fungal decomposed organic matter, which is determined by the difference between the initial substrate dry mass and the residual substrate dry mass. The OML was evaluated according to Sturion (1994), expressed by the following formula:

$$\text{OML}\ (\%) = \frac{\text{Residual substrate dry mass (g)}}{\text{Dry mass of the starting substrate (g)}} \times 100$$

The yield (g/kg) of *P. ostreatus* was calculated using the fresh mass of mushroom produced by the fresh substrate mass used.

$$\text{R}\ (\%) = \frac{\text{Fresh mushroom pasta produced (g)}}{\text{Fresh substrate initial mass (kg)}} \times 100$$

Harvest

The limit of 45 days of cultivation was established, and the determination of the harvest point was performed visually, as described by Sturion (1994).

Statistical analysis

Bioestat 7.0 software was used to read the data. The experiment was completely randomized with twenty repetitions for each treatment. Means were compared between the same substrate types (pseudostem or stalk) and within substrates of the same banana cultivar. The ANOVA test was used to analyze variance, and the Tukey test was used to contrast means ($p < 0.05$).

RESULTS AND DISCUSSION

P. ostreatus NATB, is a native Amazonian mushroom that was grown on different substrates of two banana cultivars. It showed variation in the myceliation period and early formation depending on the substrate on which it was grown (Table 2). The substrates based on pseudostems led to a shorter myceliation period and consequent primordial formation. The difference on average between substrate types in relation to these parameters was 7 days.

Biological efficiency

The biological efficiency recorded for *P. ostreatus* in banana substrates ranged from 66.98% (PSPA) to 33.88% (ENTM). Analyzing Figure 1, the highest percentage of biological efficiency was achieved in pseudococcus substrate of silver-dwarf cultivar (66.98%), followed by the pseudostem of the thap-maeo cultivar (46%). The biological efficiency in the stems of these cultivars were similar: silver dwarf with 35.32%, followed by thap-maeo with 33.88%.

There was a statistical difference at the 95% probability level based on the Tukey test between the biological efficiencies of *P. ostreatus* cultivated in the pseudostems of the two cultivars tested and the difference in biological efficiency between the substrates of the silver-dwarf cultivar. The difference was not significant between the

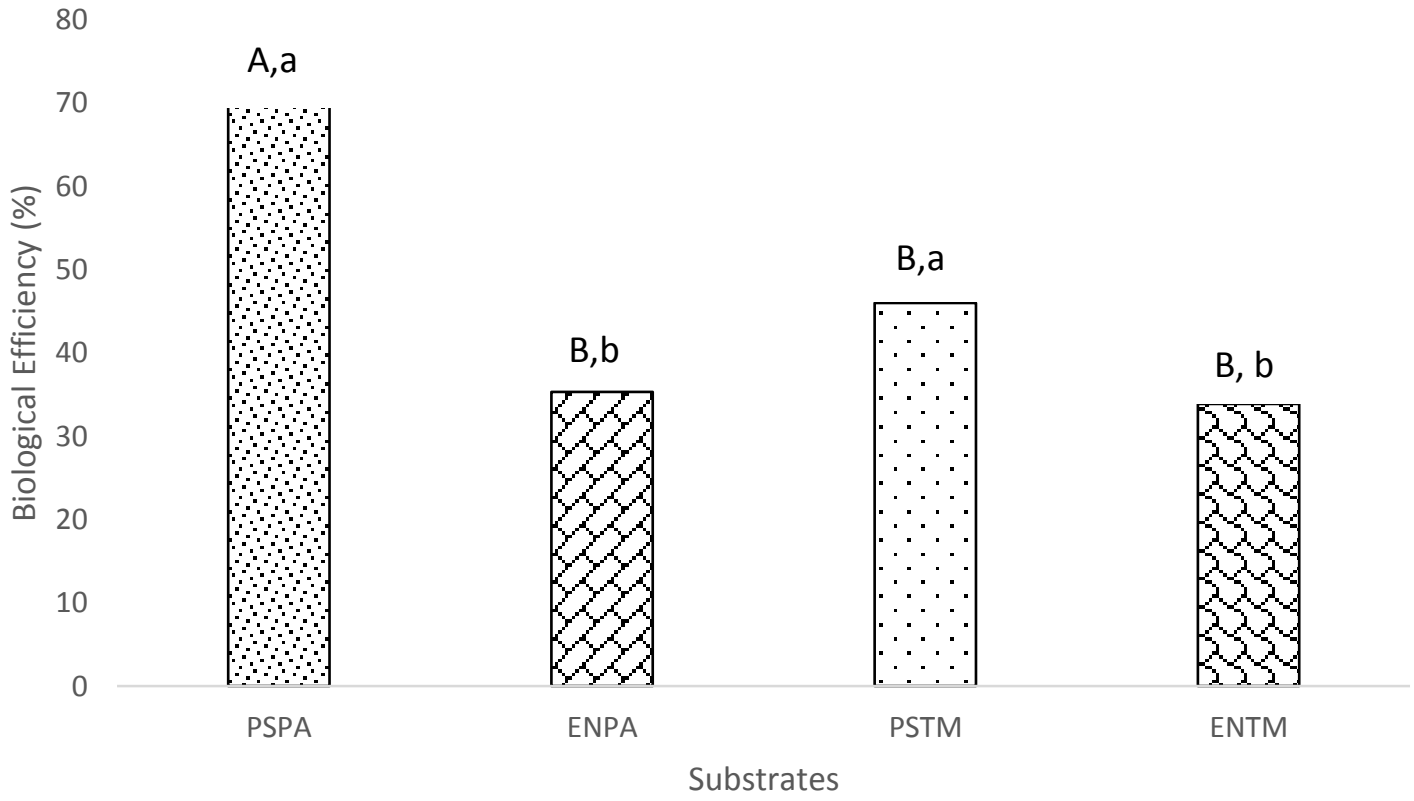

Figure 1. Average biological efficiency of the different substrates used in the cultivation of *Pleurotus ostreatus* NATB. PSPA: Dwarf silver pseudostem substrate; PSTM: Thap-maeo pseudostem substrate; ENPA: Thap-maeo stalk substrate; ENTM: Thap-maeo stalk substrate. Capital letters compare averages within the same type of banana substrate; Lower case letters compare averages within substrates of the same cultivar. Average of three repetitions. Means followed by equal letters do not differ from each other (Tukey, 5%).

pseudostem and thap-maeo stems ($p > 0.05$).

It was noted that the substrate composition influenced the productive parameters. According to Pedra and Marino (2006), this can be explained by the availability of nutrients that can be assimilated by the fungus. The treatments with pseudostems had the highest means and a positive effect of the substrates as a function of the analyzed parameter, indicating a greater dry mass bioconversion in carpophores especially for dwarf silver pseudostem production.

In a study carried out by Carvalho et al. (2012) that tested different cultivars and banana parts for *P. ostreatus* strain 09/100 production, the highest yield was observed in another substrate, thap-maeo of pseudostem (61.5%), leaving the dwarf silver pseudostem substrate at only 28% productivity.

Bonatti et al. (2003) used banana leaves to achieve much lower percentages for *P. ostreatus* (6.34%). Furlan et al. (2008) obtained 5.3 and 4.1% EB by cultivating *P. ostreatus* in cotton waste from the textile industry and in banana straw, respectively (Figure 1).

There are several factors that influence mushroom BE. Although the genus *Pleurotus* is quite versatile due to its ability to adapt to different substrate types and temperatures, substrate composition is important. Cultivation conditions or even lineage are parameters that can result in different production percentages. This study confirms the influence of the banana cultivar and its different substrates on *P. ostreatus* biological efficiency.

Yield

According to Figure 2, the highest average yield (g/kg) occurred on dwarf silver pseudostem substrate (139.95%). It was found that the average yield varied with the type of banana cultivar substrate used, and it was higher in pseudostem substrates.

The Tukey test ($p < 0.05$) showed a statistically significant difference between the types of residues and among the types of residues of the same cultivar.

Studies by Sales-Campos et al. (2010) that tested agroindustrial and timber residues for *P. ostreatus* production achieved higher percentages compared to our study. They obtained higher averages in crushed peach palm strain substrate (451.80 g/kg), followed by sugarcane bagasse substrate (250.40 g/kg) and marupá sawdust (242.80 g/kg). Yield data from a study using

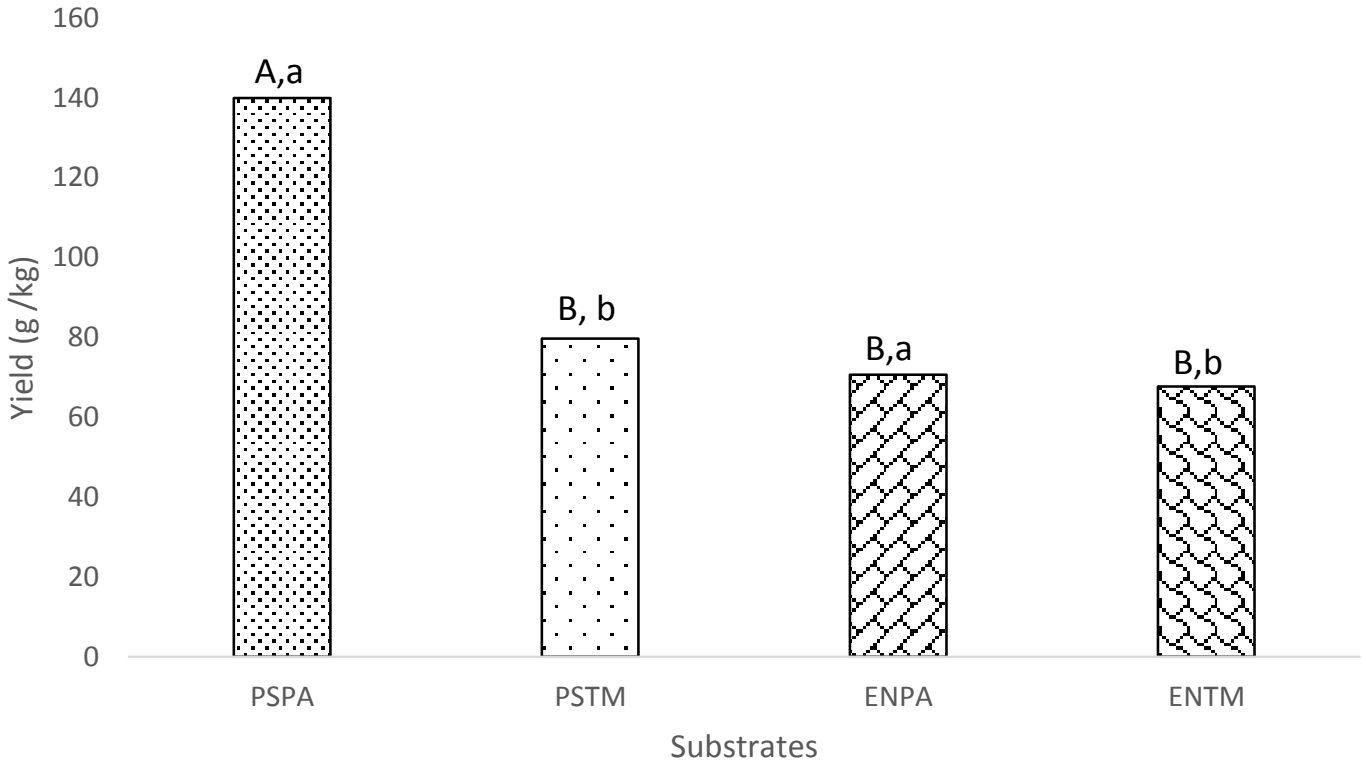

Figure 2. Average yield (g/kg) of the different substrates used in the cultivation of *P. ostreatus*. PSPA: Dwarf silver pseudostem substrate; PSTM: Thap-maeo pseudostem substrate; ENPA: Thap-maeo stalk substrate; ENTM: Thap-maeo stalk substrate. Capital letters compare averages within the same type of banana substrate; Lower case letters compare averages within substrates of the same cultivar. Average of three repetitions. Means followed by equal letters do not differ from each other (Tukey, 5%).

balsa wood sawdust was similar to those found for the dwarf silver pseudostem substrate (161.40 g/kg) (Figure 2). Rampinelli et al. (2010) tested banana straw in *Pleurotus djamor* cultivation, reaching yield percentages of 79.96% with 10% inoculum. Santos et al. (2000) also used banana straw to cultivate *P. sajor-caju*, reaching 93.03% yield in relation to dry substrate mass.

In this research, a positive correlation was observed between biological efficiency and average yield. Similar behavior was observed in studies by Sales-Campos (2008) using Amazonian timber and agroindustrial residues for the cultivation of *P. ostreatus.*

Loss of organic matter

Figure 3 shows the data on organic matter loss (PMO) of the different substrates used in *P. ostreatus* NATB cultivation. There was a statistical difference at the 95% probability level based on the Tukey test ($p < 0.05$) in organic matter loss and substrate type between the stems of the referred cultivars. When comparing the OML averages, there was a difference between the two types of residues of the same cultivar for both dwarf silver and Thap-maeo (Figure 3).

Duprat (2012) used peach palm leaf and rice bran supplementation as substrate for *P. ostreatus* DSM 1833 cultivation, achieving a percentage of 28.90% of OML, similar to the results found for dwarf silver pseudostem (28.92%). When the combination of substrate sheath and peach palm leaf (1:1) was tested with 20% inoculum, OML percentages of 36.8 were recorded for *P. ostreatus* DSM 1833; results that corroborate those observed for pseudostems prata-anã and thap-maeo of the present study.

Superior results were reported in a study by Carvalho et al. (2012) which tested four banana cultivars and different parts (pseudostem, leaf, pseudostem + leaf) in *P. ostreatus* 1467 cultivation. The authors obtained 61.5% of OML for the dwarf silver pseudostem substrate, followed by 57.3% for the thap-maeo pseudostem substrate. This differed from our results, registering 28.92% for dwarf silver pseudostem and 33.78% of OML for thap-maeo pseudostem substrate. This study shows that lower substrate OML correlates with higher biological efficiency BE.

Conclusion

P. ostreatus NATB successfully bioconverted plantain

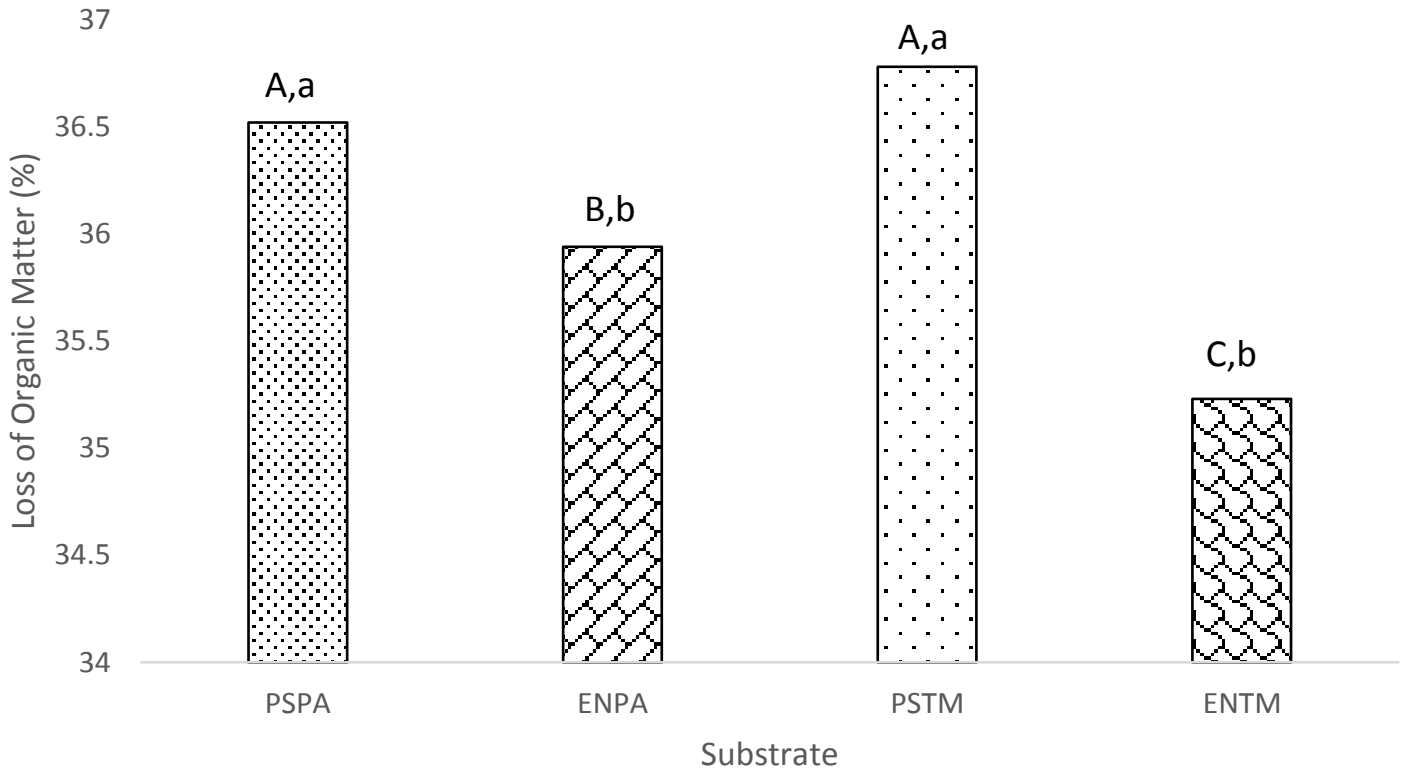

Figure 3. Loss of average organic matter from different substrates used in the cultivation of *P. ostreatus*. PSPA: Dwarf silver pseudostem substrate; PSTM: Thap-maeo pseudostem substrate; ENPA: Thap-maeo stalk substrate; ENTM: thap-maeo stalk substrate. Capital letters compare averages within the same type of banana substrate; Lower case letters compare averages within substrates of the same cultivar. Average of three repetitions. Means followed by equal letters do not differ from each other (Tukey, 5%).

residues, with emphasis on pseudostem production. The mushroom showed the best biological efficiency and yield on pseudostem substrates from the silver-dwarf cultivar. The substrates influenced the productive parameters of the evaluated mushroom.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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