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Productivity and Biological Efficiency of *Pleurotus eryngii* MMIV Cultivation at Laboratory Level

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ABSTRACT

The purpose of the study was the identification of competitive substrate formulas to sustain *Pleurotus eryngii* cultivation, in Romania, in order to recover some cellulolytic wastes easy to procure in rural areas. The experiments conducted in a laboratory greenhouse followed the basidioma size, the productivity and the biological efficiency to use the substrate. Following the addition of rice breach and pumpkin seed husk in beech sawdust, were obtained the highest values, in average by one third higher as against the hay addition values. Although, it favored the fructification, it couldn't sustain a proper productivity that led to a scanty spawn maturation.

Key words: King Oyster, substrate, sawdust, greenhouse, basidioma

INTRODUCTION

Pleurotus eryngii is a little known mushroom in Romania. It is not grown in industrial levels, being imported when found in stores and markets. It is found in spontaneous flora but little known. It is present in Asian countries, where it possess an important pharmaceutical purpose (Estrada and Royse, 2007).

Pleurotus eryngii mushroom is used for its ergothioneine content (which is an amino acid) but so for some statins (lovastatin), which prevent plasmatic cholesterol level rising and the decreasing of arterial deposits.

To all these adds a better glucose metabolism, through intervention on insulin synthesis (medicalmushrooms.net).

From current practice, the correct and efficient strategy would be single wave cultivation, whereas it produces about 80% from total productivity (Stamets and Chilton, 1983).

Currently, there are studies on obtaining new technologies or improvement of the actual ones, in order to improve the productivity and efficiency substrate exploitation (Vamanu, 2015; Estrada *et al.*, 2009). The purpose of the study was to demonstrate the ability of *P. eryngii* strain cultivation, using substrate formulas that contain easy to find components in Romania (pumpkin seed shells, rice breach and wheat straw).

MATERIALS AND METHODS

Biological material. The *Pleurotus eryngii* MMIV strain used has been provided by biologist Dr. Mihaela Ene. The spawn was isolated from the dawn spawn through PDA culture medium (made in the faculty laboratory). The pure culture has been used to obtain spawn on sterilized wheat, in glass jars of 750 mL volume, provided with sterile air filter (Vamanu, 2015).

Substrate preparation and cultivation conditions: The beech sawdust used in substrate making comes from wood workshops from Berceni town, Prahova County, Romania. The corn and the bran come from the same region. The following substrate formulas were used Standard; 2000 mL beech sawdust, 500 mL corn breach, 300 mL wheat bran, 100 mL plaster, 750 mL distilled water; SPe1: Standard formula supplemented with 500 mL pumpkin seed shells; SPe2: Standard formula supplemented with 500 mL rice breach; SPe3: Standard formula supplemented with 500 mL chopped wheat straws. After perfect mixing, the substrate is transferred into a polypropylene bag, provided with microfilter (0.2 µm). The bags filled with the substrate were sterilized in the autoclave, at 121°C, for 2 h (Vamanu, 2015).

Spawn rate was 1% for all the substrate formulas and it was done in the laminar-flow workstation. After that, each bag was sealed with an adjustable ear-type plastic collar clamp (Alananbeh *et al.*, 2014).

The propagation and the maturation phase (stabilization phase) of the spawn was done in a dark space, at a 20-23°C temperature. At the end, the bag has been removed and the substrate was kept at 18-20°C. There can be maximum two waves, at approximately 2 weeks distance in between.

It is necessary 800-1000 lumen light level, as well as minimum 90% humidity (Vamanu, 2015). The fructification phase was done in a laboratory greenhouse, Mushroom Grow Kit Mega Deluxe (Mushroom Production Center GmbH, Innsbruck, Austria). The harvesting was done when the mushroom top has started to bend. Obtained mushrooms were weighted separately for each of the substrate formulas used, in order to evaluate results. Cultivation parameters were evaluated after (Moda *et al.*, 2005).

Statistical analysis: All the parameters for antimicrobial and antioxidant activity were assessed in triplicate and the results were expressed as Mean±SD values of three observations.

RESULTS AND DISCUSSION

Substrate colonization: The substrate formula influenced directly the spawn running time (Table 1). The time obtained was superior to SPe1 and SPe2 with about 20% over the M and with 14% over the SPe3. Using the seed shells determined the fastest colonization time due to the substrate ventilation. This tendency was observed also when using the rice breach, which adds to the substrate starch, stimulating the spawn multiplication. These results were not directly correlated with previous results (Thawthong *et al.*, 2014).

The high colonization ability of the spawn in the substrate adds over all of these.

The SPe2 was superior with over 50% over all the others, except the standard. M had the most reduced propagation in the substrate with 0.4±0.1 cm over 24 h. Otherwise the propagation after 72 h exceeded 2.5±0.3 cm, the minimum being for SPe3 (data not shown). Inoculation spots from the substrate edge shown equal spawn propagation speed (p<0.5) with the ones in the interior for SPe1 and SPe2. Average propagation diameter of the spawn (starting from the inoculation spot) was 4 cm. No significant difference has been observed, even though the spawn wasn't mixed with the substrate, compared to placing it on the top, over the substrate,

where it would be in direct contact with the air provided by the bag microfilter hole. The colonization time shortening was not directly influenced by the inoculation mode in the substrate.

Productivity: First wave has appeared 10-14 days after the end of the stabilization phase. There was a 2 day difference between the M and SPe3, revealing that the wheat straws presence does not determine fructification advancing. These aspects were directly related to productivity and biological efficiency.

The SPe1 and SPe2 formulas have shown equal productivity level, of approximately 500 g each. The difference between the two formulas were less than 5% and they were similar with previous results (Hassan *et al.*, 2010). The results were approximately 20% higher than the standard substrate (M) and 23.60% higher than the SPe3. The addition of rice breach and pumpkin seed shells influence directly the biological efficiency. The highest value obtain was 47.90±4.55% for SPe2 approximately 33% higher than the wheat straw supplemented formula (SPe3). The other two formulas have shown close values, proving that these wastes are not efficient for growing *P. eryngii* MMIV (Table 1).

There was significant difference between the basidium weight (Kumla *et al.*, 2013). The minimum was registered for SPe3 substrate with value of 2.30 g and the maximum for M and SPe3 with values between 125 and 130 g.

The number of mushrooms that exceeded 100 g was minimum 4 for SPe2 and SPe3 in this case distinguished that there was a large number of mushrooms with lower weight (less than 15 g) in example 8-10 basidium. Instead, for the rest of the used formulas were determined an average mushroom amount of 42.30 g. This was consistent with the presence of double the amount of fructification spots, usually present to the edge of the substrate. The upper part usually remained idle, with a glossy appearance without any dehydration marks.

This study's results were comparable from biological efficiency perspective with anterior ones. The MMIV strain had a productive profile when using sawdust with *P. eryngii* SPe3 from Japan (Moonmoon *et al.*, 2010). Instead, the results were different from the straw usage, of which cellulosic structure is far easier to degrade, providing a faster assimilation of nutrients, first of all. This phenomenon translates into a faster fructification but not into high value productivity. During this biological process is also important the *P. eryngii* genotype strain, even though the substrate formula would be similar (Moonmoon *et al.*, 2010; Dinu and Vamanu, 2015). The results were similar with other studies that used sawdust supplemented with different additives, substrate capacity utilization values not exceeding 50% (Estrada *et al.*, 2009). Also, previous studies have demonstrated that can be obtained fruit bodies with high nutritional value by increasing the level of calcium in the substrate (Choi *et al.*, 2011).

Table 1: Effect of substrate formula on the spawn running time, productivity and biological efficiency of *Pleurotus eryngii* MMIV strain

Substrate formula	Spawn running time (days)	Productivity (g fresh mushroom harvested)	Biological efficiency (%)
M	15±1.5	405±27.21	37.75±3.70
SPe1	12±2.0	486±18.01	40.80±1.54
SPe2	12±3.0	523±8.61	47.90±4.55
SPe3	14±1.0	378±11.40	31.74±1.10

CONCLUSION

In conclusion, the tested strain holds a fickle character in a way that it has a high capacity of various substrates cultivation from waste in the food industry.

The cultivation technology is accessible and can be modified to adapt the resources present in different areas of Romania. In the future, it is envisaged also the testing of alternative methods of cultivation (casing layer), which could simplify the technological requirements of an individual mushroom.

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