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EFFICIENT RECYCLING OF FRUIT TREE WASTES THROUGH CONTROLLED CULTIVATION OF EDIBLE MUSHROOMS

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Abstract

The aim of this work was to establish the best biotechnology for efficient recycling of fruit tree wastes by using them as growing sources for mushroom species. In vitro experiments were carried out in controlled conditions for the optimal cultivation of Pleurotus ostreatus and P. eryngii to get their carpophores as food and medicinal biomass. There were set up three variants of substrates consisting of lignocellulosic wastes belonging to apricot, pear and walnut trees. After inoculation with pure cultures of mentioned species, the substrates were placed into growth chambers at constant temperature of 23 °C, during the incubation between 15-30 days. The physical and chemical parameters during the period of carpophore formation and development were set up and maintained at optimal levels, depending on each mushroom species. After a period of 30 up to 45 days, the registered results revealed a faster development of carpophores and a better productivity of P. ostreatus in comparison with P. eryngii, but the controlled cultivation of both mushroom species turned out to be an efficient recycling biotechnology to get value-added products.

Key words*: carpophores, in vitro cultivation, lignocellulose wastes, Pleurotus eryngii, Pleurotus ostreatus.*

INTRODUCTION

The woody wastes produced every year in any orchard, during the fruit tree pruning, represent in total a huge amount of redundant materials that need to be recycled through their using as main substrates for edible mushroom cultivation.

All these huge amounts of lignocellulosic materials that are coming out from the fruit trees are composed of dried trunks, branches, leaves and even fruit seeds.

However, the most part of these woody wastes are not used at all being stored in improper places or left on the fields leading to the environmental pollution (Zhang, 2008).

Sometimes, these wastes are used almost exclusively as heating sources in their rough shape or as briquettes or pellets (Soetaert and Vandamme, 2006).

The innovative procedure for recycling the fruit tree wastes through the controlled growing of mushrooms is based on the know-how of both submerged cultivation and solid-state development of such mushroom species and was carried out in specific conditions by keeping the strict correlation between both physical and chemical parameters as well as the biological factors represented by selected fungal species (Petre and Petre, 2016).

In this respect, the aim of this work was to solve this problem by recycling all fruit tree wastes through the controlled cultivation of mushroom species to get their edible carpophores, providing simultaneously the environment protection inside the orchards.

MATERIALS AND METHODS

Selected mushroom species

Following the main purpose of this research work, two mushroom species belonging to Basidiomycetes group, namely *Pleurotus ostreatus* and *P. eryngii*, from the mushroom culture collection of University of Pitesti, were selected to be used as biological tools for testing the efficiency of controlled cultivation.

P. ostreatus (Jacquin ex Fries) Kummer is a mushroom species with a high potential to grow on lignocellulose wastes and form mushroom fruiting bodies during their biological cycles (Sanchez, 2010).

P. eryngii (DC.) Quél., commonly known as "king oyster mushroom" is an edible mushroom species living saprobic or weakly parasitic on roots of herbaceous plants. *P. eryngii* is able to degrade lignin selectively when growing on non-woody lignocellulosic materials being of biotechnological interest for biopulping (Carlile and Watkinson, 1996; Camarero *et al*., 1998).

Methods used in experiments

The stock cultures were kept in viable state on malt extract agar (MEA) slants at 25ºC, for 5-7 days, and then being stored at 4ºC.

In order to get the inoculum for growing the mycelia of mentioned mushroom species, the pure cultures were inoculated in 250-mL flasks containing 100 mL of malt extract broth (MEB) as liquid medium (20% malt extract, 20% peptone and 2% yeast extract as solution in pure water up to 100%) and then kept at 23º C, on rotary shaker incubator at 110 rev min^{-1} for 12 days to get the mycelia biomass in order to be used as inoculum for mushroom cultivation (Petre *et al*., 2014).

Preparation of substrates for edible mushroom cultivation

Before starting the bioprocess of mushroom cultivation, all lignocellulosic wastes, mainly composed by trunks, branches and leaves belonging to apple, plum and apricot trees were collected from the fruit tree farms. Then, all woody dried materials were chopped and split in relatively equal sized fragments of 3-5 cm.

There were set up five variants of mushroom cultivation substrates made of lignocellulosic wastes belonging to apple, plum and apricot trees, mixed with cereal grain wastes from milling industry, such as wheat bran (5% w/w) and barley bran (5% w/w).

Beside these five substrate variants, it was used the pure cellulose (Merck) as control. All the natural ingredients were added with the role of enhancing the processes of growth and development of the mycelium of fungi used, such as, in order to stimulate enzymatic activity of fungal species, as well as processes of growth and development of mycelia biomass, according to Table 1.

Table 1. The composition of substrate variants used for controlled cultivation of mushrooms

Substrate variants	The composition of substrates
S1	Apple branches 60%, apple leaves 15%, barley bran 5%, wheat bran 5%
S2	Plum branches 60%, plum leaves 15%, barley bran 5%, wheat bran 5%
S3	Apricot branches 60%, apricot leaves 15%, barley bran 5%, wheat bran 5%
S4	Apple branches 20%, plum branches 20%, apricot branches 20%, barley bran 5%, wheat bran 5%
S5	Apple leaves 25%, plum leaves 25%, apricot leaves 25%, barley bran 5%, wheat bran 5%
Control	Pure cellulose (Merck)

Main stages of edible mushroom cultivation

In the first stage, all variants of substrates for mushroom cultivation were soaked in tap water–based synthetic medium containing 5 g/L yeast extract for 20 h at room temperature. After leaching, 1 kg of the substrate was placed in polypropylene gas-permeable bags for sterilization by autoclaving at 123°C for 50 min (Saddler *et al*., 1993; Wainright, 1992; Sanchez, 2010).

After cooling at room temperature (23-25°C), in the second phase of the experiments, the bags containing lignocellulosic wastes of apple, plum and apricot trees were inoculated with 10% (wet weight) of mycelium from pure cultures of the species *P. eryngii* and *P. ostreatus* by using the hood with sterile air laminar flow for aseptic handling of biological materials during the inoculation. Immediately after inoculation of substrates with the pure mushroom cultures of mentioned species, the inoculated plastic bags (three replicates for each strain/substrate) were placed in growth chambers type Memmert IPP 110 to be kept at a constant temperature of 23°C, during the incubation period lasting between 20 and 30 days, depending on the cultivated mushroom species (Van der Twell, 1994; Stamets, 2000).

In the next stage of cultivation, all the inoculated bags were exposed during three days at 4°C to induce a cold shock necessary for the stimulation of carpophores formation.

Then, the filled bags with inoculated substrates were kept in the fruiting room at 15-18°C, having the relative humidity around 90%, the intake air volume by 3 shifts/h and under

illumination of about 1,500 luxes (Arjona *et al*., 2009; Cohen *et al*., 2002; Stamets, 2000).
However, when the first primordia appeared, the bags were removed from the blocks formed by the mycelium colonization on the whole surface and inside the substrate volume.
Thus, the carpophores of both mushroom species emerged outside the plastic bags and they were collected during three consecutive flushes (Figures 1 and 2).

Figure 1. *P. eryngii* primordia grown on substrate S1

Figure 2. Bunch of *P. ostreatus* primordia, developed on the substrate S2

During the formation of carpophores of both mushroom species, there were registered three periods of 5-7 days, corresponding to cyclic occurrence of fruit bodies related to esch mushroom species in question (Petre *et al.*, 2014).
Data regarding the total weight of *P. ostreatus* carpophores emerged in bags containing all five types of cultivation substrates were regularly recorded and are presented in Table 2.

Table 2. The carpophore yield during the cultivation of *P. ostreatus* and *P. eryngii*, on five cultivation substrates

Mushroom species	Substrate variant	Carpophore yield (g/kg substrate)				
		Flush I	Flush II	Flush III	Total yield (g)	BE (%)
P.ostreatus	S1	570	260	145	975	97.5
	S2	510	225	135	870	87.0
	S3	470	215	145	830	83.0
	S4	450	215	125	790	79.0
	S5	420	205	120	745	74.5
P.eryngii	S1	560	250	140	950	95.0
	S2	495	230	120	850	84.5
	S3	450	250	125	825	82.5
	S4	430	220	110	760	76.0
	S5	410	190	115	715	71.5

RESULTS AND DISCUSSIONS

Regarding the controlled cultivation of both mushroom species on each one of all five substrate variants, it can be noticed in Table 2 the registered results as carpophore yield (g/kg substrate), as well as the values of biological efficiency.
Biological efficiency, often referred to as BE, is simply a way to calculate the effectiveness of a mushroom strain and substrate combination when growing mushrooms. It is a measure that was originally developed by the button mushroom industry in order to grade certain strains of mushrooms.
By definition**,** 100% biological efficiency occurs when 1 kg of fresh mushrooms is harvested from 1 kg of dry substrate, over multiple flushes.
BE = (weight of harvest/weight of dry substrate) x 100%.
Biological Efficiency (BE) was estimated as being the weight ratio between the fresh carpophores and dry substrate, multiplied by 100. In this way, the species *P. eryngii* cultivated on substrate variant S1 as well as *P. ostreatus* grown on the same substrate appeared to be the most productive mushroom/substrate variant ratio, compared with the rest of cultivation variants, the best values of BE being registered between 95% and 97.5%.
Both cultivated mushroom species were more productive especially in the first flush on the substrate S1, due to their high biological efficiency. The results regarding the weight of carpophores, revealed that the highest production of carpophores was registered in the first flushes of mushroom cultivation on the

first substrate variants, which were recorded as the most significant results (Petre *et al*., 2014). At the same time, the most productive substrates were found to be S1, S2, followed, finally, by the substrate S3. During this phase, the carpophores were harvested as being the most representative specimens of *P. eryngii* grown on all five types of substrates, as shown in the Figures 3, 4, 5 while those belonging to *P. ostreatus* are presented in Figures 6, 7, 8.

Figure 3. *P. eryngii* carpophores grown on substrate S1

Figure 4. *P. eryngii* carpophores grown on substrate S2

Figure 5. *P. eryngii* carpophores grown on substrate S2 (detail)

Figure 6. *P. ostreaus* carpophores grown on substrate S1

As it is shown in the Figures 6, 7 and 8, the carpophores of *P. ostreatus* have developed unusual shapes of their fruit bodies due to the low levels of air moisture correlated with a reduced oxygen level of the atmosphere inside the growth chamber during a period of over 30 hours, as a temporary disfunction of water sprayer.

Figure 7. *P. ostreatus* carpophores developed on substrate S2

Figure 8. *P. ostreatus* carpophores grown on substrate S3

According to the main results of this research work, the procedure of recycling the fruit tree wastes by using them as growing sources for controlled cultivation of edible mushroom species *P. ostreatus* and *P. eryngii* was established (Figure 9).

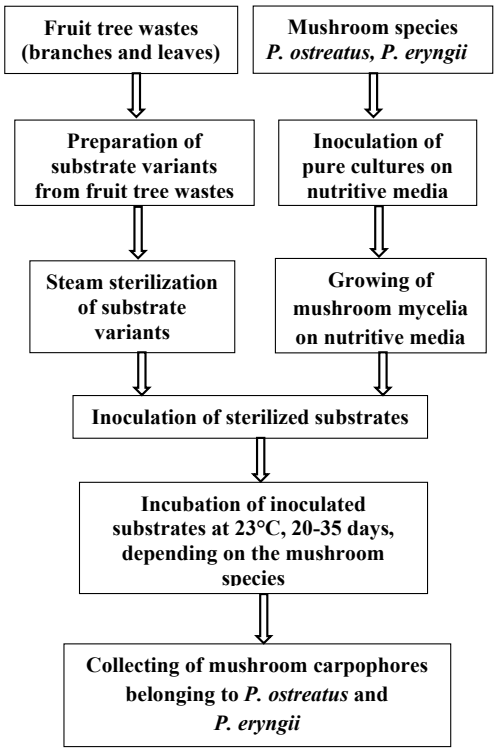

Figure 9. The scheme of efficient recycling of fruit tree wastes by controlled cultivation of edible mushroom species

CONCLUSIONS

The results regarding the weight of carpophores revealed that the highest production was registered in the first flushes of mushroom cultivation on the substrate variants.

The most significant results and most productive substrates were found to be S1, S2, followed, eventually, by the substrate S3.

Consequently, based on the carried out experiments, it was settled down the biotechnology for recycling the fruit tree wastes of apple, plum and apricot by controlled cultivation of mushroom species *P. ostreatus* and *P. eryngii*.

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