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BIOTECHNOLOGICAL RECYCLING OF FRUIT TREE WASTES THROUGH ORGANIC CULTIVATION OF MUSHROOM SPECIES

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Abstract

The excessive and long-term accumulation of large amounts of redundant lignocellulose materials, as outcome wastes from the specific activities of all fruit tree farms across the whole country in Romania, has become a huge problem which needs to be solved by using biological means for their conversion into beneficial products. Thus, the main aim of this work was to solve this problem by recycling the fruit tree wastes through organic cultivation of two mushroom species, Ganoderma lucidum and Pleurotus ostreatus. The fruit body productions of each one of these mushroom species registered the highest levels as 1,830 g for G. lucidum and 2,750 g for P. ostreatus, relative to 5 kg of substrates made of fruit tree wastes. According to these results, the suitable biotechnological procedures for recycling of apple, plum and cherry tree wastes through organic cultivation of mentioned mushroom species is presented in this paper.

Key words*: biotechnology, Ganoderma lucidum, Pleurotus ostreatus, lignocellulose wastes.*

INTRODUCTION

It is well known that every year huge amounts of redundant lignocellulose materials outcome from any orchard as fruit tree wastes which cause serious environmental troubles if they accumulate in the local fruit tree farms or they are burned on the soil of other areas (Chahal, 1993; Carlile & Watkinson, 1996).

All these natural but redundant materials, mainly composed of dried trunks and branches of fruit trees, could be recycled as main substrates for solid-state cultivation of mushroom species belonging to the group of Basidiomycetes (Stamets, 1993; Moser, 1994).

In this respect, the experiments were set up on testing and optimizing the biotechnological processes of fruit tree wastes recycling through controlled cultivation of edible and medicinal mushroom species *Ganoderma lucidum* and *Pleurotus ostreatus,* in order to get their carpophores to be used as food and nutraceuticals (Smith, 1998).

The main aim of this research work was focused to find out the best biotechnological procedure for recycling the fruit tree wastes from orchards through the organic cultivation of certain mushroom species on these wastes made of lignocellulose materials and finally get the carpophores of edible and medicinal mushrooms.

MATERIALS AND METHODS

Mushroom species used in experiments

As a mushroom species belonging to the group of white rot fungi, *Ganoderma lucidum* (Curt. Fr.) P. Karst is a wood degrading fungus, belonging to lignin decomposers. Until now, *G. lucidum* species has been cultivated mainly on wood substrates or as fungal mycelium in synthetic liquid media in small scale production processes (Stamets, 1993; Cohen et al., 2002). On the other side, *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).

In order to achieve the experiments related to biotechnological recycling of fruit tree wastes through organic cultivation of mushroom species, selected pure cultures of mushrooms from the culture collection belonging to the University of Pitesti were used. The stock cultures were maintained on malt-extract agar (MEA) slants at 25°C for 5-7 days and after that, they were stored at 4°C. To achieve the experiments, the mushroom pure cultures were transferred in 250-mL flasks containing 100 mL of MEB medium (20% malt extract, 2% yeast extract and 20% peptone solution in pure water up to 100%) and let to grow at 23°C

on rotary shaker incubators at 110 rev min^{-1} for 5-7 days (Petre et al., 2014)

Substrate variants for mushroom cultivation

For the optimal cultivation of mushroom species *G. lucidum* and *P. ostreatus*, there were set up three variants of mushroom cultivation substrates, mainly consisting of natural compounds like woody wastes made of sawdust resulted from milled branches of apple, plum and cherry, which were chopped, mixed and hydrated (24-30 h) with a solution made of following ingredients: wheat bran, yeast extract, calcium carbonate and tap water, as it is shown in Table 1.

Table 1. The composition of substrate variants for the controlled cultivation of mushroom species

Substrate ingredients	The composition of each substrate variant (w/w)		
	S1	S2	S3
Sawdust from milled apple branches	70	-	-
Sawdust from plum milled branches	-	70	-
Sawdust from cherry milled branches	-	-	70
Wheat bran	10	10	10
Yeast extract	3.5	3.5	3.5
Calcium carbonate	1.5	1.5	1,5
Tap Water	15	15	15

Then, all three variants of substrates, S1, S2, S3 were soaked in a nutritive aqueous solution made of natural ingredients, having the composition presented in Table 1, and then were placed in thermorezistant polypropylene bags with 5 kg weight, subsequently being sterilized in an autoclave at the temperature of 121^{o}C, for 50 min. After cooling, the contents of sterilized polypropylene bags containing the substrate variants were aseptically inoculated with the pure cultures of mushroom species *G. lucidum* and *P. ostreatus*. Next, all bags with the variants of substrates, previously disinfected by sterilization and inoculated with pure cultures of mentioned mushroom species, were placed into automatic growing chambers and kept at the constant temperature of 23^{o}C, for 15-30 days depending on the mushroom species used in experiments. Then, during the incubation, the whole mycelial biomass, developed inside the substrates, placed in polypropylene bags, formed the fruit bodies belonging to both mushroom species.

During the process of fruit body formation the culture parameters were set up and maintained at the following levels depending on each mushroom species used in experiments: the air temperature, 18-20^{o}C, the air flow volume, 5-7 m^3/h, air flow speed, 0.2-0.3 m/s, the relative moisture content, 95-97%, the light intensity, 500-1,000 luces for 8-10 h/day. The whole period of mushroom growing from the inoculation up to the fruit body formation lasted between 30-35 days for *P. ostreatus* and 60-70 days for *G. lucidum*.

RESULTS AND DISCUSSIONS

After the first stage of primordial formation, the carpophores belonging to both mushroom species have developed continuously in a fast growing process. Thus, during the five crop stages of such biological process, the carpophores of both species were studied regarding their body development and increasing in significant weight. In this respect, during a period of time lasting between 30 and 70 days, the mature carpophores belonging to both mushroom species were collected and after that, they were weighted.

The results regarding the harvest of carpophores for each one of the mushroom species were registered and assessed during a period of time lasting from 35 up to 70 days, depending on the mushroom species used in experiments, as it is shown in Tables 2 and 3.

Table 2. The mushroom harvest variation, depending on each crop stage and substrate variant, during the cultivation of *G. lucidum*

Crop stage	Mushroom harvest on substrate S1* (w/w)	Mushroom harvest on substrate S2* (w/w)	Mushroom harvest on substrate S3* (w/w)
I	610	490	530
II	475	350	450
III	350	270	320
IV	240	210	250
V	155	140	120
Total weight (g)	1,830	1,460	1,670

*The average of harvest which were registered during three repeated cultivation cycles

Table 3. The mushroom harvest variation, depending on each crop stage and substrate variant, during the cultivation of *P. ostreatus*

Crop stage	Mushroom weights on substrate S1* (w/w)	Mushroom weights on substrate S2* (w/w)	Mushroom weights on substrate S3* (w/w)
I	730	770	590
II	650	630	430
III	510	530	310
IV	370	470	250
V	310	350	210
Total weight (g)	2,570	2,750	1,790

*The average of weights which were registered during three repeated cultivation cycles

Regarding the registered results, it is of great importance to take into consideration that each value of weight, presented both in Table 2 and Table 3 means the average of weights which were registered during three repeated cultivation cycles, by keeping constant the environmental factors (the air temperature at 18-20^{o}C, the air flow volume, 5-7 m^{3}/h, the air flow speed, 0.2-0.3 m/s, the relative moisture content, 95-97%, the light intensity, 500-1,000 luces for 8-10 h/day).

Comparing the values of registered amounts of mushroom carpophores belonging to *G. lucidum* species, it has to be mentioned that the highest total weights were noticed when it was used the substrate variant S1, followed by the substrate variant S3, and finally, by the substrate variant S2.

Significant differences of mushroom weights between the crop stages were noticed in the case of *G. lucidum*, respectively between the first three crop stages corresponding to the mushroom harvest on substrate variants S1 and S2, as they are shown in Table 2.

At the same time, there were determined significant differences between the crop stages of *P. ostreatus*, mainly between the first three crop stages related to the mushroom harvest on substrate variants S2 and S3, like they were presented in Table 3.

In Figures 1, 2 and 3, the carpophores belonging to *G. lucidum* mushroom species, which have developed on substrate variants S1, S2 and S3, are displayed.

Figure 1. *G. lucidum* carpophores, developed on the substrate S1

Figure 2. *G. lucidum* carpophores, grown on the substrate variant S2

Figure 3. *G. lucidum* carpophores, grown on the substrate variant S3

The collected carpophores belonging to the mushroom species *P. ostreatus* are illustrated in the Figures 4, 5 and 6.

Figure 4. Bunches of *P. ostreatus* carpophores, developed on the substrate variant S1

Figure 5. A bunch of *P. ostreatus* carpophores, developed on the substrate variant S1

Figure 6. *P. ostreatus* carpophores, grown on the substrate variant S3

Regarding the results of *P. ostreatus* harvesting, almost the same significant differences between the crop stages were noticed, but the highest total weights of mushroom carpophores were registered for the substrate S2, followed by S1 and the last one being the substrate S3.

According to the registered results, the optimal biotechnology for recycling fruit tree wastes by using *G. lucidum* and *P. ostreatus* mushroom species was established and it is shown in Figure 7:

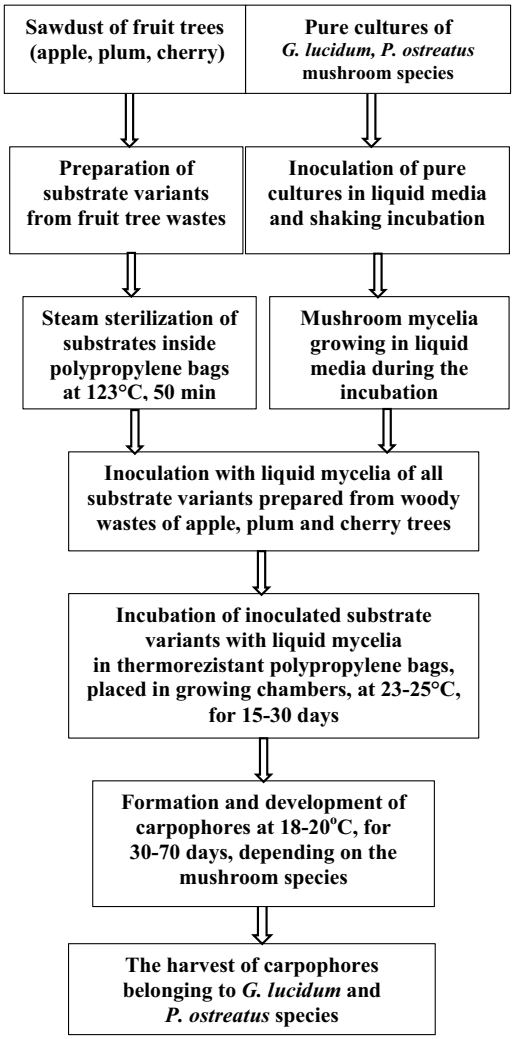

Figure 7. Biotechnology for recycling the fruit tree wastes by using *G. lucidum* and *P. ostreatus* mushroom species, through organic cultivation

The most important advantage of using such biotechnology for recycling the fruit tree wastes by using *G. lucidum* and *P. ostreatus* is that the carpophores which were collected from the substrate variants are 100% natural food products, being obtained through the organic cultivation of these mushroom species.

It is expected to implement this biotechnology for recycling the fruit tree wastes, by following the controlled process previously presented, by decomposing such lignocellulose materials and getting significant amounts of carpophores, as well as providing the environment protection in orchards designed for growing apple, plum and cherry trees.

CONCLUSIONS

According to the registered results, the best substrate variant for *G. lucidum* cultivation was determined as being S1 and for *P. ostreatus* mushroom species it was proven to be the substrate variant S2.

By comparing the amounts of carpophores belonging to *G. lucidum* mushroom species harvested on the substrate variants which have been used in experiments, it has to be mentioned that the highest total weights were noticed when it was used the substrate variant S1, followed by the substrate variant S3, and finally, by the substrate variant S2.

Speaking about the mushroom species *P. ostreatus*, there were registered the highest total weights of mushroom carpophores when the substrate variant S2 was used, followed by the substrate variant S1 and the last one being the substrate S3.

Significant differences of mushroom weights between the crop stages were noticed in the case of *G. lucidum*, respectively between the first three crop stages corresponding to the mushroom harvest on substrate variants S1 and S2. At the same time, there were determined significant differences between the crop stages of *P. ostreatus*, mainly between the first three crop stages related to the mushroom harvest on substrate variants S2 and S3.

The fruit body productions of each one of these mushroom species have registered the highest levels as 1,830 g for *G. lucidum* and as 2,750 g in the case of *P. ostreatus*, relative to 5 kg of substrate variants S1 and respectively S2, mainly made of fruit tree wastes.

However, in-depth experiments regarding the optimal valorizing of different types of lignocellulose wastes coming every year from fruit tree growing works through controlled cultivation of mushroom species like *G. lucidum* as well as *P. ostreatus* are going to be carried out in the next period of time.

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