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ECOTECHNOLOGY FOR FULLY RECOVERY OF FRUIT TREE WASTES THROUGH CONTROLLED CULTIVATION OF EATABLE MUSHROOMS

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Abstract

Year by year, large amounts of fruit tree wastes, such as etiolated leaves, woody wastes (old branches, dried trunks, unproductive shoots) resulting from annual pruning or cleaning of fruit trees are produced in many orchards becoming in the end huge sources of pollution. The main objective of this research work was focused on the development, implementation and testing of experimental model for ecological recycling of fruit tree wastes through controlled cultivation of the eatable mushroom species Lentinus edodes, Pleurotus ostreatus and P. eryngii on substrates made of leaves, branches and trunks of apple, plum and apricot trees. All three mushroom species were used as pure culture to be grown on different variants of substrates made of fruit tree wastes, namely etiolated dried leaves, shoots and trunks as well as distilled marc of fruits after their use as raw matter for alcohol fermentation and distillation. All substrates were steam sterilized at 123 °C, 50 min, and after that they were inoculated with the pure mushroom cultures. After 30-50 days of incubation at 23 °C, depending on the mushroom species, the first buttons emerged and after one or two days they developed the mature fruit bodies. After a period of 120 days mushroom growing on the substrates made of fruit tree wastes the results showed a fast development of Pleurotus species, respectively P. ostreatus was faster than P. eryngii, and had registered a better productivity than L. edodes. The implementation of such an experimental model of green technology will determine the full recovery of all fruit tree wastes produced in orchards (leaves, branches, wood stems) and their fast recycling through the natural food chains of the whole organic matter. Inside the newly formed food chains, the fruit tree wastes were the basic link on which the eatable mushroom species, such as L. edodes, P. ostreatus and P. eryngii that decompose lignin, hemicellulose and cellulose have synthesized natural organic compounds through the carpophores that were finally used as food products by the human consumers.

Keywords: *ecotechnology, fruit tree wastes, mushroom cultivation, L. edodes, P. ostreatus, P. eryngii*

INTRODUCTION

Agricultural works related to the fruit tree growing as well as fruit processing have generally been matched by a huge formation of wide range of cellulosic and woody wastes that accumulate every year in the most orchards all over the world (Beguin and Aubert, 1994; Verstraete and Top, 1992).

Many of these lignocellulose wastes cause serious environmental pollution effects, if they are allowed to accumulate in the orchard or much worse to be burned on the soil. So far, the most recent approaches on lignocellulose-degrading procedures have been directed to the applications of environmental protection, with emphasize on the bioremediation technology of lignin and cellulose decomposition, including their biodegradation and bioconversion through the controlled cultivation of eatable mushrooms (Carlile and Watkinson, 1996; Smith, 1998).

In this respect, the main aim of this work was to find the best eco-technology of recycling the fruit tree wastes by using them as growing sources for eatable mushrooms in order to extend the food chain in orchard ecosystems (Moser, 1994).

MATERIALS AND METHODS

Eatable mushroom species and culture media

According to the main purpose of this work, three fungal species from Basidiomycetes, namely *Lentinus edodes*, *Pleurotus eryngii* and *P. ostreatus* from University of Pitesti culture

collection were used as pure cultures in experiments. 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. The fungal cultures were grown 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%) at 23 °C on rotary shaker incubators at 110 rev min^{-1} for 5-7 days (Petre and Petre, 2008).

Methods used in experiments

Preparation of liquid fungal inoculum

The mushroom cultures for experiments were prepared by inoculating 100 mL of culture medium with 3-5% (v/v) of the seed culture and then cultivated at 23-25 °C in rotary shake flasks of 250 mL. The experiments were conducted under the following conditions: temperature, 25 °C; agitation speed, 90-120 rev min^{-1}; initial pH, 4.5–5.5. The seed cultures were transferred on culture medium and grown for 7–12 days (Petre and Petre, 2013a).

Incubation of mushroom cultures

The experiments of this research project were achieved by growing all the mushroom species in special growing chambers, where all the culture parameters were kept at optimal levels in order to get the highest production of fruit bodies (Raaska, 1990; Chahal and Hachey, 1990). During the experiments, the influence temperature, pH level, inoculum size and volume and incubation time on mycelia net formation and especially, on fruit body induction were investigated (Petre et al, 2012; Stamets, 1993; Chahal, 1994).
All the culture substrates for mushroom growing were inoculated using liquid inoculum with the age of 5–7 days and the volume size ranging between 3-7% (v/w). During the period of time of 18–20 days after this inoculation, all the mushroom cultures had developed a significant biomass on the culture substrates made of fruit wastes (Petre and Petre, 2012).
The optimal temperatures during the incubation for mycelia growing were maintained between 23–25 °C. The whole period of mushroom growing from the inoculation to the fruit body formation lasted between 30–60 days, depending on each fungal species used in experiments (Petre et al, 2012).

Preparation of mushroom culture substrates

After inoculum preparation, the seed cultures were cultivated on special growing media made of lignocellulosic materials resulted from apple, plum and apricot wastes that were used as substrates for mushroom development (Petre and Petre, 2013b). These fruit tree wastes were mechanical pre-treated to breakdown the lignin and cellulose structures to be more susceptible to the enzyme actions (Leahy and Colwell, 1990; Glazebrook et al., 1992). All these pre-treated wastes were disinfected by steam sterilization at 123^{o} C for 50 min. The final composition of culture substrates used in experiments is presented in Table 1.

Table 1. Composition of substrate variants (%) made of fruit tree wastes

Substrate ingredients (w/v)	Composition of substrate variants (%)		
	Apple wastes (S1)	Plum wastes (S2)	Apricot wastes (S3)
Fruit marc collected after alcohol distillation	35	25	30
Fruit tree sawdust of milled branches	20	15	17
Etiolated dried leaves of fruit trees	10	17	15
Wheat bran	4.5	3.5	3
Chalk	3	3	3.5
Gypsum	2.5	1.5	1.5
Pure Water	25	35	30

As control samples for each variant of culture substrates, used for the experimental growing of all mushroom species were used only woody chops of oak kept in pure water three days before the experiments and then sterilized by steam at 123 °C, 50 min. in order to be disinfected, inoculated with liquid mycelia of the same mushroom species and incubated up to 60 days at 23 °C.

Preparation of Mushroom Spawn

A significant example to describe the mushroom spawn preparation is the following:

3,000 g of fresh apple marc collected after alcohol distillation, 2,000 g of apple tree sawdust made of milled branches and 1,000 g of etiolated dried leaves belonging to apple trees were mixed in 3,000 L of pure water by adding the following supplements: 500 g wheat bran, 300 g of chalk, 200 g of gypsum, in order to obtain three variants of natural substrates for mushroom spawn growing. The mixed ingredients were loaded in 1,000 mL glass jars and 10 kg heat resistant polyethylene bags, being then steam-sterilized at 123 °C, for 50 min. After cooling, when the temperature decreased below 35°C, all recipients containing sterilized substrate were inoculated with different amount of inoculum developed in liquid medium (Petre and Petre, 2013).

Then, all glass jars and heat resistant polyethylene bags filled with sterilized and inoculated substrates were incubated at 23-25 °C, until the spawn fully colonized the whole growing contents, prepared as it was previously shown (Figures 1 and 2).

Figure 1. Glass jars filled with *P. ostreatus* mycelia grown on apple wastes

Figure 2. Polyethylene bags filled with *L. edodes* mycelia grown on apple wastes

RESULTS AND DISCUSSION

During the whole processes of fruit body formation and development, the culture parameters were set up and maintained at the following levels depending on each mushroom species: air temperature 15–17 ^{o}C, the air flow volume 5–6 m^3/h, air flow speed 0.2–0.3 m/s, the relative moisture content 80–85%, light intensity 500–1,000 luces for 8–10 h/d.

According to the main results of this research work, the eco-technological procedure of recycling the fruit tree wastes by using them as growing sources for eatable mushrooms *L. edodes, P. ostreatus* and *P. eryngii* was established as it can be seen in Figure 3.

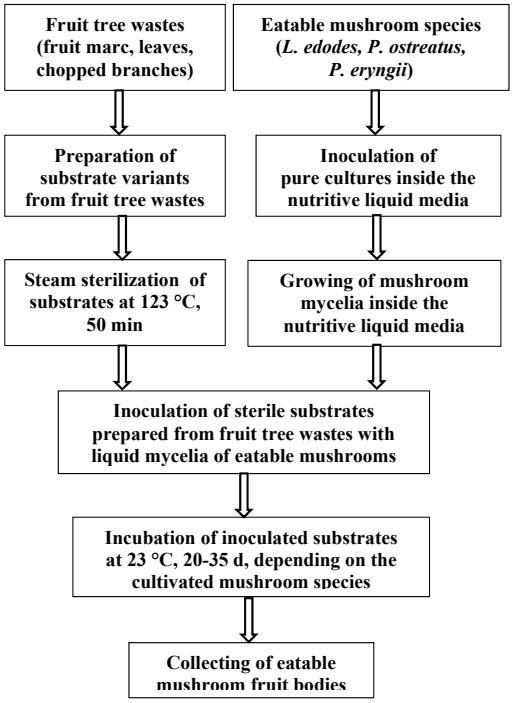

Figure 3. The ecotechnology of recycling the fruit tree wastes by using eatable mushrooms

Amongst certain physiological properties, the incubation temperature correlated with the pH value, as well as the age and volume of mycelia inoculum may play an important role in mushroom hyphae development as well as in fruit body formation (Stamets, 1993; Ropars et al., 1992; Carlile and Watkinson, 1992; Wainwright, 1992).

In order to study the effects of initial pH correlated with the incubation temperature upon fruit body formation, *P. ostreatus*, *P. eryngii* and *L. edodes* were cultivated on substrates made of fruit tree wastes at different initial pH values (4.5–6.0). To find the optimal incubation temperature for mycelia growth, these eatable mushrooms were cultivated at different temperatures ranging from 20-25 °C (Table 2).

To test the influence of inoculum age as well as inoculum volume during cultivation cycles, the same eatable mushroom species *P. ostreatus*, *P. eryngii* and *L. edodes* were grown on substrates made of fruit tree wastes during different time periods between 30 and 60 days, varying the inoculum volume (5-7 v/w), as it is shown in Table 3 and Table 4.

Table 2. The influence of initial pH and temperature upon fruit body formation of *P. ostreatus*, *P. eryngii and L. edodes*

pH (pH units)	Temperature (^{o} T)	Final Weight of the Fresh Fruit Body (g / kg substrate)		
		P. ostreatus	*P. eryngii*	*L. edodes*
4.5	18	175±0.23	191±0.10	180±0.02
5.0	21	193±0.15	203±0.05	297±0.14
5.5	23	198±0.10	195±0.15	351±0.23
6.0	26	181±0.12	179±0.12	280±0.03
6.5	29	173±0.09	105±0.23	257±0.15

Table 3. The effect of inoculum age upon fruit body formation of *P. ostreatus*, *P. eryngii and L. edodes*

Inoculum Age (h)	Final Weight of the Fresh Fruit Body (g /kg substrate)		
	P. ostreatus	*P. eryngii*	*L. edodes*
264	123±0.14	128±0.05	135±0.23
240	141±0.10	150±0.28	157±0.17
216	154±0.12	195±0.90	193±0.15
192	155±0.23	221±0.25	215±0.05
168	169±0.37	235±0.78	241±0.07
144	210±0.20	248±0.03	259±0.12
120	230±0.15	253±0.05	264±0.21
96	215±0.09	230±0.15	253±0.10
72	183±0.05	205±0.23	210±0.05

Table 4. The effect of inoculum volume upon fruit body formation of *P. ostreatus*, *P. eryngii and L. edodes*

Inoculum Volume (v/w)	Final Weight of the Fresh Fruit Body (g /kg substrate)		
	P. ostreatus	*P. eryngii*	*L. edodes*
7.0	234±0.12	215±0.20	220±0.05
6.5	245±0.15	248±0.23	251±0.20
6.0	253±0.15	257±0.07	280±0.15
5.5	243±0.12	235±0.03	247±0.07
5.0	255±0.23	215±0.15	235±0.03

All data are the means ± S.D. of triple determinations.

Analysing the registered results of the initial pH and temperature upon the mushroom fruit body formation, the optimal pH levels were determined as being 5.0–5.5. It can be noticed that the highest values of mushroom production was obtained for *L. edodes* species, when it was grown on the fruit waste substrates having the initial pH 5.5 at 23 °C.

The inoculum age of 120 h as well as an inoculum volume of 6.0 (v/w) had beneficial effects on the fungal biomass production. The whole period of mushroom growing from the inoculation to the fruit body formation lasted between 20–35 days depending on each mushroom species used in experiments. The most significant influence of the volume as well as age inoculum upon the final weight of mushroom fruit bodies was registered for the cultures of *L. edodes*, on the next places being *P. ostreatus* and *P. eryngii* species.

Regarding the whole duration of the mushroom cultivation cycles from all the tested species during present experiments, *P. ostreatus* was registered as being the fastest mushroom culture (20–25 days), followed by *P. eryngii* (25–30 days) and finally, *L. edodes* as the longest mushroom culture (30–35 days).

CONCLUSIONS

1. From all eatable mushroom species which were tested in our experiments, *P. ostreatus* was registered as the fastest mushroom culture (25–30 days), then *P. eryingii* (35–45 days) and finally, *L. edodes* as the longest mushroom culture (45–60 days).
2. The inoculum age of 120 h as well as an inoculum volume of 6.0 (v/w) have beneficial effects on the fungal biomass production.
3. The optimal pH values were between 5.0–5.5 correlated with the best temperature levels of 21-23° C for higher mushroom fruit body production.
4. After a period of 120 days mushroom growing on the substrates made of fruit tree wastes the results showed a fast development of *Pleurotus* species, respectively *P. ostreatus* was faster than *P. eryngii*, and had registered a better productivity than *L. edodes*.
5. The implementation of such an experimental model of green technology will determine the fully recovery of all fruit tree wastes produced in orchards (marc, leaves, branches, wood stems) and their fast recycling through the natural food chains of all organic matter.

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