BIOTECHNOLOGY FOR AGRICULTURAL WASTES RECYCLING BY USING *IN VITRO* CULTURES OF EDIBLE AND MEDICINAL MUSHROOMS

BIOTEHNOLOGIE DE RECICLARE A DEŞEURILOR AGRICOLE PRIN CULTURI *IN VITRO* DE CIUPERCI ALIMENTARE ŞI TERAPEUTICE

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Rezumat. *Principalul scop al acestei lucrari a fost acela de a stabili biotehnologia optima de protectie a mediului prin reciclarea deseurilor viticole si vinicole, prin utilizarea acestora drept substraturi corespunzatoare pentru cultivarea ciupercilor alimentare si medicinale. Avand in vedere faptul ca ciupercile alimentare si medicinale necesita un micromediu specific, ce include un complex de nutrimente, precum si factori fizici si chimici care influenteaza productia de biomasa fungica si formarea corpurilor de fructificare, experimentele au fost concentrate asupra studierii unei noi biotehnologii de reciclare integrala a deseurilor lignocelulozice care sunt produse anual, atat in podgorii, cat si in industria de vinificatie. In conformitate cu principalul scop al acestei lucrari, in experimentele efectuate au fost utilizate doua specii de ciuperci alimentare si terapeutice apartinand grupului Basidiomicetelor, si anume: Lentinus edodes si Pleurotus ostreatus, sub forma de culturi pure.*

Abstract. *The main aim of this work was to establish the optimal environmental biotechnology of recycling the winery and vine wastes by using them as appropriate substrata for edible and medicinal mushrooms growing. Taking into consideration that any edible or medicinal mushrooms requires a specific micro-environment including complex nutrients, as well as all physical and chemical factors that have an influence upon fungal biomass production and fruit body formation, the experiments were focused on the study of a new biotechnology for total recycling of all the lignocellulose wastes which are released annually both in vineyards and wine producing industry. According to the main purpose of this research work, two edible and medicinal mushroom species of Basidiomycetes group, namely Lentinus edodes as well as Pleurotus ostreatus were used as pure mushroom cultures*

The agricultural works as well as the industrial activities related to vine crops and wine processing have generally been matched by a huge formation of wide range of waste products. Many of these lignocellulosic wastes cause serious environmental pollution troubles if they are allowed to accumulate in the vineyards or much worse to be burned on the soil inside the vineyard sites (Vournakis & Runstadler 1989). The main aims of the presented experiments were to find out the best biotechnology of recycling the winery and vineyard wastes by using them as a growing source for edible mushrooms and protect the vineyard

ecosystems by valorising all the wastes of vineyard ecosystems and wine producing industry (Petre, 2002; Petre & Petre, 2006).

MATERIALS AND METHODS

Fungal culture media

The stock cultures were maintained on malt-extract agar (MEA) slants (20% malt extract, 2% yeast extract, 20% agar-agar). Slants were incubated at 25°C, 120-168 h and stored at 4°C. The pure mushroom cultures were expanded by growing in 250-ml flasks containing 100 ml of liquid malt-extract medium at 23°C on rotary shaker incubators at 110 rev min^{-1} for 72-120 h (Petre, 2006).

Methods used in experiments

In order to prepare the inoculum for the spawn cultures of *Pleurotus ostreatus* and *Lentinus edodes,* the pure mushroom cultures were inoculated into 100 ml of liquid malt-yeast extract medium with 3-5% (v/v) and maintained at 23-25°C in 250 ml rotary shake flasks (Ropars et al., 1992; Wainwright, 1992). The experiments of inoculum preparation were set up under the following conditions: constant temperature, 25°C; agitation speed, 90-120 rev min -1; initial pH, 5.5–6.5. All the seed mushroom cultures were incubated for 120–168 h (Chang & Hayes, 1978). The seed cultures of these mushroom species were inoculated in liquid culture media (20% malt extract, 10% wheat bran, 3% yeast extract, 1% peptone) at pH 6.5 previously distributed into 1000 ml rotary shake flasks. During the incubation time period, all the spawn cultures were maintained in special culture rooms at 25^0 C (Fig. 1).

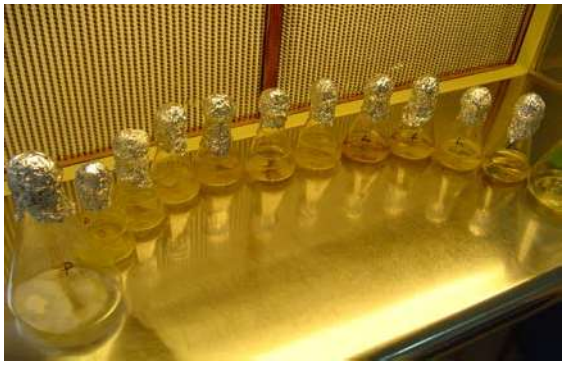

Fig. 1 – Mycelia cultures of *Pleurotus ostreatus* and *Lentinus edodes* mushrooms developed in liquid media

The culture composts dedicated to mushroom production were prepared from the lignocellulosic wastes resulted from: vineyard cuttings and grapes pommace. In this respect, there were prepared three variants of culture compost made of grapes pommace and vineyard cuttings, after they have already been mechanical pre-treated by grinding in order to make them more susceptible to the enzyme actions (Carlile & Watkinson, 1996; Stamets, 1993). All the culture compost variants made of ground vineyard and

winery wastes were transferred into 1000 ml glass jars and disinfected by steam sterilization at 120^0C for 60 min. When the jars filled with composts were chilled they were inoculated with the liquid mushroom spawn already prepared. After 10 to 15 days of incubation, the mycelia have grown inside the culture composts covering the whole their surfaces (Fig. 2).

Fig. 2 – Jars with *Pleurotus ostreatus* spawn grown on composts made of vine cuttings and grapes pommace

RESULTS AND DISCUSSIONS

The effects induced by additional ingredients upon the mycelia growing during the incubation were investigated (Fig. 3, 4, 5):

- carbon sources (maltose, glucose, sucrose, xylose),
- nitrogen sources (what bran, malt extract, peptone, tryptone, yeast extract);
- minerals ($CaCO_3$, $CaSO_4$, $MgSO_4 \cdot 5\ H_2O$, K_2HPO_4, KH_2PO_4)

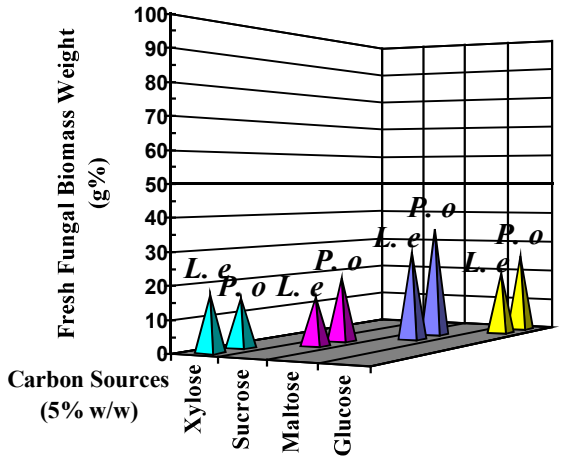

Fig. 3 - Comparative effects of carbon sources upon mycelia growing of *Pleurotus ostreatus* (P. o) and *Lentinus edodes* (L. e)

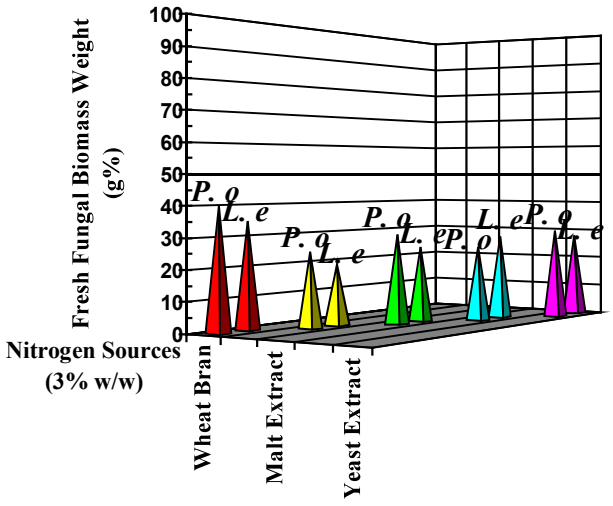

Fig. 4 - Comparative effects of nitrogen sources upon mycelia growing of *Pleurotus ostreatus* (P. o) and *Lentinus edodes* (L. e)

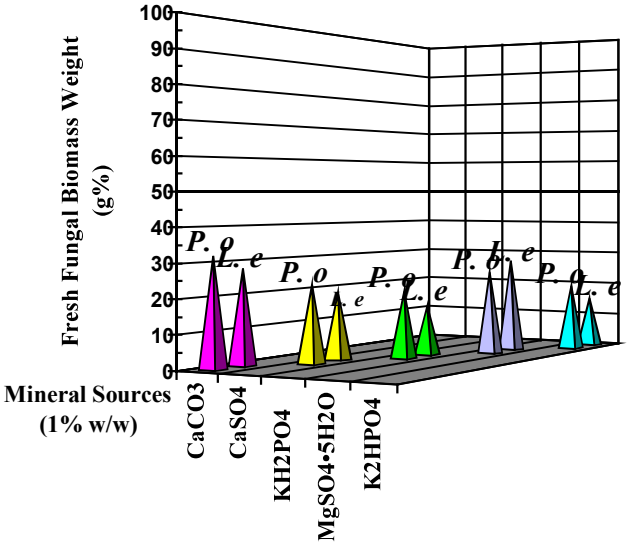

Fig. 5 - Comparative effects of mineral sources upon mycelia growing of *Pleurotus ostreatus* (P. o) and *Lentinus edodes* (L. e)

The research experiments were achieved by growing all these fungal species in special rooms, where the physical, chemical and biological parameters of mushroom cultures (temperature, inoculum age and size, pH level) were kept at optimal levels to get the highest production of fruit bodies (Fig. 6, 7).

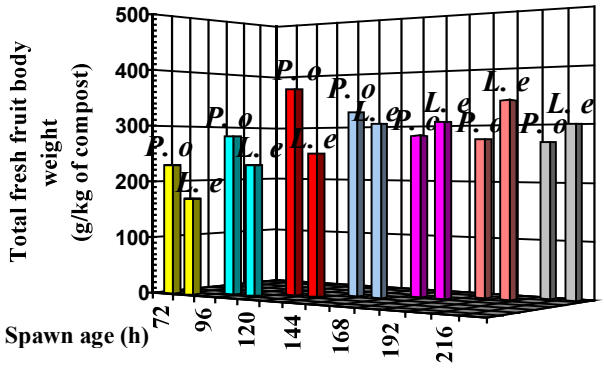

Fig. 6 - The influence of spawn age upon fruit body formation of *Pleurotus ostreatus* (P. o) and *Lentinus edodes* (L. e)

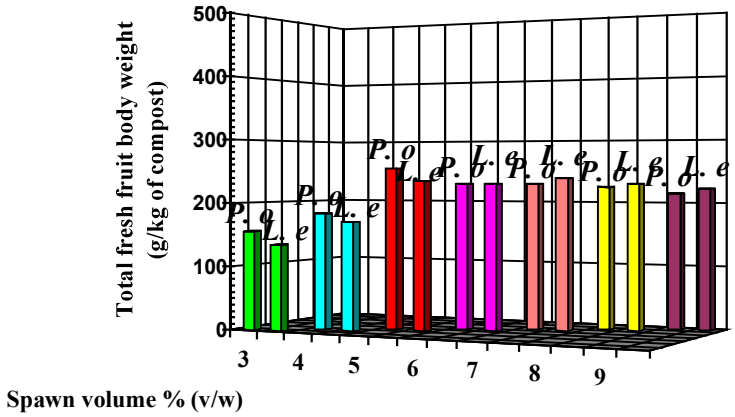

Fig. 7 - The influence of spawn volume upon fruit body formation of *Pleurotus ostreatus* (P. o) and *Lentinus edodes* (L. e)

Studying the effects of physical and chemical factors that could influence the mycelia growing as well as fruit body formation and development of *Pleurotus ostreatus* and *Lentinus edodes*, there were registered the following representative results:

- maltose, as one of tested carbon sources, had shown the highest influence upon the mycelia growing and fresh fungal biomass production about of 28 – 35g%;

- from those five nitrogen sources, wheat bran was the most efficient nutrient upon the mycelia growing and fungal biomass production of *L. edodes* and *P. ostreatus*, by 35-40 g% fresh fungal biomass weight, being closely followed by the malt extract at 25–30 g%;

- $CaCO_3$ yielded the best mycelia growing as well as fungal biomass production at 28-32 g% and for this reason it was registered as the optimal mineral source;

- the best pH levels for the mushroom fruit body production were 6.5–7.0 for both mushroom species, registered at the temperature level of 17°C;

- concerning *P. ostreatus*, the spawn age of 144 h and its volume of 5% (v/w) are the best variants and for *L. edodes* species the highest fruit body production was registered at the spawn age of 192 h and the volume of 7% (v/w).

CONCLUSIONS

Registered data revealed that by applying this biotechnology, the winery and vineyard wastes could be recycled as useful raw materials for mushroom compost preparation in order to get significant mushroom production.

The final fruit body production of the cultivation of these two mushroom species was registered between 1.5 – 2.8 kg relative to 10 kg of composts made of vineyard and winery wastes.

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