Scientific Bulletin. Series F. Biotechnologies, Vol. XX, 2016
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

# CULTIVATION OF FLORIDA OYSTER MUSHROOM ON VARIOUS TYPES OF SUBSTRATE

**Denisa STĂNESCU, Emanuel VAMANU**

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: email@emanuelvamanu.ro

***Abstract***

*Valorisation of agricultural wastes is one of the main objectives for activity optimization in agriculture industry. One method for waste utilization implies their use as substrate for obtaining edible mushrooms, which are a raw material of interest in current food industry. The aim of the paper was the cultivation of Pleurotus ostreatus var. florida species, on diverse common plant wastes. The species had good fructification, resulting numerous pins on the surface of the substrate, the cap diameter reaching a maximum of 4 to 5 cm. The color of the basidium was brighter, because the cultivation temperatures were above 20°C most of the time. The fructification time decreased with the increase of inoculation rate. Although major differences haven't been determined for morphological characteristics of the fructification body, the supplementation of the substrate formula with other components determined approximately 10% increase in productivity. The study proved that applying supplements to the substrate formula lead to the optimization of valorisation of plant wastes taken into consideration. Also, the use of supplements did not stimulate infection rate of the substrate.*

***Key words****: mycelium, Pleurotus, pins, productivity, substrate.*

## INTRODUCTION

*Pleurotus ostreatus* is a mushroom industrially cultivated for over 50 years. Due to the composition rich in biologically active substances it is commercialized in food markets. In Romania, it is often used to replace meat, because the texture, after cooking, is relatively similar (Yang et al., 2013). Usually only the cap is consumed. The stipe of the fungus is slightly difficult to digest and it is not consumed directly.

Currently, agricultural research is being carried out in order to exploit diverse plant wastes (Zervakis et al., 2013). Also, different species are tested, for the implementation in industrial mushroom farms: *P. ostreatus* var. *florida*, *P. djamor* or *P.citrinopileatus*. In Romania, partial experiments were realized so far in order to adapt *P. ostreatus* var. *florida* (*Florida Oyster Mushroom*), also known as *Hiratake* (Alananbeh et al., 2014). In our country, there are cultivated (excepting summer) common species, known as Winter Oyster Mushroom (Rahi & Malik, 2016). In both cases productivity is directly dependent on temperature (Vamanu, 2012). The purpose of the paper was the adaptation of the species *P. ostreatus* var. *florida* M 2125 for valorization of household plant wastes (straw from different types of cereals), in the farm of Denisa Stănescu student's family. There were used wheat, oats, sorghum straw, clover leaves and chopped corn stalks.

## MATERIALS AND METHODS

**Biological material**: *P. ostreatus* var. *florida* M 2125 was obtained from Mycelia BVBA, Belgium.

The mycelium was stored on wheat grains, at -20˚C in glycerol. The revitalization was achieved by cultivation on PDA medium. Wheat grains were previously sterilized at 121˚C and complete colonization was achieved in approximately 10 days at 25˚C in a LabTech thermostat (Dinu & Vamanu, 2015).

**Obtaining the substrate**: The raw material was obtained from Teleorman County, Romania. The substrate formulas (Exp. 1) were supplemented with broken rice (Exp.2) and grain mixture (Exp.3) (**Table 1**). The substrates were sterilized with hot water, 60 - 80˚C. The experiments were carried out in plastic bottles of at least 5 liter. Inoculation was 2 - 3%. Colonization of the substrate was performed at

20 - 24˚C in the dark. Fructification phase took place after 10 to 14 days, humidity 50-60%, 800-1000 lumens (Zervakis et al., 2013). Humidity was maintained by regular spraying with a water (Konan et al., 2014, Dinu & Vamanu, 2015).

**Determining productivity.** The following parameters were calculated:

Productivity = total amount of harvested mushrooms into a wave,

Biological efficiency (% ) = (amount of harvested mushrooms / substrate weight ) × 100 (Yang et al., 2013; Dinu & Vamanu, 2015).

**Table 1.** Substrate formulas

| No. | Formula |
|---|---|
| **Experiment 1** | Control: 100% wheat straw |
| | 100% clover |
| | 100% dried leaves stalks |
| | 100% dried oat |
| | 100% dried sorghum |
| **Experiment 2** | Formula Experiment 1 supplemented with 10% broken rice |
| **Experiment 3** | Formula Experiment 1 supplemented with 10% grain mixture |

**Statistical analysis.** All experiments were assessed in triplicate, and the results were expressed as mean ±SD values of the three sets of observations.

## RESULTS AND DISCUSSIONS

The minimum time of colonization was nine days for the control realized on wheat straw and sorghum (Exp. 1). For dried clover and dried oat straw the average colonization time was by five days longer. Finally, FS 2 substrate was infected (for all experiments carried out) and was not taken into further consideration for interpreting the results. FS 5 formula determined a colonization period by one third longer compared to FS 1. From our point of view, the infection of all clover samples was determined by inadequate sterilization procedure, which was not compatible with this raw material. For such a situation it is necessary to use autoclavable bags with microfilter. The introduction of these stages makes clover unsuitable for cultivation oyster species. This behavior has been observed since the presentation of partial results (Stănescu & Vamanu, 2015).

**Figure 1.** Fructification phase

Substrate colonization had a medium propagation rate of 0.5 cm/24 h. Generally, after this period, the advance of mycelium into the substrate was of minimum 0.4 cm/24h. The trend was the increase of propagation rate by approximately 20 % in 24 hours. FS 4 substrate had a medium propagation rate which was constant in 24 h (data not shown). These values were not directly proportional to the total productivity (**Table 2**).

There were obtained up to six flushes, and calculated productivity had medium values that exceeded 50 g (**Figure 1**). From **Table 2** it is observed that the first two flushes had similar productivity, regardless of the used substrate formula. Mushrooms obtained after flush no. 3 cannot be used, but it is significant that, in constant environmental conditions, tested species may use the substrate at a maximum level. The mushrooms were inadequate because of the small size. The cap was below 5 cm in diameter. This productive behavior was not observed in previous studies on *P. ostreatus* M 2175 (Dinu & Vamanu, 2015).

Maximum productivity exceeded 350 g for substrate FS 3. This was similar to the control, FS 1, but also to the M 2175 species, when using poplar sawdust (Dinu & Vamanu, 2015). Biological efficiency was 45-50% for FS 3 being a novelty in the composition of substrate for the species cultivation (**Figure 2**). Productivity in this case was approximately

15% higher compared to other used formulas. The fastest fructification (**Figure 3**) was registered for flush 1, once the substrate was fully colonized.

**Table 2.** The average productivity obtained for M 2125 species

| Substrate formula | Flush | Productivity (g) | Medium colonization time (days) |
|---|---|---|---|
| FS 1 | 1 | 313.33±34.99 | 9±3 |
| | 2 | 310.33±38.50 | |
| | 3 | 242±85 | |
| | 4 | 164±14 | |
| | 5 | 115±15 | |
| | 6 | 63±10 | |
| FS 2 | - | - | - |
| FS 3 | 1 | 373.33±50 | 14±5 |
| | 2 | 364.33±48 | |
| | 3 | 293.33±100 | |
| | 4 | 203.5±33.5 | |
| | 5 | 142.5±2.5 | |
| | 6 | 70.5±17.5 | |
| FS 4 | 1 | 307±50 | 15±4.5 |
| | 2 | 305±47.5 | |
| | 3 | 239.66±110.5 | |
| | 4 | 145±5 | |
| | 5 | 115±0.00 | |
| | 6 | 62±0.00 | |
| FS 5 | 1 | 297.66±8.32 | 12±3 |
| | 2 | 294.33±55.83 | |
| | 3 | 238.66±82 | |
| | 4 | 166±29 | |
| | 5 | 114±0.00 | |
| | 6 | - | |

**Figure 2.** Fructification phase on different substrates

It was noted that at least some of primordia appeared before this moment, which corresponded with substrate formulas FS 1 and FS 3. The transition from one flush to another generally decreases the fruitification time. Reduction of fructification time does not exceed two days. Also, from flush no. two, relatively constant period, of nine days was observed, regardless of substrate formula.

In the case of supplements (broken rice, for example) productivity was by 8% higher, which lead to a product formula for industrial cultivation (**Figure 2**), with the following composition: wheat straw and dried leaves stalks (1:1), supplemented with broken rice (10%).

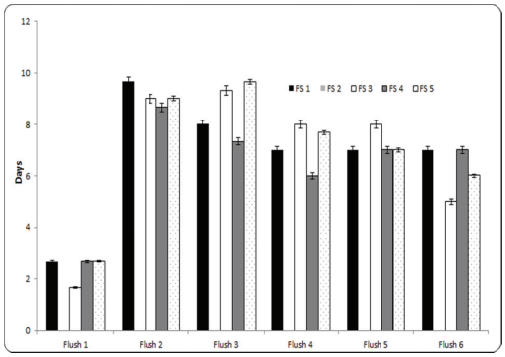


**Figure 3.** The medium fructification periods for M 2125 species

If using bags of 20 kg substrate in the first flush, an average of 1 kg of mushrooms was obtained. The first flush appeared after a period of 12 days from the substrate inoculation. In this case the first three flushes could be valorified. Primordia number significantly decreased by over 50 % after the third flush. Keeping adequate humidity was a parameter difficult to maintain in the absence of industrial cultivation system (**Figure 4**).

**Figure 4.** Bags cultivation

## CONCLUSIONS

The substrate of dried leaves stalks lead to the best productivity (30 %), being used to create a new formula for industrial cultivation. Cultivation conditions proved that the species is a competitive one for the valorization of raw vegetal materials from agricultural industry in Romania. In addition, the species may be cultivated during periods of high temperatures. The substrate was also used as inoculum for colonization of stumps during winter time (**Figure 5**).

**Figure 5.** Substrate valorisation


## ACKNOWLEDGEMENTS

This work was partially supported by the project PNCDI II UEFISCDI — Human Resources, Theme TE 61, Project 102/1.10.2015 (www.robiomush.ro). Vegetal material was provided by the student, Denisa Stănescu (Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine, Bucharest), for his university graduation thesis.


## REFERENCES


Alananbeh K.M., Bouqellah N.A., Al Kaff N.S., 2014. Cultivation of oyster mushroom *Pleurotus ostreatus* on date-palm leaves mixed with other agro-wastes in Saudi Arabia. Saudi Journal of Biological Sciences, 21:616–625.

Dinu M., Vamanu E., 2015. Growing species *Pleurotus ostreatus* M 2175 on different substrates under household. Scientific Bulletin. Series F. Biotechnologies, Vol. XIX:364 – 368.

Konan Y.K.F., Kouassi K.M., Kouakou K.L., Koffi E., Kouassi K.N., Sekou D., Kone M., Kouakou T.H., 2014. Effect of methyl jasmonate on phytoalexins biosynthesis and induced disease resistance to *Fusarium oxysporum* f. sp. vasinfectum in cotton (*Gossypium hirsutum* L.). International Journal of Agronomy, vol. 2014, Article ID 806439, 11 pages.

Rahi D.K., Malik D., 2016. Diversity of mushrooms and their metabolites of nutraceutical and therapeutic significance. Journal of Mycology, vol. 2016, Article ID 7654123, 18 pages.

Stănescu D., Vamanu E., 2015. Cultivarea speciei *Pleurotus ostreatus* var. *florida* M 2125 pe diferite formule de substrat, în regim gospodăresc. Simpozionul Național Studențesc – „Biotehnologiile - prezent și viitor", 26 iunie, București.

Vamanu E., 2012. Biological activities of the polysaccharides produced in submerged culture of two edible Pleurotus ostreatus mushrooms. Journal of Biomedicine and Biotechnology, vol. 2012, Article ID 565974, 8 pages.

Zervakis G.I., Koutrotsios G., Katsaris P., 2013. Composted versus raw olive mill waste as substrates for the production of medicinal mushrooms: An assessment of selected cultivation and quality parameters. BioMed Research International, vol. 2013, Article ID 546830, 13 pages.

Yang W.J., Guo F.L., Wan Z.J., 2013. Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. Saudi Journal of Biological Sciences, 20:333–338.