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# GROWING SPECIES *PLEUROTUS OSTREATUS* M 2175 ON DIFFERENT SUBSTRATES UNDER HOUSEHOLD

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***Abstract***

*Cultivation of winter oyster mushroom is realised in an increasing number and variety of ways, due to minimum environmental requirements, and its high productivity. The research was made on different substrates, in order to identify an efficient formula for the Pleurotus ostreatus M 2175 species cultivation under household. The sawdust, available in Nehoiu, Buzău was mainly used. Heat-sterilized substrate was placed in heat-resistant plastic bags. The inoculum was obtained on sterilized wheat seeds, at a ratio of 1 - 2%. The average temperature of colonization/fructification varied between 17 – 20$^0$ C. It was noted that all substrate formulas could support cultivation of the P. ostreatus M 2175 species, including formula partially containing softwood sawdust. The number of primordia was comparable to controls developed exclusively on beech sawdust and chopped straw. The study proved that this mushroom species has a significant adaptability to different types of substrate.*

***Key words**: mushroom, substrate, sawdust, productivity.*

## INTRODUCTION

The benefic effects of mushrooms consumption are due to significant content in protein, dietary fibers, mineral salts and various bioactive compounds (phenolic compounds, flavonoids, vitamins, carotenoids, tocopherols, essential amino acids). They have a low fat content generally up to 2.5%. Among the mushroom species chemical composition differs significantly, wild edible mushrooms having a more complex composition in bioactive compounds. For cultivated species, the biological value of fruit bodies depends on the substrate formula (Tudor, 2007).

In Romania, Oyster mushroom has a consumption similar to *Agaricus bisporus* species because it is considered to have a more pleasant aroma and the biological value is higher (Vamanu et al., 2012). *P. ostreatus* cultivation is facilitated by the isolation of species adapted to atmospheric conditions, thereby growing during the year without requiring a particular technological effort. Normal substrate (straw) is a much cheaper and readily available, fact that eliminates imports. It can be obtained without investment in equipment or special processing conditions in any individual household. Spawn necessary for substrate inoculation is also cheap (Kwon and Kim, 2004).

Since sawdust is the most valuable substrate for the cultivation of the species, the aim of the paper was the identification of original formulas to valorize woody biomass in Nehoiu area, Buzau county, Romania to support the obtaining of high productivity of Winter Oyster Mushroom (*P. ostreatus* M 2175), under household. Another objective was to obtain a fresh production for personal use.

## MATERIALS AND METHODS

*Culture preparation.* The species *P. ostreatus* M 2175 was obtained from Mycelia BVBA, Belgium.

The mycelium was stored on wheat grains, in glycerol, at -20$^0$ C. Revitalizing was realised by the cultivation on PDA medium (http://en.wikipedia.org). Other Petri dishes with PDA medium for spawn preparation (sterilized wheat grains) were inoculated with this culture. The wheat grains were sterilized at 121$^0$ C, for two hours, in a glass jar with air filter. In both cases, the propagation of mycelium was realised for 7-10 days, at 23$^0$ C, in the dark in a Labtech laboratory incubator (Moonmoon et al., 2010; Pathmashini et al., 2008).

**Table 1.** Substrate formulas

| No. Crt. | Substrate formula |
|---|---|
| M1 | Straw 1000 mL, 25 mL gypsum. |
| M2 | Cobs 1000 mL, 25 mL gypsum. |
| S1 | Beech 1000 mL, cobs 1000 mL, 50 mL gypsum |
| S2 | Poplar 1000 mL, cobs 1000 mL, 50 mL gypsum |
| S3 | Poplar 1000 mL, cobs 1000 mL, husked rice 500 mL, 50 mL gypsum |
| S4 | Poplar and resinous mixture (1:1), cobs 1000 mL, 50 mL gypsum |
| S5 | Poplar and resinous mixture (1:1), cobs 1000 mL, husked rice 500 mL, 50 mL gypsum |
| S6 | Acacia 1000 ml, cobs 1000 mL, 50 mL gypsum |
| S7 | Spruce 1000 mL, cobs 1000 mL, 50 mL gypsum |

*Substrate preparation.* Vegetal biomass (sawdust) came from individuals who process timber in Nehoiu, Buzau county, Romania. To obtain the substrates there was used sawdust of the following species: beech, aspen, mixed conifer, poplar, acacia, spruce (**Table 1).** As control, straw and chopped stalks (with a shredders OK, bauMax Romania) were used.
Incubation during the colonization of the substrate was at 20 – $24^0$ C, in the dark, while the fruiting period was maintained constant at $17^0$ C, humidity of 50 - 60%, light 800 lumen. The moisture was maintained by means of a sprayer, up to 2-3 watering / day. Natural ventilation was used. The flushes successive were at an interval of 7-10 days, depending on the substrate. The required environmental conditions were made in a sterile enclosure inside a house in Nehoiu, Buzau County, Romania **(Figure 1)**.
*Determining productivity*. The productivity of the species during the 3 or 4 flushes was calculated using the following formula: Productivity (%) = (amount of fresh mushrooms / weight of fresh substrate) × 100. Biological efficiency was also, determined by the following formula: BE (%) = (amount of fresh mushrooms / weight of dry substrate) × 100 (de Andrade et al., 2007).

**Figure 1.** The fructification enclosure under domestic condition

*Determination of total protein content of mushrooms*. The Lowry method was used, and the results were expressed as mg protein / g fresh mushroom (Vamanu et al., 2010).
*Statistical analysis*. The results are the mean of at least three separate experiments.

## RESULTS AND DISCUSSIONS

The most rapid colonization was observed for the control, exclusively containing straw, while the two substrates from coniferous species showed a higher period for colonization of approximately 40%. For the straw mycelium run was of 1.5 - 2 cm /day, respectively maximum 0.5 cm /day for substrate formulas containing coniferous species.
In the first flash, the productivity of the two controls had significant differences, over 50% (**Table 2**). Chopped stalks led to a reduction in productivity, and in biological efficiency on average of about two thirds. Using beech and poplar sawdust determined an average increase of about 50% of productivity compared to M2 and 25% lower than that achieved in the case of wheat straw (M1 – **Figure 2**). The biological effectiveness was approximately 10% lower than that obtained in the case of M1. The biological effectiveness was proved superior in the case of using poplar sawdust to the beech. Substrate supplementation with rice caused an increase of biological efficiency and productivity on average 10%. Thus, the average

value of the substrate S3 productivity was of 30.00 ± 1.00% and 52.00 ± 2.00% of biological efficiency (**Figure 3** and **Figure 4**).

**Figure 2.** Fruiting substrate M1

**Figure 3.** Fruiting substrate S2

**Figure 4.** Fruiting substrate S3

**Figure 5.** Fruiting substrate S6

**Figure 6.** Fruiting substrate S7

It was noticed that the exclusive use of poplar sawdust lead to four flushes, which was interpreted as the maximum utilization of the substrate, even if the average value of productivity was lower than in the presence of husked rice (substrate S3). The last flush had a low average productivity of 5.00 ± 1.00%. In general, it was noted that the presence of a single type of sawdust, without supplements, causes a slower and more efficient use, even if such behavior decreases productivity in favor of biological efficiency. The study is supported by previous research that interprets the behavior of *P. ostreatus* mycelium as one response to the content of lignin present in the substrate. Although the enzymatic mechanism is not clear yet, it seems to be dependent on the metabolic activity of the species used (Ruiz-Rodríguez et al., 2011).
A reduced time of colonization and fructification (first flush) was registered for the use as a substrate of straw and stalks, with an average of 10 days. For use in substrate of deciduous species the first flush was determined after 15 days. For husked rice supplementation an average decrease of 2 to 3

day of theperiod of the first fructification was observed. There was recorded instead, that use of spruce and acacia determined first fruiting after about 20 days, the period being divided equally between colonization and the emergence of primordial stage. If mixed with aspen, spruce sawdust use had not negatively affected the range of colonization and fructification. For the rest of the flushes (max three) the interval between harvest and appearance of the first primordia averaged 7 days. The fourth flush occurred after an interval of 10 to 15 days and was not obtained when using resin substrate formula. This last flush, at the use of poplar sawdust, was directly influenced by temperature because the increase of 2 to 3 degrees had a direct influence through the absence of the fourth fructification. The results proved that sawdust supports a final competitive production cost (**Figure 5** and **Figure 6**). Although wheat straw is the main component in Romania, the mixture of different species of hardwood and softwood causes high productivity. This substrate as compared to the straw is used directly, whereas the straw requires a further stage consisting in grinding and special equipment. Using sawdust competes with another use that got more ground in recent years, with the advent of more sophisticated heating systems, pellet manufacture. They are considered, a renewable green fuel.

**Table 2**. Effect of substrate formula on the productivity and the average value of the biological efficiency of *P. ostreatus* M 2175 species

| Substrate formula | Productivity (%) | Biological efficiency (%) |
|---|---|---|
| M1 | 32.00 ± 3.70 % | 50.35 ± 2.80 % |
| M2 | 11.50 ± 2.00 % | 17.00 ± 1.00 % |
| S1 | 24.00 ± 0.80 % | 44.70 ± 1.00 % |
| S2 | 26.50 ± 1.00 % | 46.75 ± 6.00 % |
| S3 | 30.00 ± 1.00 % | 52.00 ± 2.00 % |
| S4 | 23.00 ± 2.00 % | 39.00 ± 3.00 % |
| S5 | 20.66 ± 1.90 % | 42.96 ± 1.60 % |
| S6 | 12.50 ± 5.00 % | 30.50 ± 8.50% |
| S7 | 07.00 ± 1.00% | 18.00 ± 1.50% |

Another observation was that the maximum protein content was obtained when substrate formulas were supplemented with husked rice. Instead, the resinous substrate causes a reduction in the protein content of mushrooms, about 25.00 ± 0.30% compared to the control (wheat straw) - M1 substrate. The values do not exceed 5.00 ± 0.60 mg /g mushroom.

## CONCLUSIONS

In conclusion, the study proved the efficiency of softwood sawdust in cultivation of *P. ostreatus* M 2175 species, especially if supplemented with poplar sawdust. Husked rice increases with minimum 10% substrate utilization, expressed by the value of biological efficiency.

Poplar sawdust was found to be more effective than the beech one, which is commonly used for the production of mushrooms. This sawdust is at least as effective in obtaining Winter Oyster mushrooms in household arrangements. The biological value of this sawdust is demonstrated by the fact that the combination with resin can sustain high productivity. These findings result in a higher valorification of woody biomass species of conifers.

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