Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

44

Print ISSN: 0367-4444

Online ISSN: 2232-7266

UDC: _____

Original Scientific Article

53-58

Mycelial growth rate and yield of oyster mushroom - *Pleurotus ostreatus* fruitful part (Jacquin: Fr.) Kumm at different temperatures

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Article info
Received: 21/4/2015
Accepted: 9/6/2015

Keywords:
mycelium,
oyster mushroom,
temperature,
nutrient medium,
yield,
substrate

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Abstract: In this paper are presented possibilities for production of oyster mushroom, its mycelium and compost, as well as how different temperatures affect the mycelial growth and yield of these mushrooms. Mycelium was cultivated on the PDA media, which was later transferred onto the sterilized wheat grains and at the end in the compost made from beech sawdust and hay. The ratio of sawdust and hay was 1:1. Compost can be prepared from 50% wheat straw + 50% soybean straw, 50% wheat straw + 50% corn stems, 50% wheat straw and 50% sunflower stems (Bugarski et al, 2005).

The mycelium which was growing at an average temperature of 20 °C after 15 days was ready for the next grafting, while the mycelium, which was kept at 4 °C for the same period, had very slow growth rate. Mycelium was then inoculated onto sterile grain of wheat, which at a temperature of 20 °C grown very quickly. Two seedlings on compost were made, summer and autumn. The average air temperature during the summer seeding was 19.96 °C and in autumn 13.85 °C. It was found that the yield from the summer seeding was 17.76%, while the yield of autumn seeding was 7.2% relative to the weight of the wet substrate. Low temperatures, around 4ºC have inhibitory influence on the mycelium growth and in such conditions mycelium can be stored up to one year. The average temperature of 19.96 °C is ideal for the growth of both mycelium and mushrooms, as well as the expected yield.

INTRODUCTION

Today in controlled conditions about 30 species of fungi can be successfully cultivated. The basic preconditions for the artificial production of mushrooms are: the choice of substrate for the cultivation, selection of the mushroom type and control of growth conditions. Majority of world mushroom production is dedicated to the noble mushrooms. In second place, in terms of produced mushrooms quantity, are oyster mushrooms followed by a shiitake (Novak, 1997). To be able to cultivate specific sort of mushroom it is necessary to know its life cycle. A relatively simple method of production and high nutritional value are sufficient reasons for this type of research. During cultivation when the parameters, such as light, temperature and humidity, are controlled yields are much higher. Given the above facts as well as economic importance of the complete production of this mushroom, the following basic goals of this study are set: to master the technique of growing mycelium in vitro, to produce a substrate suitable to inoculate already produced mycelium, to monitor the growth rate of mycelium in laboratory conditions, to follow temperature during growth, record the changes and get healthy fertile mushroom body. For the production of oyster mushroom the substrate is prepared from herbal residues like: wheat, soya, rice, straw, bean, pea, cotton stems, and other waste parts of the industry such as: sugar cane, sunflower husks and stems, etc. (Bugarski et al, 2002).

Oyster mushroom, which is produced on various types of residues from the cellulose, has a high proportion of protein, vitamin C, D and B complex. Vitamin A is absent (Jonathan et al, 2012).
In nature, the oyster is widespread in many different trees, often as a parasite. It usually grows on beech, warm, walnut, willow, black locust and oak trees and rarely on conifers and maple (Focht, 1990). Oyster mushroom grows in cups, in the autumn until the first frosts, and even in winter, if it is mild; on stumps and trunks of harvested trees (Pace, 1981).

MATERIAL AND METHODS

Parent mycelium was isolated from individual oyster mushroom from the surrounding area of Konjic cityand used in this study. The mycelium was transported in sterile test tube on a PDA (potato dextrose agar) nutritive surface (Figure 1). All the containers and instruments were sterilized in an autoclave. Sterilization was performed at a temperature of 135 ºC and a pressure of 2 Atm for 60 minutes from the moment of achieving the above mentioned parameters. Nutrient media were also sterilized in an autoclave. After sterilization substrate was placed into Petri dishes and test tubes, while sterile nutrient medium was inoculated with obtained mycelium in sterile chamber (Vukojevic, 2000).

Figure 1. A parent mycelium

When an inoculum mycelium grown through the nutrient medium it is ready for further grafting, or to be shift to another carrier media. In this study, a sterile wheat grain was used as a second carrier. Wheat grains were prepared to plant mycelium on them in the manner that they were first dipped in hot water and left for 24 hours, during which time the wheat swell. This stage is important because the water from the swollen wheat is used by mycelium for its normal growth and development. After 24 hours, the wheat was thoroughly washed and sterilized in an autoclave. Sterilization was performed in duration of 30 minutes at a temperature of 114 ºC and a pressure of 1 atm. In this study, as containers for sterilization of mycelia carriers were used glass jars with volume of 0.3L and 0.2L with polypropylene lid and the jar of larger volume without polypropylene lids. The lids on jars during sterilization were not completely closed due to the equalization of pressure inside and outside of the jars during the sterilization process. Upon completion of the sterilization lids were quickly and completely closed and allowed to cool. When the temperature of the grain in the pots decreased below 30 ºC, followed the phase of mycelium transplantation from the Petri dish with substrate on wheat grain in sterile conditions.
In this study, for the preparation of compost was used hay and beech sawdust in the 1:1 ratio. Compost was cooked for two hours with the lid closed in order that inside a pot, in which it was cooked, increased pressure also increase the effect of pests' destruction. After cooking the pot remained closed to gradually cools and thus prolonging thermal treatment. When the substrate was cooled below 30 ºC the seeding started.
Seeding of compost with mycelium is the most sensitive stage in the production, because of frequent infections by competitive species of mushrooms and some types of bacteria. After the technical preparation of compost, it was seed with mycelium and packed in nylon bags with volume of 10L. Seeding is done by mixing compost with mycelium on nylon. The substrate was seeded with mycelium in ratio 5-7% by weight of the wet substrate (Smith, 1997). After seeding and packaging of compost, each bag weighed 15 kg.

RESULTS

After inoculation of the sterile substrate with a piece of mycelium its growth was followed at room temperature (20 °C) and in the refrigerator (4 °C). Average daily growth (ADG) of the mycelium at 20 °C was approximately 3 mm on all edges of the seeded surface. On the seventh day after inoculation mycelium covered surface of 18 mm, extending concentrically around the inoculum. At this rate of growth, after 15 days the mycelium was ready for the next grafting (Figure 2).
Mycelium best develop at a temperature of 25 °C and fully outgrowth substrate mycelium on the 15th day after inoculation. Growth is optimal at temperature of 25 °C, followed by growth rate at a temperature of 20 °C, while growth at 30 °C was the weakest (Bugarski et al, 1997).

In case of the mycelium which was left in the refrigerator immediately after seeding the first sign of growth was noticed seven days after inoculation in a form of off white thickening at the edge of the inoculums (Table 1).

Figure 2. The substrate covered with mycelium

As can be observed the low temperature (4 °C) has an inhibitory activity on the mycelium growth by slowing down physiological processes, which slow use of nutrient medium, so that mycelium in these conditions (4 °C) can survive for up to one year. Mycelial growth at the stage when it was placed on sterile grain was at the same rate as in the case with Petri dishes (at a temperature of 20 °C) and after 15 days the mycelium was ready for the next grafting.

Table 1. Growth of mycelium at a temperature of 4 °C and 20 °C.

Day	Temperature 4^0 C	Temperature 20^0 C
1.	---	---
2.	---	White thickening of the inoculum edge
3.	---	2 – 3 mm
4.	---	5 – 6 mm
5.	---	10 mm
6.	---	15 mm
7.	White thickening of the inoculum edge	18 mm

Having examined the influence of temperature on growth, two experimental seedlings were performed. In each experiment 10 bags were sown. First seeding was done in the summer and the other in the autumn. The average temperature during incubation and fructification in the two cycles varied considerably as shown in Figure 3.

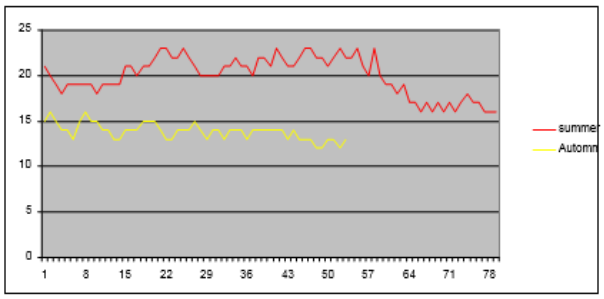

Figure 3. Temperature variations according to seasons

Temperature of the room for outgrowth (incubation) should be between 18 ^{0}C and 20 ^{0}C (Novak, 1997). The temperature of the room in which the incubation took place for the first experiment, and which lasted for 79 days, ranged from 16-23 ^{0}C (with an average temperature of 19.96 ^{0}C), which does not represent a radical deviation from the optimum temperature as reported by Novak (1997). Reported incubation duration is 15-25 days (Novak, 1997). In this experiment, incubation lasted 29 days, which also does not represent significant deviation from the above mentioned literature references.

After incubation began fruiting phase (fructification) or its first cycle (Figure 4). The first cycle usually provide about 70% of the total yield of mushrooms, in the second cycle can be expected 20 to 25% and in the third 10 to 15% of the total yield of mushrooms. The yield is calculated based on the weight of the wet substrate, ranging from 15 to 30% from weight of the wet substrate. Weight of moist substrate in this experiment was 15 kg for each of the 10kg bags. Expected yields on the above literature data, cited by Novak (1997), would amount to 2.25 to 4.50 kg per bag (15-30 % of 15 kg, which was the weight of the wet substrate).

Figure 4. Start of fructification

Weight of the mushrooms in the first cycle of the first experimental seeding can be seen in Table No. 2 for each bag separately while the average yield of the first cycle was 1.77 kg per bag.

In the second cycle the mushrooms began to produce yield after 49 days from the day of compost inoculation. The yield of mushrooms harvested in this cycle is shown in Table 2. In this cycle of harvesting mushrooms average yield per bag was 0.58 kg. The harvest was short, only two to three days, so that the third cycle of fruiting started 55 days after inoculation. The third cycle of fruiting was mixed in the sense that all the bags did not yield simultaneously. This cycle is completed 69 days after inoculation, when the harvest was performed on the last bag. The yield of the third fructification cycle was slightly smaller than the yield from others, as shown in Table 2. The average number of yield in the third harvest was 0.325 kg per bag.

Adding up all the yields from all three cycles of fruiting we obtained information that the total yield per bag was 2.665 kg, which is 17.76% of the moist compost weight. The values obtained agree with the data cited by Novak (1997), which was expected because the parameters (temperature, mycelium-compost ratio) did not significantly differ from the optimal value.

The incubation at the room temperature in the second experimental seeding, which was done in the autumn, ranged from 12 to 16 °C. The average incubation room temperature was 13.85 °C, which is significantly lower than the optimum temperature (18-20 °C). The lower temperature has the effect of extending the incubation time and reducing the strength and vitality of the mycelium (Novak, 1997). Incubation in the second experimental seeding lasted 44 days, when first primordia appeared. Only 53 days after inoculation the mushrooms were ready for harvesting. Due to the low temperatures, fruiting after the first cycle was no longer existent. The yield was much lower than the yield of the first experimental seeding (Table 3) and amounted to only 7.2% relative to the weight of the wet substrate.

The average yield per bag from another experimental seeding was 1.080 kg, which represents 40.52% of the yield obtained in the first experimental seeding.

Table 2. The weight of yield of summer seeding per cycle (kg)

bag	1	2	3	4	5	6	7	8	9	10	∑	X
1. cycle	1.75	1.45	1.90	1.65	1.90	1.50	1.85	1.70	2.20	1.75	17.65	1.77
2. cycle	0.55	0.50	0.70	0.45	0.65	0.40	0.65	0.50	0.70	0.65	5.75	0.58
3. cycle	0.35	0.30	0.40	0.30	0.35	0.25	0.45	0.30	0.35	0.20	3.25	0.33
∑	2.65	2.25	3.00	2.40	2.90	2.15	2.95	2.50	3.25	2.60	26.65	2.67

Table 3. The weight yield of autumn seeding (kg)

Bag	1	2	3	4	5	6	7	8	9	10	∑	X
Yield	1.50	1.20	0.90	0.80	1.30	1.20	1.10	1.00	0.60	1.20	10.8	1.08

CONCLUSION

When examining the effect of different temperatures on the mycelial growth we found that: low temperature (4 °C) slows down the growth of the mycelium; at a temperature of 20 °C, the mycelium grows relatively quickly and the mycelium grow was at the same rate on a sterile wheat grain as well as the culture medium, at the same temperature (20 °C), with incubation at a temperature which varied between 16-23 °C and with an average of 19.96 °C. The incubation was completed after 29 days, at a temperature which varied between 12 and 16 °C (the mean value 13.85 °C) after incubation of 44 days. Yield of the first (summer) experimental seeding was 17.76 % of the wet substrate mass. The yield of the second (autumn) experimental seeding was 7.2 % based on the weight of the wet substrate. In general, we can conclude that in the production of oyster mushrooms it is required to ensure during all phases of the growth the temperature of about 20 °C (without major deviations) in order to obtain optimum yield.

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Summary/Sažetak

Mogućnosti proizvodnje gljive bukovače, njenog micelija i komposta, te kako različite temperature utiču na rast micelija i prinos gljive, predstavljene su u ovom radu. Na PDA podlogama razmnožen je micelij, koji se kasnije prebacivao na sterilisana zrna pšenice, te na kraju u kompost od bukove piljevine i sijena. Omjer piljevine i sjena bio je 1:1. Kompost se može pripremiti i od pšenične slame 50% i sojine slame 50%, pšenične slame 50% i stabljike kukuruza 50%, pšenične slame 50% i stabljike suncokreta 50% (Bugarski i ostali, 2005).

Micelij je brzo rastao na prosječnoj temperaturu od 20 ^{o}C i nakon 15 dana je bio spreman za naredno presađivanje, dok je micelij koji je držan na 4 ^{o}C za to vrijeme rastao zanemarivo malo. Micelijem je dalje inokulisano sterino pšenično zrno, gdje je na temperaturi od 20 ^{o}C rastao veoma brzo. Na kompostu su bila dva zasijavanja, ljetno i jesensko. Prosječna temperatura zraka u toku ljetnog zasijavanja je iznosila 19,96 ^{o}C, a a kod jesenskog 13,85 ^{o}C. Ustanovljeno je da prinos u ljetnom zasijavanju je bio 17,76%, dok je prinos jesenskog zasijavanja bio 7,2% u odnosu na težinu vlažnog supstrata. Niske temperature, oko 4 ^{o}C inhibitorno utiču na rast micelija, te se na takav način micelij može čuvati i do jednu godinu. Prosječna temperatura, 19,96 ^{o}C je idealna za rast i razvoj micelija i gljive, te daje očekivane prinose plodonosnih tijela.