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STIMULATION OF GROWTH OF SPECIES OF THE FUNGUS OF THE GENUS *Pleurotus* (Fr.) P. Kumm. AT A GLUCOSE NUTRITION

I. V. Pereima
T. V. Ivanova
National University of Life and Environmental Science of Ukraine, Kyiv
E-mail: tivanova1@ukr.net

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The purpose of this study was to evaluate the optimal nutritional conditions for macromycetes micelles of the *Pleurotaceae* family. The factors influencing mycelium growth on the example of two species of *Pleurotus* were investigated.

To determine the effects of nutrient components five variants of agar medium were used, containing:potato, sweet potato, yam, oak bark and malt extractswith the addition of peptone and glucose. The pure culture of *Pleurotus* species, in the form of agarics blocks, was placed on the nutrient media. Hyphae germination was observed on the eighth day of incubation.

The temperature influence was evaluated in the range from 16 to 36 °C. The maximum growth of mycelium of both species was observed at a temperature of 28 °C.

It was determined that nutrient media containing potato, sweet potato, yam and malt extracts with the addition of glucose and peptone were the most favorable for growth of mycelium of oyster mushroom. They all had mandatory presence of a source of carbohydrates — glucose. At the same time, the media containing extracts of oak, yam, and partially of potato extract, were optimal for the growth of maple oyster. According to the experiment, the mycelial growth efficiency of the maple oyster was much higherin comparison to the oyster mushroom 1.

Key words: *Pleurotus*, mycelium, nutrient medium, temperature.

Every year in our country there are increasing man-made and recreational loads in forest plantations, which are the main places for collecting edible mushrooms.

This situation leads to a significant reduction. Due to the above reasons, the consumption of these valuable products by the population drastically decreased. The experience of many countries of the world, testifies to increase this indicator, can only be due to the cultivation of mushrooms in special and adapted buildings throughout the year. This will increase the amount of valuable high protein products and will prevent food poisoning caused by the consumption of wild mushrooms.

The development of modern mushroom growing in Ukraine has revealed the need to create new optimal conditions for growth and nutrition of mycelium that would meet modern requirements: qualitative improvement of mycelium growth, increase in its volume, high productivity and commodity quality [1,2].

Materials and Methods

We conducted laboratory studies during 2014—2017 at the Department of Ecobiotechnology at the Laboratory of Industrial Biotechnology. We used strains of *Pleurotus ostreatus* NK-35 *(PO)* and *Pleurotus cystidiosus (PC)*, which we received from the

Table 1. **Passport strains of oyster mushroom**

Strain	Country of origin, year	Fruiting temperature, °C	Color of the fetal body of the mushrooms
Pleurotus ostreatus НК-35	Hungary, 1984	5–22 (opt 14–16)	Grey
*Pleurotus cystidiosus*1726	Israel, 2000	5–22 (opt 15–17)	Cream-white

Fig. 1. **Fruit bodies of oyster mushroom, obtained by artificial growing conditions**

collection of the Mushroom Scientific and Production Enterprise "RKS" (Kyiv).

The characteristic features of *Pleurotus ostreatus* are the presence of small darker strips extending from the center to the edge of the cap, high yields and a relatively small number of spores [1–2]. The mushroom is indescribable to light. It reacts well to the substrate, prepared by pasteurization. In keeping with the technology, yields will be at least 22%. The collected mushrooms are well kept at +2 °C, perfectly withstand transportation and are suitable for any processing [2].

Pleurotus cystidiosus is characterized by small cream-white chops, coinciding with the center of the lamellae and a short central or centrifugal leg.

Ideally, this species is grown on straw and some agricultural wastes with a low content of lignin.

The getting a clean culture. Mycelium disks (1 cm in diameter) of each mushroom with a sterile microbiological loop were placed in Petri dishes with a nutrient medium under aseptic conditions. Then they were incubated at 28 °C in the dark. The diameter of mycelial growth was measured every 2 days for 8 days [3–4].

Nutrient media

We used five nutrient media including potato glucose agar, sweet potato + agar, yams + agar, oak + agar and malted extract + agar + peptone [5].

For the preparation of potato glucose agar in 1 l of distilled water was added a decoction of 200 g of potatoes, 20 g of glucose and 15 g of agar, if necessary, adjusted to pH 5.0–6.0. The resulting medium was brought to a boil under constant stirring on a magnetic stirrer. The hot medium was filtered through a glass of filter was added and flocculated for coking.

In order to prepare the medium of sweet potato + agar, we added 1 l of distilled water to a decoction of 200 g of sweet potatoes, 20 g of glucose and 15 g of agar, adjusted to pH 5.0–6.0, if necessary. The resulting medium was brought to a boil under constant stirring on a magnetic stirrer.

The hot medium was filtered through a cotton gauze filter and rinsed in flacons for sterilization. Preparation of the medium Yams + agar was similar to the previous cooking. The difference was only in the equivalent constituent nutrient media. The preparation of medium malt extract + agar. For 1 l of distilled water, 20 g of malt extract, 20 g of glucose and 15 g of agar and 15 g of peptone were added. The resulting medium was constantly stirred to boil. Having filtered it, it was poured into sterilization bottles.

The media was left in an autoclave at 121 °C for 40 minutes. Then the hot nutrient medium was poured into sterile Petri dishes for 20 ml each, and they were waiting for a full thickening.

Results and Discussion

The growth of mycelium of two types of common plain, including *Pleurotus ostreatus* and *Pleurotus cystidiosus*, was exposed to various temperature conditions and nutrient media.

Influence of different nutrient media on growth of mycelium

The data show (Table 2, 3) that the mycelium of oyster mushrooms was significantly different in four different media ($P < 0{,}05$), and the diameter of the colonies of the mycelium of oyster mushroom maple did not differ significantly. The density of the mycelium of both species was compact in all media. Their average diameter of the mycelium varied from 3.92 to 9.00 cm for 8 days after inoculation. Growth of mycelium of oyster mushrooms on potato and yams agar was better than on sweet potato and mildew media for 2, 4, 6 days after inoculation. However, it did not differ substantially from the eighth day after inoculation. The result showed that these five nutrients are the best for maple oyster, while potato and yam agar are more suitable for the growth of mycelium oyster mushrooms. The medium with an extract of oak bark showed indirect results for 8 days after inoculation in both species.

Table 2. **Influence of components of nutrient medium for the growth of mycelium oyster maple**

Nutrient media with extract	Diameter of the colony, sm							
	Days after inoculation							
	2		4		6		8	
	Strains of oyster mushroom							
	I**	II***	I	II	I	II	I	II
Control	1.96	1.36	4.80	1.92	8.20	3.02	9.00	3.92
Batat	1.64*	1.40	4.52*	2.36*	7.54*	3.10*	9.00	3.96*
Malt	1.80*	1.46*	3.96*	2.34*	7.12*	3.06	9.00	3.94
Yams	2.12*	1.44*	4.98*	2.24*	8.46*	3.24*	9.00	3.98*
Oak bark	2.01*	1.38	4.39*	2.10	8.38	3.13*	9.00	3.94

Note: * — $P \leq 0.05$ in comparison with control (potato agar); I** — *Pleurotus ostreatus*, II*** — *Pleurotus cystidiosus.*

Table 3. **Influence of temperature on growth of mycelium Pleurotus grown on potato agar nutrient media**

Temperature, °C	Diameter of the colony, sm							
	Days after inoculation							
	2		4		6		8	
	*I***	*II****	*I*	*II*	*I*	*II*	*I*	*II*
16	1.12*	1.00	1.70*	1.16*	2.64*	1.32*	4.00*	1.66*
20	1.24	1.00	2.56*	1.28*	4.08*	1.78*	6.76*	2.38*
24 (control)	1.40	1.02	4.10	1.88	6.60	2.68	9.00	3.46
28	2.12*	1.48*	4.80*	2.34*	8.08*	3.34*	9.00	4.58*
32	1.66*	1.20*	4.24	2.24*	7.04*	2.98*	9.00	4.08*
36	1.68*	1.00	2.70*	1.00*	2.96*	1.02*	3.12*	1.26*

Note: * — $P \leq 0.05$ in comparison with control (temperature of growth of mycelium +24 °C); I** — *Pleurotus ostreatus*, II*** — *Pleurotus cystidiosus.*

The results of this study also showed that mycelial growth of *Pleurotus ostreatus* was superior to Pleurous cystidiosus in all media. These results were similar to the results presented by scientists [5–6]. They noted that potato and yams-glucose agar are favorable environments for the better accumulation of biomass mycelium. In Ukraine, the potatoes are much cheaper than yam yam because potato agar is effective for use as an alternative to the culture of two kinds of mushrooms.

Effect of temperature on mycelium growth

The trend of mycelial growth, depending on temperature, was very similar between the two species. The optimum temperature for both types of fungi was determined (Table 4). Growth of the mycelium of mushrooms of the common clay was much more intense than that of coarse maple in each temperature regime.

Pleurotus cystidiosus did not seem to grow after 8 days of inoculation at 36 °C . The density of the mycelium of the PC fungus was very low at 16 °C and 36 °C. To our experiments, researchers [5] determined that the optimum temperature for the growth of mycelium glial royal was 25 ~ 30 °C.

This temperature result showed that both types of gloves were able to grow better in the summer and in the autumn season. The results are very similar to those presented by scientists [6].

The influence of carbon sources and sucrose concentration on mycelium growth

The carbohydrates, which play a key role as structural and storage compounds in the cell, are isolated as mono-, di- and polysaccharides. The mycelium of many fungi will grow somewhat better to a certain extent with a wide range of carbon sources. Different types of carbon sources (2% concentration) including

glucose, dextrose, fructose, maltose, sucrose, and molasses were added to the potato glucose agar to find the optimum level of carbon. The result presented in Table 4 shows the suitability of various carbon sources for the growth of mycelium of gladiolus.

The result has shown that there is a significant difference in the growth of mycelium of ordinary clay and gluvent maple, grown on different sources of carbon. Among all the sources of carbon — glucose, sucrose and molasses, glucose, sucrose and molasses were favorably favorable for mycelium growth (diameter and density of mycelium), whereas glucose, sucrose and molasses also showed high growth rates of mycelium of maple shale (Table 4). The largest diameters of the mycelium of the colony of the eccentric gloves were obtained from 6 days after inoculation, containing glucose (8.60 cm), sucrose (8.54 cm), and molasses (8.34 cm), while the largest diameters of colon micelles Maple layers were determined on a medium containing glucose (3.50 cm), sucrose (3.54 cm), and dextrose (3.44 cm). Conversely, the slowest growth was the mycelium of both species (observed 6 days after inoculation containing lactose as a carbon source.

The scientists report that sucrose was the best source of carbon for growth of mycelium *Villosiclava virens*. Glucose is the best source of carbon for growth of mycelium *Pleurotus florida* [6]. It serves as the optimum source of carbon for the production of cordycepin from traditional Chinese medicinal mushrooms, *Cordyceps militaris*. In addition, glucose was also identified as the best source of carbon for the production of edible fungal exopolysaccharides, such as *Phellinus*. Data on the fact that the environment with glucose, sucrose, molybdenum and glial maple glue glucose, sucrose, dextrose is the optimum medium for glucose, due to the ease of metabolizing processes for the production of cellular energy that promotes the growth of the body. The ability of the species *Pleurotus* to use different sources of carbon may be expressed by physiological differences between species or strains, since different strains of the same species can produce different results.

The among the sources of carbon (glucose, dextrose, sucrose, molasses, etc.) for the two strains, sucrose and molasses are the cheapest sources. Sucrose gave a considerably high index of the diameter of the colonies of mycelium for both species, while the molasses gave a significantly higher mycelium growth for oyster mushrooms. Sucrose also gave a compact density of mycelium in both species. It was chosen as a source of carbon in the next experiment.

The among the concentrations of sucrose 1~5% (10~50 g/l), there was no significant difference in the diameter of the mycelium of colonies of oyster mushrooms. However, for 6 days after inoculation, with a concentration of sucrose from 1% to 5%, a high ratio of mycelium colony (8.28~8.62 cm) was obtained and significantly higher than that of maple oyster on the control medium (potato agar) and other concentrations of sucrose. The high colonization rate of mycelial *Pleurotus cystidiosus* (3.22~3.44 cm) was also achieved at a sucrose concentration of 1 ~ 3%. However, high concentrations of sucrose (more than 3% for *Pleurotus cystidiosus* and more than 5% for

Table 4. **Influence of carbon sources on the growth of mycelium of oyster**

Diameter of the colony, cm						
Days after inoculation						
Sources of carbon	**2**		**4**		**6**	
	*I***	*II****	*I*	*II*	*I*	*II*
Control (potato agar)	1.66	1.10	4.54	1.68	6.88	2.50
Glucose	1.94*	1.24*	5.26*	1.96*	8.60*	3.50*
Lactose	1.44*	1.18*	1.94*	1.72	3.36*	2.50
Dextrose	1.70*	1.18*	4.52	1.98*	7.96*	3.44*
Fructose	1.52	1.12	4.76*	1.48*	7.98*	2.52
Saccharose	1.82*	1.20*	4.86*	2.24*	8.54*	3.54*
Molasses	1.78*	1.22*	4.62*	1.80*	8.34*	3.06*

Pleurotus ostreatus) failed to increase the diameter of mycelial colonies. This is due to the fact that the osmotic pressure caused by high concentration of sucrose can be harmful to biosynthesis of metabolites.

On the other hand, too high a concentration of carbon also gives a high ratio of carbon/nitrogen, which will suppress the growth of mycelium.

Influence of sources and concentration of nitrogen on growth of mycelium

Nitrogen is a necessary element important for all mushrooms to synthesize nitrogen-containing compounds such as purines, pyrimidines, proteins, and the cell wall component, chitin [6]. The chemical composition of the nutrient medium, especially the nitrogen concentration, is a means of physiological control and regulation of the metabolism of microorganisms. In this study, inorganic nitrogen sources were used to evaluate their effect on the growth of mycelium of PO and PC mildew.

We noticed a significant difference in the growth of mycelium in both species. At 6 days after inoculation, significantly enlarged mycelium diameters of the colonies *Pleurotus ostreatus* and *Pleurotus cystidiosus* (8.84 cm and 3.70 cm respectively) were obtained in media containing ammonium chloride (NH_4Cl) as a source of nitrogen. Ammonium sulfate ($N_2H_8SO_4$) is the second best nitrogen source for both types of mycelium growth, but the diameter of the mycelial colonies of *Pleurotus ostreatus* did not differ significantly from that of the control environment. At 6 day after inoculation, we observed significantly lower micelles diameter *Pleurotus ostreatus* and *Pleurotus cystidiosus* (5.06 cm and 3.16 cm respectively), which were obtained in a medium with urea addition (Table 6, Fig. 4). It was an increase in the density of mycelium in all procedures for the addition of various sources of nitrogen (except for urea). However, the diameters of colonies of mycelium of both mushrooms cultivated in environments containing some sources of nitrogen, such as malt extract, soy, yeast extract, have not improved. The density of mycelium in a medium containing organic nitrogen was more compact than in an environment using inorganic nitrogen, with the exception of the medium containing ammonium chloride. Ammonium chloride supports the growth of mycelium *P. ostreatus* better than sodium nitrate and calcium nitrate, because nitrate ions contained inhibitors of some basidiomyces, which can be difficult to transport through a mushroom that promotes growth.

The ammonium chloride and ammonium sulfate are the most suitable sources of nitrogen for the growth of mycelium Villosiclava virens and in comparison with sources of nitrate nitrogen, sources of ammonium nitrogen were favorable for the growth of mycelium [6].

Based on the above results, ammonium chloride was selected as the appropriate nitrogen source for further studies. In Table 6 summarizes the initial effects of ammonium chloride concentrations on the growth of mycelium oyster.

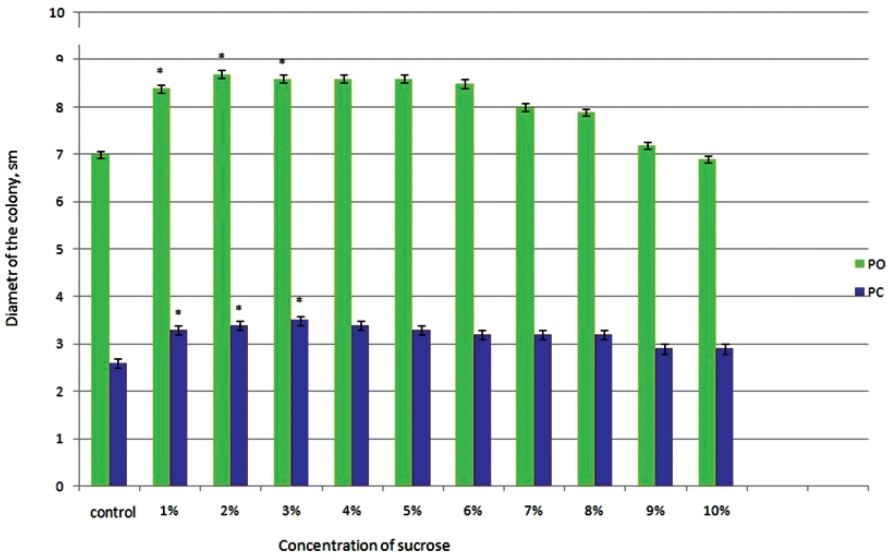

Fig. 2. **Growth of mycelium *Pleurotus ostreatus*(PO) and *Pleurotus cystidiosus* (PC) on potato agar depending on sucrose concentration**

Table 5. **Influence of nitrogen sources on mycelium growth in potato-glucose media**

Diameter of the colony, cm						
Days after inoculation						
Nitrogen sources	2		4		6	
	I**	II***	I	II	I	II
Control(potato agar)	1.60	1.24	4.68	2.36	8.40	3.36
Ammonium chloride NH_4Cl	2.12*	1.50*	5.04*	2.52*	8.84*	3.70*
Ammonium sulfate $N_2H_8SO_4$	2.08*	1.50*	4.88*	2.40	8.52	3.56*
Peptone	1.64	1.22	4.40*	2.28*	7.02*	3.50
Urea	1.90*	1.32	4.58	2.10*	8.32	3.16*

The data presented in Table 5 show that the growth of the mycelium of ordinary clay increases with an increase in the concentration of ammonium chloride from 0.01% to 0.09% in potato-glucose agar media. The growth of mycelium of maple maple glue is also increased with a concentration of ammonium chloride from 0.01% to 0.05%.

High rates of colonization of mycelium colony *Pleurotus ostreatus* are achieved at a concentration of ammonium chloride from 0.03% to 0.09%, while the parameters of the colony of mycelium *Pleurotus cystidiosus* reached the highest value with a concentration of ammonium chloride from 0.03% to 0.05%. The growth of mycelium *Pleurotus ostreatus* and *Pleurotus cystidiosus* is reduced at a concentration of ammonium chloride greater than 0.09% and 0.05%, respectively (Fig. 3, 4, 5).

This may be due to the high concentration of nitrogen source, which leads to a too low carbon/nitrogen ratio, which suppresses the growth of mycelium.

The growth of mycelium of two types of common plain, including *Pleurotus ostreatus* and Pleurotus cystidiosus, was exposed to various temperature conditions and nutrient media.

Also after analyzing the growth of mycelium on different nutrient media, it can be said that the agar medium with potato, sweet potato, yam and malt extracts was the most favorable for the growth of mycelium of the fungus *Pleurotus cystidiosus* whereas potato and yams-glucose agar were the best for *Pleurotus ostreatus.*

After studying the growth of mycelium in different temperature regimes, we can state

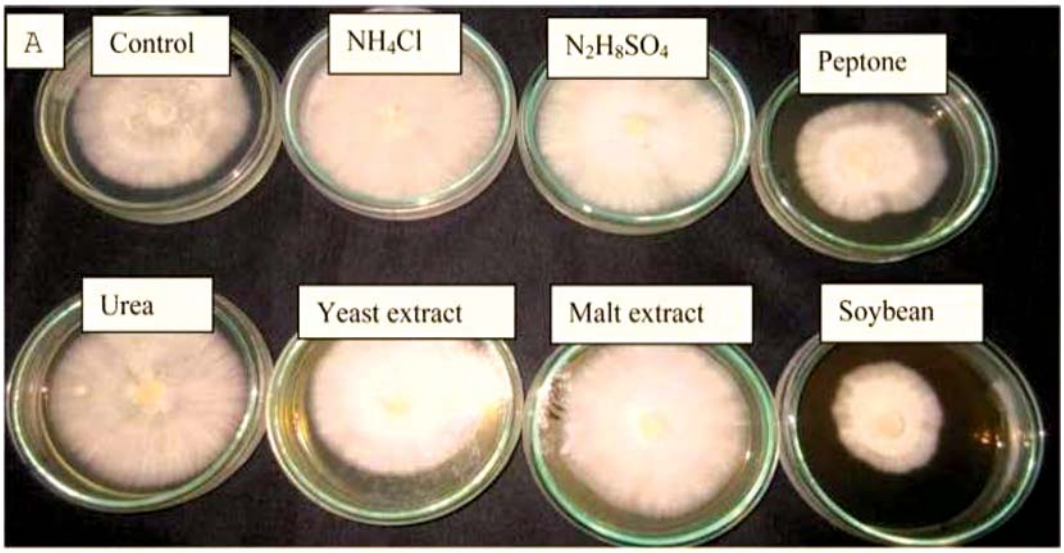

Fig. 3. **Growth of mycelium *Pleurotus ostreatus* grown on different sources of nitrogen for 6 days after inoculation at 28 °C**

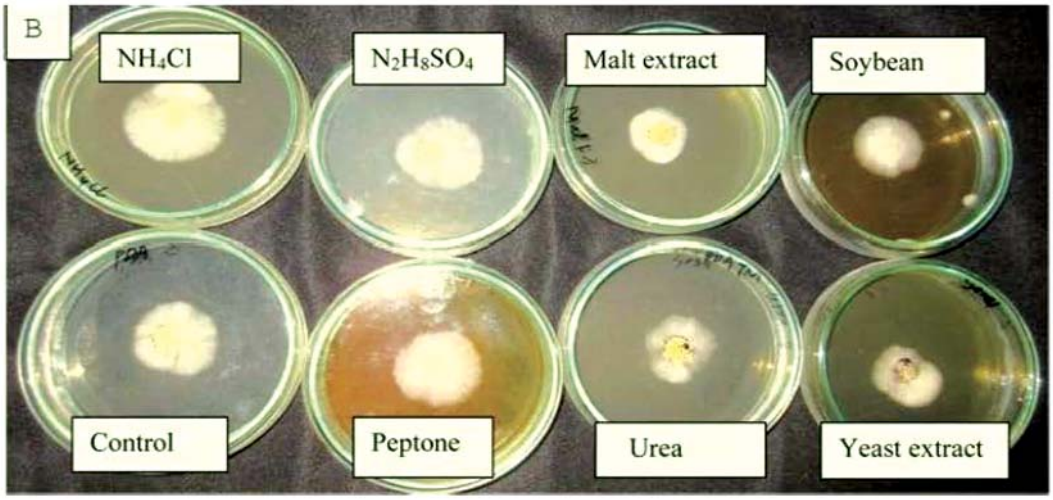

Fig. 4. **Growth of mycelium *Pleurotus cystidiosus* grown on different sources of nitrogen for 6 days after inoculation at 28 °C**

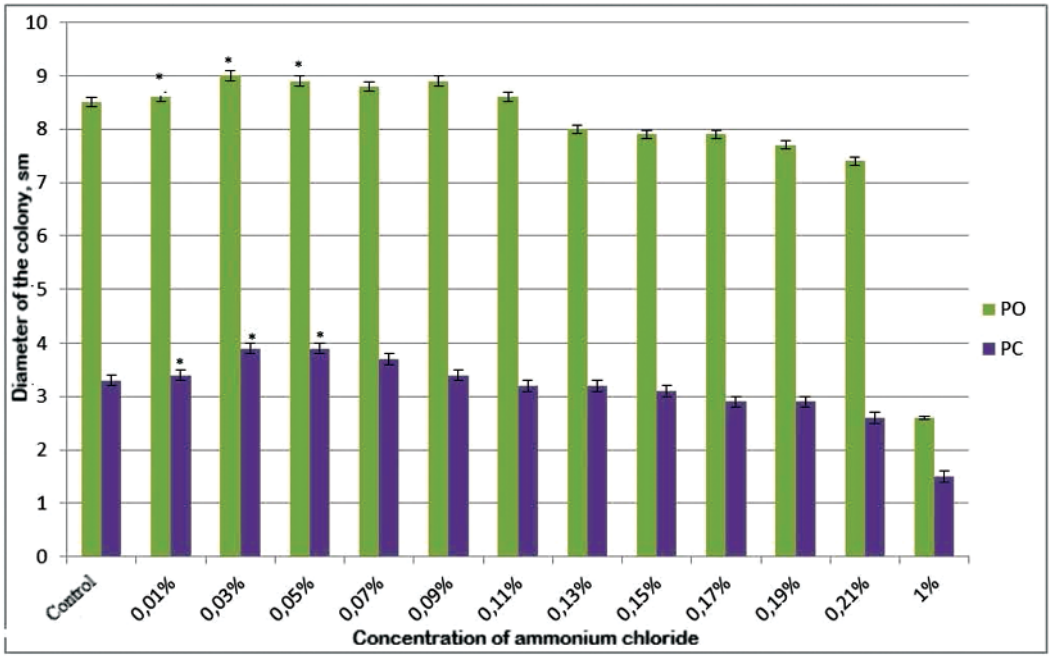

Fig. 5. **Influence of concentration of ammonium chloride in PDA medium on growth of mycelium *Pleurotus ostreatus* (PO) and *Pleurotus cystidiosus*, grown within 6 days after inoculation at 28 °C**

that the tendency of mycelium growth to vary according to temperature was very similar between the two species of glue. The optimum temperature for both types of fungi, from 28 °C to 32 °C and 24 °C.

In determining the optimal composition of nutrient media relative to the source and concentration of carbon *Pleurotus ostreatus* in potato-glucose agar with 1~5% sucrose concentration, while the concentration of 1~3% sucrose gave a good growth of mycelium Pleurotus cystidiosus. The experiment showed that the concentrations of ammonium chloride at 0.03~0.09% and 0.03% 0.05~ give the highest values of the diameter of the mycelium *Pleurotus ostreatus* and *Pleurotus cystidiosus*, respectively.

In determining the optimal temperature regime for the growth of mycelium, we fixed that the maximum growth of mycelium for both species was achieved due to their cultivation at +28 °C.

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СТИМУЛЯЦІЯ РОСТУ ВИДІВ ГРИБА РОДУ *Pleurotus* (Fr.) P. Kumm. ЗА ГЛЮКОЗНОГО ЖИВЛЕННЯ

Перейма І.В.
Іванова Т.В.

Національний університет біоресурсів і природокористування України, Київ

E-mail: tivanova1@ukr.net

Метою цього дослідження є оцінювання оптимальних умов живлення міцелію макроміцетів родини *Pleurotaceae*. Було досліджено фактори, що впливають на ріст міцелію на прикладі двох видів гливи.

Для визначення дії компонентів живлення використовували п'ять варіантів агаризованих середовищ, які містили екстракти картоплі, батату, ямсу, кори дуба та солоду з додаванням пептону і глюкози. Чисту культуру видів *Pleurotus*, у вигляді агаризованих блоків, поміщали на досліджувані живильні середовища. Проростання гіф спостерігали на восьму добу інкубації.

Оцінювання впливу температури проводили у діапазоні від 16 до 36 °С. Максимальний ріст міцелію обох видів спостерігали за температури 28 °С.

Виявлено, що середовища з екстрактами картоплі, батату, ямсу та солоду з глюкозою і пептоном були найсприятливішими для росту міцелію гливи кленової. Усіх їх об'єднує обов'язкова присутність глюкози як джерела вуглеводів. Водночас середовища з екстрактами кори дуба, ямсу і частково картоплі були оптимальними для росту гливи звичайної.

За даними експерименту ріст міцелію гливи звичайної був значно кращим порівняно з гливою кленовою.

Ключові слова: *Pleurotaceae*, міцелій, живильне середовище.

СТИМУЛЯЦИЯ РОСТА ВИДОВ ГРИБА РОДА *Pleurotus* (Fr.) P. Kumm. ПРИ ГЛЮКОЗНОМ ПИТАНИИ

Перейма И.В.
Иванова Т.В.

Национальный университет биоресурсов и природопользования Украины, Киев

E-mail: tivanova1@ukr.net

Целью данного исследования является оценка оптимальных условий питания мицелия макромицетов семейства *Pleurotaceae*. Были исследованы факторы, влияющие на рост мицелия на примере двух видов вешенки.

Для определения действия компонентов питания использовали пять вариантов агаризованных сред, содержащих экстракты картофеля, батата, ямса, коры дуба и солода с добавлением пептона и глюкозы. Чистую культуру видов *Pleurotus*, в виде агаризованных блоков, помещали на исследуемые питательные среды. Прорастание гиф наблюдали на восьмые сутки инкубации.

Оценивание влияния температуры проводили в диапазоне от 16 до 36 °С. Максимальный рост мицелия обоих видов наблюдался при температуре 28 °С.

Выявлено, что среды с экстрактами картофеля, батата, ямса и солода с глюкозой и пептоном были наиболее благоприятными для роста мицелия вешенки кленовой. Всех их объединяет обязательное присутствие глюкозы в качестве источника углеводов. В то же время среды с экстрактами коры дуба, ямса и частично картофеля были оптимальными для роста вешенки обыкновенной.

По данным эксперимента рост мицелия вешенки обыкновенной был значительно лучшим по сравнению с вешенкой кленовой.

Ключевые слова: *Pleurotaceae*, мицелий, питательная среда.