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EFFECTS OF MEMBRANOTROPIC MICROFERTILIZERS TO GROW THE MYCELIUM OF *LENTINULA EDODES*

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ABSTRACT

Microfertilizers is an important factor for good production of the mycelium of Lentinula edodes mushrooms. The commercial cultivation of mushrooms depends on the correct adjustment of the components of the nutrient media. Three formulas of nutrient media, including decoctions of oak, oatmeal and potatoes. Most mushrooms grow and function well at a pH close to neutral or light basic. Method of light microscopy. Biotechnological (obtaining and subcultuvating of strain 3667 in vitro), microbiological (obtaining pure mushroom culture, studying the cultural properties of the colonies, determining the hydrogen index (pH) of the nutrient medium), mycological (measurement of growth, growth density and dry weight of mycelium, determination of mycelial radial growth, analysis for the presence of buckles and hyphae), light microscopy and statistical methods were used. Performed experiments showed that acceleration of mycelial growth and the greatest yield of mycelium L. edodes were observed on a nutrient medium that contained microfertilizer «Avatar-1». The performed experiments showed that the acceleration of mycelial growth and the greatest yield of mass of mycelium L. edodes were observed on nutrient media with microfertilizer «Avatar-1». During the experiment, it was found that the maximum overgrowth of the medium by mycelium occurs at 7 days. It has been proved that in the «Avatar-1» environments, there was an increase and consolidation of bifurcated hyphae and buckles. The dependence of growth rate on the type of nutrient medium, the administration of doses of the drug, which effectively influences and reduces the technology of obtaining primary mycelium L. edodes, is demonstrated.

Keywords: mycelium, mushroom, nanopreparation, microfertilizer

INTRODUCTION

In recent years, artificial mushroom cultivation has become popular in many countries around the world. Undisputed leaders of mushroom production in the world are China, USA, Holland, France and Poland. The reasons for such success are primarily due to a correct investment policy, a broad raw material base, and a constant revision of mushroom production technologies. In Ukraine, in production terms, in addition to cultivating common and oyster mushrooms, growing of the lesser-known delicacy basidial mushrooms – shiitake is being gradually implemented. It is due to the confirmed therapeutic and prophylactic properties of the macromycetes. Nowadays, there is a question of using fungi as a source of biologically active substances. Functional mushroom-based preparations are being produced on a large scale around the world. These days, medicine pays high attention to fungal therapy, the main reason is to find a cure for complex diseases – cancer, HIV and AIDS. Shiitake mushroom is famous for such properties. The composition of the fungus includes polysaccharide – lentinan, which inhibits development of cancer cells and is characterized by antiviral properties, as well as lentinin – a protein that provides an inhibitory effect on development of leukemia. It is well – known, that shiitake in dried form contains compounds blocking carcinogens formation (**Rashydov, N., Kliuchnikov, O., Gorovyy, L., et al. 2012, Kraft, D., 2017)**.

Medicinal properties are confirmed by a long-standing practice of using shiitake mushrooms in fungal therapy, as well as by the latest clinical trials conducted in South-East Asia, Europe and the USA. China, Japan, and the United States are leaders in shiitake consumption. Shiitake mushroom combines high nutritional properties, as well as synthesizes a wide range of proteins, lipids, vitamins and other physiologically active compounds, nutritional value is representing **(Kraft, D., 2017, Peter Amwoga Ayeka, 2018).**

Protein additives are used today to increase yields and improve the quality of spawning mycelium. However, mushroom organism requires special care not only in terms of high concentrations of proteins and carbohydrates in the composition of the nutrient medium, but also in terms of nutritional elements, particularly of trace elements. Trace elements themselves do not participate in protein molecules formation, but stimulate the enzymatic reactions for their synthesis, i.e. significantly accelerate the production. Over past five years, the preparation was studied on the growth parameters of the common mushroom. It was established that solution of metals has a number of positive factors for its application: an increase in mycelial growth, yield, obtaining of more solid fruit bodies; an increase in the content of irreplaceable mineral trace elements **(Chen L., Gong Y., Cai Y., et al., 2016, Scola G., Scariot F.J., Dillon A. J. P., Moura, S., Echeverrigaray, S., Henriques, J.P., Roesch-Ely M., 2018).**

Microfertilizer «Avatar-1» possesses high bioavailability for mycelium – 98% (membranotropic effect), high chemical purity – 99,9% and has 4th class of danger (low hazard substances). Ingredients of the preparation perform a trophic function, by compensating for the nutrients scarcity; as well as a regulatory function, by activating all biochemical processes. It contains: Cu (800.0 mg/L), Zn (70.0 mg/L), Mg (800.0 mg/L), Mn (50.0 mg/L), Co (25.0 mg/l), Mo (25.0 mg/l), Fe (80.0 mg/l) (according to TU U 24.137033728-001: 2010) **(Bisko, N.A., 2015)**. «Avatar-1»was created by means of the joint work of the Ukrainian Scientific-Production Company «Avatar» and a group of scientists from N.G. Kholodny Institute of Botany of the NAS of Ukraine. This microfertilizer is widely used for growing cereals, sunflower, corn and soybeans **(Dimchev, V.A., Romanenko O.T., 2013, Kapitans′ka, O. M., 2014)**. In 2012, the preparation was tested in the cultivation process of a common mushroom *Agaricus bisporus* **(Bisko, N.A., 2015).**The effect of «Avatar-1»components on the intracellular processes of mushrooms: Mg is a functionally irreplaceable element that plays a predominant role in metabolism and growth of fungi; Zn is a part of the enzymes that participate in carbohydrate metabolism, increasing mycelium mass in relation to digested nutrients from the nutrient medium; Fe is an element that can be found in catalase, peroxidase, etc. components that convert components of the nutrient medium into available nutrient sources; Mn is involved in the nucleic acid synthesis within mycelium cells; Mo is vital for the enzymes involved in the processes of fruit bodies growth; Co is a part of vitamin B_{12}, which is necessary for the synthesis of nucleic acids within mycelium cells **(Ivanova, 2015, Yong Zhang, Wei Liu, Chunping Xu, Wei Huang, Peixin He, 2017)**.

Nowadays, the impact of «Avatar-1» nanocomplex on mushroom cultures was investigated only on a common mushroom, so the timeliness of our experiment is undoubted. The need for new experiments with the subsequent investigation of their cultural-morphological and physiological-biochemical indicators, has great prospects for the selection of industrial mushroom crops in the future.
The purpose of this work was to investigate the effect of microfertilizer «Avatar-1» on *L. edodes*.

MATERIALS AND METHODS

In this work, domestic nanopreparation «Avatar-1» was used – a micronutrient complex of carboxylates solution of especially pure biogenic metals, provided by the Ukrainian Research Institute of Nanobiotechnology and Resource Saving of the State Agency of Ukraine's Reserve. The object of the study was *L. edodes* strain 3776. Various agar and liquid broth nutrient media were used for the study of mycelial growth: oat meal, oat meal and oak bark, potato dextrose agar (PDA) – in pure form, with addition of microfertilizer «Avatar-1» and sodium selenite solution.
In experiment we used basidial culture *Lentinula Edodes* (Berk.) Sing) strain 3776 of the Catalogue of mushroom culture collection of Institute of Botany name Kholodny of the NAS of Ukraine **(Bisko N.A., Lomderg M.L., Mykchaylova O.B., N.Yu. Mytropolska, 2016).**
Optimization of nutrient medium was carried out using an oak bark agar. Oat grains and oak bark were drenched in boiling water and were left for 8 hours in a dark place. After this, the extracts were combined and filtered through a cotton-gauze filter, then were heated for 15 minutes, and, finally, the solution was brought to an initial volume and transferred to flasks (each flask filled by ½). To study the vegetative growth of the fungus, microbiological agar was added to the medium and transferred to Petri dishes, 20 ml into each **(Pereyma, I., Ivanova, T., 2017)**. To study the biomass growth, similar liquid nutrient medium was used. Sterilization was carried out in an autoclave at 120 ° C, at pressure of 0.12 mPa for 40 minutes.
After cooling, the medium was inoculated with *L. edodes* strain 3776 pure culture, under sterile conditions, in a quantity of 2% of the medium volume. Petri dishes with the inoculum were incubated in a thermostat at 23˚C for 7 days.
Scheme of experiment using «Avatar-1»: the preparation was added to the nutrient medium using laboratory pipette, following steps of the nutrient medium preparation followed after stirring. Monitoring was conducted after 3, 5 and 7 days. Used equipment: laboratory pipette, laboratory stirrer, autoclave, and thermostat. Microfertilizer rate: 20 ml /l.
Scheme of the experiment using Na_2SeO_3 solution: similar to the previous one. Monitoring was conducted after 3, 5 and 7 days. Used equipment: laboratory pipette, laboratory stirrer, autoclave, and thermostat. Microfertilizer rate: 20 ml/l.
Investigation of colonies' cultural properties, mycelial growth speed and density parameters, determination of hydrogen index (pH) of nutrient medium were carried out in accordance with generally accepted methods **(Ramkumar, L., Ramanathan T., Nedumaran Emir. J., 2011,).**
We described morphological and cultural features of the colonies on the 7, 10 and 15th day of observation, after complete overgrowth of the fungus with the mycelium of the nutrient medium. The measurement of radial velocity was carried out according to the formula given in the methods section: V, mm/day): V=a–b/t, a – the radius of the colony at the end of linear growth, mm, b – the radius of the colony at the start of linear growth, mm, t – duration (number of days) linear growth, days.
At the end of active growth stage, mycelium was separated from a culture fluid using dense tissue, receiving a culture filtrate. Obtained mycelium was washed three times with distilled water, dried with filter paper, and completely dry biomass was determined (**Pereyma, I., Ivanova, T., 2017**).
The integral value of specific growth rate was calculated according to the formula (Dudchik, 2009): $\mu = (\ln m_1 - \ln m_0) / t_1 - t_0$. Results for different nutrient composition are given in Table.
Mycelium examination was carried out by light microscopy methods, aseptically selected micellar sections were transferred to the substrate by a microbiological loop. Investigations were carried out with 40x100 zoom using XS-5520 MICRO med microscope. Selected area with dense septate mycelium was observed and characteristics of mycelium cultivated on different media were compared **(Ayeka, P.A., 2018).**
We have been processing data using Microsoft Office Excel.

RESULTS AND DISCUSSION

The following nutrient media were used in the experiment: oatmeal agar, oatmeal and potato dextrose agar (Fig. 1, 2, 3) with their modifications in the form of adding an «Avatar-1» microelement complex and Na_2SeO_3 solution as the mineral fertilizer and on other nutrient media.

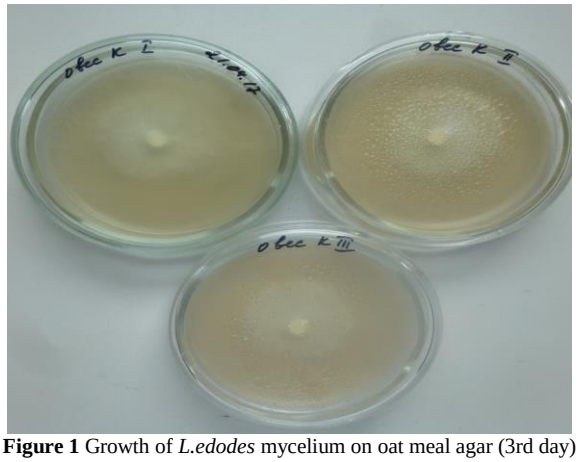

Figure 1 Growth of *L.edodes* mycelium on oat meal agar (3rd day)

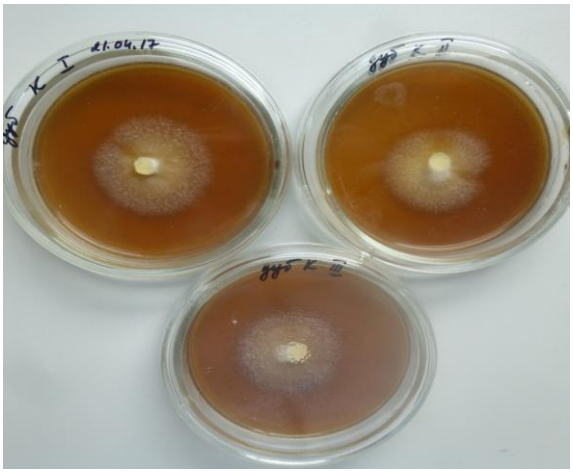

Figure 2 Growth of *L.edodes* mycelium on oak bark agar (3rd day)

Figure 3 Growth of *L.edodes* mycelium on PDA (3rd day)

Primary mycelium was incubated for 7 days in order to reach the expositional phase. Then, visual control of purity was performed **(Ivanova, T., Otkidach, I., Kuziomko, N. Mamontova, 2015, 2016)**. Methods of cultivation of basidiomycetes are described in detail in materials and methods.
Mycelium *L. edodes* strain 3776 growth was measured on the third, fifth and seventh day. The density of overgrowing of the medium was calculated on a 3-point scale (1 – liquid mycelium, transparent, 2 – medium density mycelium, transparent, 3 – thick mycelium, the medium is not translucent) on the 5th day of cultivation.
During the assessment of shiitake growth, we conducted a daily measurement of the colony diameter in two mutually perpendicular directions. The average daily growth rate of fungus was 3.6-4 mm/day within the studied period.
It should be noted, that the growth rate of fungus was not constant, it varied according to the age of culture (day) and the composition of the nutrient medium.
At the initial stage of development, the density of mycelium was low – up to 1 point, for 5 days it increased to 2-3 points and was 3-4 mm/day. Complete overgrowth of the nutrient medium occurred at 7 days, at a temperature of 23 ° C and a relative humidity of 60%.

To compare the effect of the nanopreparation on an object, a solution of sodium selenite Na_2SeO_3 as a control fertilizer was used in the experiment, which indices significantly differed by visual characteristics of colonies on the substrate and density of mycelium, as well as the number of buckles and hyphae discovered during light microscopy analyses of the colonies.

In this case, we observed a positive dynamics of mycelium growth on the medium, where «Avatar-1»was added, in the past two days the gain was more than 8 mm. Sodium selenite did not have a significant effect.

We noticed an active growth of mycelium on the second version of the nutrient medium – oatmeal and oak bark agar, with the addition of «Avatar-1». Mineral fertilizer (sodium selenite) did not produces a desired result.

Addition of nanopreparation to potato extract glucose agar compared with other two substrates showed a positive trend. Addition of sodium selenite to PDA inhibited the growth of mycelium.

The use of «Avatar-1»preparation with the norm of applying – 2% of the volume, positively influenced the growth dynamics by 2-6 mm compared with the control, and by 2-4 mm compared with sodium selenite. High rates were observed on the PDA medium. The parameter of mycelium height showed a characteristic dependence on the type of used medium. Nutrient medium with oak bark had the lowest results from 3.6 to 9.3 mm. The greatest growth was on potato extract glucose agar – 5,6-15,3 mm.

During the experiment on obtaining primary mycelium of *L. edodes*, it was found that the addition of the nanopreparation contributed to an increase in the yield of biomass and a great reduce of cultivation time. In percentage terms, this figure is 22-30%. The growth rate at a temperature of + 23-25 ° C was up to 5 mm/day.

The measurement of radial velocity was carried out according to the formula given in the methods section: V, mm/day): V=a–b/t,

According to the data, and the calculations were carried out. The obtained data on radial growth rate of mycelium is summarized in Table 1.

Table 1 Radial growth rate L. edodes on different agar nutrient media

Name	**Indicator $R_{med,}$ mm/day**
Oat meal (control)	2,1 ±0,18
Oat meal + «Avatar-1»	2,9 ±0,17
Oat meal + Na_2SeO_3	2,7±0,14*
Oat meal and oak bark (control)	2,6±0,19
Oat meal and oak bark + «Avatar-1»	3,4±0,19
Oat meal and oak bark + Na_2SeO_3	2,9±0,15*
PDA (control)	3,3±0,17
PDA + «Avatar-1»	3,5±0,21*
PDA + Na_2SeO_3	1,3±0,12*

Note*:* * – P ≤ 0,05 comparing to control (nutrient media without microfertilizers).

This table characterize radial growth of mycelium *L. edodes*. Application of microfertilizer «Avatar-1» gave a positive trend in growth. The indexes for different nutrient media are: oatmeal agar + «Avatar-1» – 2,9 mm/day, oatmeal and oak bark agar + «Avatar-1» – 3,4 mm/day, PDA + «Avatar-1» – 3,5 mm/day. At the end of the active growth stage of mycelium, measurements of dry mass growth were made (9th day of cultivation). Measurements were made according to the materials and methods described above. For this experiment, mycelium and culture filtrate were used. The hydrogen index of the culture filtrate was determined by potentiometric method by «pH-150 MIO» pH-meter (Table 2).

Table 2 Indicator of pH of the medium during mycelium deep cultivation

Nutrient medium name	**pH indicator**	
	Beginning of cultivation	**End of cultivation**
Oatmeal (control)	6,5	5,3
Oatmeal + “Avatar-1”	6,5	3,6*
Oatmeal a + Na_2SeO_3	5,6*	3,4*
Oatmeal and oak bark (control)	6,6	4,8
Oatmeal and oak bark + “Avatar-1”	6,6	3,6*
Oatmeal and oak bark + Na_2SeO_3	5,6*	3,8*
Potato dextrose (control)	6,6	5,2
Potato dextrose “Avatar-1”	6,5	4,5*
Potato dextrose + Na_2SeO_3	5,8*	3,6*

Note.: * - P ≤ 0,05 comparing to control (nutrient media without microfertilizers)

We tested the dry mass of mycelium on liquid nutrient media. The dry biomass measurement depends on the purpose of the experiments and cultivation conditions there is 1-50 days. We carried the first growth dimension after 18-24 hours of sowing. For research we used a culture filtrate of mycelium. We received a culture filter at the end of the cultivation of the mycelium. Active growth phase of mycelium was 9 days.

Mycelium was washed three times with distilled water, dried with filtration paper, and absolutely dry biomass (ADB) was determined, dried at 60 °C to constant weight (80 min), and measured on electronic weights (Fig.4, 5, 6 Table 3).

Figure 4 L. edodes mycelium on nutrient media potato dextrose + Avatar-1

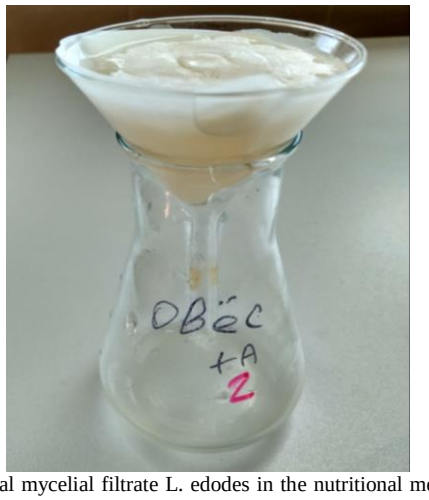

Figure 5 Cultural mycelial filtrate L. edodes in the nutritional medium Oatmeal +Avatar-1

Table 3 Dry biomass of *L. edodes* mycelium on different media

Absolutely dry biomass, g/l	
Oat meal broth (control)	2,8±0,02
Oat meal broth + «Avatar-1»	9,8±0,03*
Oat meal broth + Na_2SeO_3	8,6±0,03*
Oat meal and oak broth (control)	3,6±0,02
Oat meal and oak broth + «Avatar-1»	13,2±0,03*
Oat meal and oak broth +Na_2SeO_3	6,0±0,04*
Potato dextrose (control)	10,8±0,02
Potato dextrose + «Avatar-1»	14,2±0,10*
Potato dextrose + Na_2SeO_3	12,0±0,02*

Note*:* * – P ≤ 0,05 comparing to control (nutrient media without microfertilizers)

Figure 6 Dry biomass of *L. edodes* mycelium

The results of increase in mycelium fluctuate were within 0,3 ± 0,05 g/l. As it can be seen from the data, a significant increase in mass occurred on the PD+ Avatar-1 medium, indicating a positive effect of the preparation on the mycelium growth.

After maximal growth of the colony (complete overgrowth of the Petri dish), a visual analysis of the purity of mycelium was performed, followed by a microscopic analysis. The analyses were carried out using the crushed-drop method described in methods and materials. For these analyses, selected area of mycelium was aseptically transferred onto a piece of glass by microbiological loop. A micro-preparation was prepared and investigated with an increase of 40x100 using XS-5520 MICromed microscope. The area with dense septate mycelium was investigated, buckles were found, and characteristic of mycelium cultivated on different media were summarized. Mycelium grown on all three types of nutrient medium was involved in the experiment (Fig. 7).

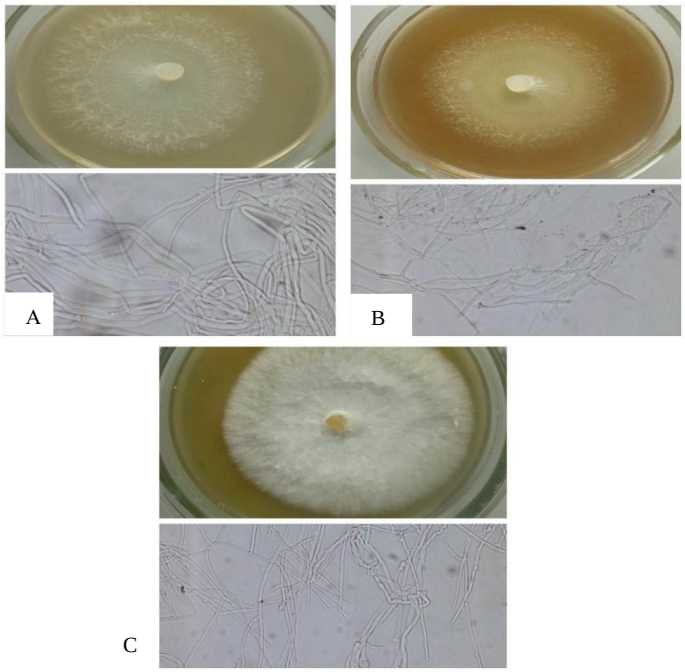

Figure 7 Microphotography of mycelium on the 5th day of cultivation: a) agar + oats + "Avatar-1", b) PDA + "Avatar-1", c) agar + oats and oak + "Avatar-1", 40x100

Table 4 Influence of agar nutrient media and preparations on mycelium morphology L. edodes

Nutrient medium	Morphology of mycelium
Control samples	Density is normal. Available buckles. Uniform growth.
With «Avatar-1»	Thickening and enlargement are present in branched hyphae. Increase the number of buckles. Uniform growth. The absence of too thin or thick hyphae.
With Na_2SeO_3	The Mycelium is wavy, thin twisted in a tangle. Many buckles.

Note*:* control – nutrient media without microfertilizers

Characterization of shiitake structure under light microscope detection. On control samples, mycelium was of normal density, visible buckles and performed uniform growth. With addition of «Avatar-1» to the medium an increase and consolidation of twisted hyphae and buckles was observed. Uniform germination of mycelium was observed, there were no too thin or too thick hyphae. Significant growth stimulation was detected. Media with Na_2SeO_3 – a micellar was wavy, thin and twisted in a tangle, a lot of buckles and a hyphae were observed. The mycelial growth was restrained, the fungus remains in the long stage of adaptation. It should be noted that differences of mycelium grown on different medium types were not observed, a general characteristic was established: *L. edodes* mycelium has a homogeneous dense structure, with a size of hyphae up to 1.0-1.5 μm.

Measurement of mycelium height

According to the above listed method. Growth characteristics of basidiomycetes were determined by the height growth factor. The research was conducted on a solid agar medium, the age of culture – 9 days.

Figure 8 Cross-section of mycelium on medium PDA + «Avatar 1»

The height of mycelial hyphae, that completely overgrown the Petri dish, was measured with a ruler at the edge of Petri dishes from the upper layer of an agar medium. Then the medium was cut with a sterile scalpel and the measurements were carried out from the middle of the diameter of the initial growth point of the mycelium (Fig. 8). According to the received data, mycelium on PDA was more dense and higher (Table 5).

Table 5 The height of mycelium *L. edodes* on different types of nutrient medium

Name of nutrient medium	The height of mycelium on the edge of the Petri dish, mm	The height of mycelium in the center of the Petri dish, mm
Oatmeal agar (control)	7,6	0,3
Oatmeal agar + «Avatar-1»	11,0*	0,5
Oatmeal agar + Na_2SeO_3	5,3*	0,2
Oatmeal and oak bark agar (control)	9,3	0,6
Oatmeal and oak bark agar + «Avatar-1»	8,0*	1,2*
Oatmeal and oak bark agar + Na_2SeO_3	3,6*	0,9
PDA (control)	15,3	1,1
PDA + «Avatar-1»	16,2*	1,5*
PDA + Na_2SeO_3	5,6*	0,8

Note: * - P ≤ 0,05 in comparison with control (medium without added microfertilizers)\

DISCUSSION

According to the stated purpose of the study, the experiment was conducted to investigate the effect of «Avatar-1» nanopreparation on the growth of mycelium. In accordance with literary sources, the drug was introduced into the nutrient medium (2%) before the autoclaving stage. The mycelia of the mushrooms were cultivated on the specified media with the addition of the micronutrient complex. According to obtained results (Fig. 1,2,3, Table 3), mycelial growth accelerated to 5 mm/day and dry mass increased by 0,3 g on the medium with the addition of microfertilizer «Avatar-1». The colonies differed visually by a denser structure (fig. 7), thickening of the hyphae and increased number of buckles, indicating an increase in the processes of genetic material exchange (Fig. 8).

Apart from «Avatar-1», sodium selenite Na_2SeO_3 (1.0 mmol/L) was used in the experiment. The solution was added to all the prepared nutrient media used in the work. It should be noted, that cultivation with addition of sodium selenite solution caused gloss and high density of mycelium. The fungus culture had a snow-white coloring, a very dense structure. During the cultivation stages, there

was a negative reaction, as well as a significant difference in the investigated aspect between different types of media.

There was a quantitative difference between culture reactions to «Avatar-1» and the addition of sodium selenite to the indicated media, expressed in growth rate, especially for culture growth on the 5th day of cultivation. The results of conducted experiments allow us to affirm that the drug «Avatar-1» carries out essential actions in the fungus metabolism, the vivacity of *L. edodes*, which is demonstrated in parameters changes of mycelial growth, both on liquid and agar media. The growth rate and biomass growth were remarked on all used media, preferably on a medium rich for carbohydrates – potato extract glucose agar.

Sodium selenite almost does not stimulate the growth rate of shiitake but increases the growth of biomass.

The best growth was observed on PDA, the biomass growth had high rates on the oatmeal agar, the height of the mycelium was well shown on oatmeal and oak bark agar. The positive effect of nanopreparation was noted at all stages of culture development during 7 days of cultivation.

In the study of growth characteristics, microfertilizer – sodium selenite was involved. Samples with addition of sodium selenite developed more slowly but had higher values as of control samples. Suspended growth of mycelium in selenium-rich media was likely to be due to the content of the limiting components, that is, with the increase of the medium volume, the limiting components content increases, which provides the mycelial growth duration during the exponential phase.

While studying the dependence of biomass growth on the amount of spawning material (volumetric/%, V/V), a specific tendency was observed, similar to that described for the dependence of biomass growth on the amount of nutrient medium. For further experiments, 10% inoculum dose in 50 ml of medium was defined as optimal ratio.

The dynamics of growth as well as the accumulation of biomass of Shiitake mycelium was established. According to the results of the study, an optimal cultivation period was confirmed as 7 days, when the cultures are in the logarithmic stage of growth.

After series of experiments on the selection of nutrient media for the cultivation of Shiitake spawning with addition of «Avatar-1», using a comparative analysis, it can be concluded that the «Avatar-1» nanoparticle with a concentration in the nutrient medium of 2% is recommended for use. Potato extract glucose agar is an optimal media for further experiments.

In literary sources, it is reported that with increasing concentration of the selenite solution to 4 moles/L and its addition to the nutritional medium results red pigmentation of *L. edodes* mycelium, this may indicate on destruction of sodium selenite to a free element Se. Researchers – J. Turlo, **(Turlo, J., Gutkowska B., Herold F., 2010)** studying the mechanisms of Na_2SeO_3 effect on the shiitake culture, as well as E. Vetchinkina **(Vetchinkina, E., Loshchinina, E, Kurskyi, V., Nikitina, V. (2016)**, investigating the effect of diacetophenonyl selenite on the growth of the shiitake mushroom – confirm red pigmentation of mycelium in the presence of Se.

CONCLUSIONS

Summing up the obtained data, we can conclude that the use of the microelement complex is perspective, especially combining cultivation with carbohydrate-rich nutrients. In addition to accelerating growth rates, we discovered an increase in mycelium biomass in an environment with added microfertilizer. We also recommend the use of micronutrient fertilizers to produce mycelium for spawning in order to accelerate or minimize the phase of lag phase of fungal mycelium development, by increasing the biochemical processes in the cell. It can also be assumed, that the addition of micronutrient solutions accelerates enzymatic reactions and plays a role of a metabolic regulator in the fungus cell. In general, series of experiments have shown that the acceleration of mycelial growth and the highest yield of biomass of primary mycelium *L. edodes* strain 3667 occurred on nutrient media with the addition of «Avatar-1»microfertilizer. Investigated features of the shiitake fungus growth on the media enriched with nanopreparation – «Avatar-1», that were discussed in this paper, can be used for further research in industrial and biotechnological laboratories and mushroom plants.

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