

THE EFFECT OF CULTIVATION CONDITIONS ON GROWTH AND THERAPEUTIC ACTIVITY OF *PLEUROTUS ERYNGII*

Krupodorova T. A., Barshteyn V. Yu.

INTRODUCTION

Since ancient time mushrooms play an important role in traditional medicine. The attention of researchers in recent decades focuses on the comprehensive study of different genus of Macromycetes: *Agaricus*, *Ganoderma*, *Pleurotus*, *Trametes*, *Lentinus* and others, and their biologically active secondary metabolites. Of considerable interest is one of the important species of edible mushrooms *Pleurotus eryngii* (DC.) Quél., often referred to as the King Trumpet Mushroom, French Horn Mushroom, King Oyster Mushroom, King Brown Mushroom and Xingbaogu (China). Studies have shown that extracts from *P. eryngii* fruit bodies possesses numerous medicinal properties as anticancer, antiviral, antioxidant, antimicrobial, anti-allergic, antiinflammatory, immunomodulating, hypolipidemic, estrogen-like activity. Various biologically active compounds have been isolated from *Pleurotus eryngii*: polysaccharides (β -1,3-glucans), lovastatin, protease (pleureryn), eryngin, ribonuclease, eryngeolysin, ergothioneine, 17 β -estradiol, protein xb68AB, acidic-glycosphingolipids^{1,2}. The major problem to its availability and using is limited harvests of this fungus in wildlife and their seasonality. These factors contributed to the introduction of this fungus into culture and the search for simple methods of cultivation³. Despite the numerous articles about *P. eryngii* growth on agro-industrial wastes on solid state cultivation^{4,5,6,7,8,9,10} a limited number of works has been

¹ Zhiming F., Liu Y., Zhang Q. A Potent Pharmacological Mushroom: *Pleurotus eryngii*. *Fungal Genomics and Biology*. 2016. № 6. P. 139. DOI: 10.4172/2165-8056.1000139.

² Staji M., Vukojevi J. and Duleti-Lauševi S. Biology of *Pleurotus eryngii* and role in biotechnological processes: a review. *Critical Reviews in Biotechnology*. 2009. № 29 (1). P. 55–66. DOI: 10.1080/07388550802688821.

³ Gregori A., Švagelj M., Pohleven J. Cultivation Techniques and Medicinal Properties of *Pleurotus* spp. *Food Technology and Biotechnology*. 2007. № 45 (3). P. 238–249.

⁴ Gregori A., Švagelj M., Pohleven J. Cultivation Techniques and Medicinal Properties of *Pleurotus* spp. *Food Technology and Biotechnology*. 2007. № 45 (3). P. 238–249.

⁵ Barshteyn V., Krupodorova T. Utilization of agro-industrial waste by higher mushrooms: modern view and trends. *The Journal of Microbiology, Biotechnology and Food Sciences*. 2016. № 5 (6). P. 563–577. DOI: 10.15414/jmbfs.2016.5.6.563-577.

⁶ Haasan F.R.H., Medany G.M., Hussein A.S.D. Cultivation of the King Oyster Mushroom (*Pleurotus eryngii*) in Egypt. *Australian Journal of Basic and Applied Sciences*. 2010. № 4 (1). № 99–105.

carried out on investigation of some factors on mycelium growth in pure culture. It was established a relationship between various geographical location and optimum temperature and vegetative mycelium growth^{11,12}. Also effect of culture media, alternate substrates and pH on mycelial growth of some *Pleurotus eryngii* strains has been studied^{13,14}. Few works has been carried out on investigation of mycelium growth in submerged cultivation. It was found that 10-oxo-trans-8-decenoic acid can improve the mycelial growth of King Oyster Mushrooms both on agarplates and in liquid culture¹⁵. Czapek's medium and static conditions were preferred for mycelium biomass production of *Pleurotus eryngii*¹⁶. It is obvious that the influence of cultivation conditions on mycelium biomass production and the therapeutic properties of mushroom preparations is very important¹⁷. So, the main factors that affected on *P. eryngii* mycelium growth and its therapeutic effect, such as cultural media, temperature, carbon and nitrogen sources, pH, have been studied fragmentary. Thus, the question of optimizing the

⁷ Moonmoon M., Uddin Md.N., Ahmed S., Shelly N.J., Khan Md.A. Cultivation of different strains of king oyster mushroom (*Pleurotus ostreatus*) on sawdust and rice straw in Bangladesh. *Saudi Journal of Biological Sciences*. 2010. № 17. P. 341–345.

⁸ Choi U.K., Lee O.H, Kim Y.C. Effect of calcinated oyster shell powder on growth, yield, spawn run, and primordial formation of king oyster mushroom (*Pleurotus eryngii*). *Molecules*. 2011. № 10. 16 (3). P. 2313–2322. DOI: 10.3390/molecules16032313.

⁹ Xie C., Yan L.,Gong W., Zhu Z., Tan S., Chen D., Zhenxiu H., Peng Y. Effects of Different Substrates on Lignocellulosic Enzyme Expression, Enzyme Activity, Substrate Utilization and Biological Efficiency of *Pleurotus Eryngii*. *Cellular Physiology and Biochemistry*. 2016. № 39. P. 1479–1494.

¹⁰ Atila F. Determining the Effects of Container Types on Yield and Fruitbody Features of *Pleurotus eryngii* Strains. *International Journal of Crop Science and Technology*. 2017. № 3 (1). P. 7–14.

¹¹ Alavi A., Goltape E.M., Kashy A., Arzani K., Asadi E. Investigation on Cultivation of Wild King Oyster Mushroom (*Pleurotus eryngii* DC: Fr. Quel) of Charmahal va Bakhtiari Province (Iran). *Proceedings of The Fourth International Iran & Russia Conference*. P. 692–696.

¹² Soylu M.K., Boztok K., Esiyok D. Mycelial growth performance of the *Pleurotus eryngii* species complex strains on different temperatures. *ISHS Acta Horticulturae 1123: XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): International Symposium on High Value Vegetables, Root and Tuber Crops, and Edible Fungi Production, Supply and Demands*. DOI: 10.17660/ActaHortic.2016.1123.29.

¹³ Zăgrean V., Sbirciog G., Buzatu M.-A., Mândru I. Effect of Nutritive Media and pH on Mycelial Growth of some *Pleurotus eryngii* Strains in vitro. *Bulletin UASVM Horticulture*. 2016. № 73 (2). P. 276–278.

¹⁴ Nguyen T.M. and Ranamukhaarachchi S.L. Effect of different culture media, grain sources and alternate substrates on the mycelial growth of *Pleurotus eryngii* and *Pleurotus ostreatus*. *Pakistan Journal of Biological Sciences*. 2020. № 23. P. 223–230.

¹⁵ Mau J.-L., Ma J.-T. Effect of 10-oxo-trans-8-decenoic acid on Mycelial growth of *Pleurotus eryngii*. *Fungal Science*. 2002. № 17 (1, 2). P. 1–9.

¹⁶ Janpoor J., Pourianfar H.R., Shahtahmasebi S. Study on effect of culture medium and growth conditions on Liquid Spawn king oyster mushroom (*Pleurotus eryngii*). *The International Journal of Farming and Allied Sciences*. 2017. № 6 (6). P. 154–156.

¹⁷ Krupodorova T.A., Barshteyn V.Yu., Kizitska T.O., Pokas E.V. Effect of cultivation conditions on mycelial growth and antibacterial activity of *Lentinula edodes* and *Fomitopsis betulina*. *Czech Mycoljgy*. 2019. № 71 (2). P. 167–186.

conditions of this mushroom species growth and synthesis of therapeutically active metabolites is relevant.

The aim of the current work was to investigate the effect of cultivation conditions on mycelial growth and therapeutic activity of *Pleurotus eryngii*.

1. Materials and methods

Source of basidiomycetes. *Pleurotus eryngii* (DC.) Quel. 2015 was kindly supplied by the Culture Collection of Mushrooms (IBK) of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine¹⁸. Stock cultures were maintained on beer-wort-agar slants at 4°C.

Cultivation of mycelia on soil media agar. Several media were chosen to determination growth rate: Czapek Dox Agar (CZA, Sigma Aldrich), Malt Extract Agar (MEA, Difco), Potato Dextrose Agar (PDA, Difco), Glucose-Peptone-Yeast Agar (GPYA) composed of (g/L): 25.0 glucose, 3.0 yeast extract, 2.0 peptone, 1.0 K₂HPO₄, 1.0 KH₂PO₄, 0.25 MgSO₄·7H₂O, and 20.0 agar. Prepared media were autoclaved at 121°C for 20 min. The media were dispensed into the 9 cm sterile Petri dishes. The plate dishes were inoculated with mycelial agar discs (diameter – 5 mm) of mushroom cut from the Petri dishes with GPYA using a sterile borer at the mycelial active growth stage. Cultivation on solid media was carried out at temperature 26±2°C in the dark. The vegetative growth of the mycelium of the mushroom on different media was assessed by measuring the diameter of mycelium in the Petri dish every 2 days until mushroom covered the whole plate.

Effect of incubation period on biomass production. Two liquid media were used for growth investigation in dynamics: GPY and natural media with 60 g amaranth flour per 1 l H₂O. The basis of natural cultivation medium was the waste after CO₂-extraction of flour prepared from the *Amaranthus hybridus* L. grains (hereinafter referred to as amaranth flour). *A. hybridus* species was a variety “Ultra” (Mykolaiv Oblast, Ukraine, 2011). CO₂-extraction conditions: pressure – 7.2 MPa; temperature – 24°C; duration of extraction – 2 hours.

Fully colonized Petri dish with GPYA was sterile homogenized and used for 10% (v/v) inoculation of 250 ml Flasks with 50 ml respectively liquid medium sterilized by autoclaving for 20 min at 121°C and then were submerged cultivated at 26±2°C in a rotary shaker (120 rpm).

Separation and biomass determination. Mycelium was separated from the medium by filtration through Whatman’s filter paper № 4, washed with distilled water, dried to a constant weight at 105°C. Fungi biomass was estimated by absolutely dry weight (a.d.w.).

¹⁸ Bisko N.A., Lomberg M.L., Mytropolska N.Y., Mykchaylova O.B. The IBK mushroom culture collection. *National Academy of Sciences of the Ukraine*. Kyiv : Alterpres. 120 p.

Exopolysaccharides determination. The culture liquid after submerged cultivation was treated with 96% ethanol at a volume ratio of 1:1 to obtain exopolysaccharides (EPS) and left overnight at 4°C. The precipitate (polysaccharide fraction) was harvested by centrifugation at 8 000 g for 15 min, and the precipitate of crude EPS was dried to a constant weight at 60°C¹⁹. Exopolysaccharides were absent in GPY medium. However, the content of exopolysaccharides was determined in amaranth flour in traces amount.

Effect of temperature and initial pH on biomass production. The GPY medium was employed to determine optimum temperature for mycelia growth (from 20 up to 30°C), and pH values of the growth medium from 4.5 up to 6.5. The pH was adjusted using 1M HCl and 1M NaOH. Flasks (150 ml) with 50 ml liquid medium sterilized by autoclaving for 20 min at 121°C and then were inoculated with three mycelial plugs of 8 mm diameter cut from the Petri dishes with GPYA using a sterile borer at the mycelial active growth stage. Mushroom was grown as static cultures (without agitation and in the dark) in flasks for 7 days at 26±2°C.

Effect of carbon and nitrogen sources on biomass production. Glucose-asparagine medium (g/l): 10.0 glucose, 0.4 asparagine, 1.0 KH₂PO₄, 0.5 MgSO₄·7H₂O, 11 H₂O has been used to determine the optimum carbon and nitrogen sources for mycelia growth. In this medium glucose was replaced by monosaccharides (arabinose, fructose, galactose, xylose), disaccharides (maltose, sucrose), polysaccharide (soluble starch) and mannitol in terms of 12 g of C/l (where pure carbon per liter calculated by percentage of carbon element in the molecule). Different concentrations of arabinose (10, 15, 25, 30, 35 g) were dissolved in 1000 ml in glucose-asparagine medium at pH of 5.5. Similarly, asparagine was replaced by different nitrogen sources (ammonium sulphate, peptone, sodium nitrate, yeast extract, urea) in terms of 0.21 g of N/l (where pure nitrogen per litre calculated by percentage of nitrogen element in the molecule). Different concentrations of asparagine 0.4, 0.8, 1.2, 1.6, 2.0 g were dissolved in to 1000 ml in glucose-asparagine at pH of 5.5. Flasks (150 ml) with 50 ml respectively liquid medium sterilized by autoclaving for 20 min at 121°C and then were inoculated with three mycelial plugs of 8 mm diameter cut from the Petri dishes with GPYA using a sterile borer at the mycelial active growth stage. Mushroom was grown as static cultures (without agitation and in the dark) in flasks for 7 days at 26±2°C.

Antibacterial activity. The bacterial cultures, *Bacillus subtilis* UCM B-901 (ATCC 6633), *Escherichia coli* UCM B-906 (ATCC 25922), and *Staphylococcus aureus* UCM B 4001 (ATCC 65388) were kindly supplied by Ukrainian Collection of Microorganisms (UCM, Institute of microbiology and virology NAS of Ukraine). Tested microorganisms were cultured on

¹⁹ Бабицкая В.Г. Базидиальные грибы – субстанция для получения функциональных препаратов. *Материалы международной конференции «Современное состояние и перспективы развития микробиологии и биотехнологии»*. Минск, 2004. С. 230–232.

Mueller-Hinton agar (MHA) (37°C, 24 h). Each microorganism was suspended in sterile saline and diluted to 10⁶ Colony Forming Units (CFU) per ml.

Antibacterial activity was determined by the agar disc diffusion method²⁰. Sterile paper discs (8 mm diameter) with mycelium suspension (grounded into native homogenized mycelium by using mortar and pestle) or ten times evaporated cultural liquid (using the sand bath) were placed into the Petri dishes with MHA previously inoculated with the bacterial suspensions. The inoculated Petri dishes were incubated overnight at 37°C. Antibacterial activity was evaluated by measuring the zone of inhibition against the tested organisms (in mm, using the ruler graduated to 0.5 mm). Antibacterial activity was determined to be present where the zone of inhibition was larger than 8 mm (exceeding diameter of the inserted paper disc). Distilled water was used as a negative control.

Antiviral activity. Cell lines and viruses, cytotoxicity assay and antiviral assay consistent with those set out in our article²¹.

Antifungal activity. The Ascomycetes cultures: *Issatchenkia orientalis* Kudryavtsev 301, *Candida albicans* (C.P. Robin) Berkhout 17/138 and clinical strains of *C. albicans* 311, 315, 319 were from the Culture Collection of Microorganisms of the Institute of Epidemiology and Infectious Diseases of the National Academy of Medical Sciences of Ukraine. *Aspergillus niger*, *Mucor* sp. and *Penicillium* sp. were from the Collection of strains of microorganisms and plant lines of the Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine. Stock cultures were maintained on potato dextrose agar slants at 4°C.

All Ascomycetes were transferred from stored cultures to Petri dishes with PDA and cultured at 26±1°C to obtain mycelial colonies. In the phase of active growth of cultures, mycelial discs of *P. eryngii* with a diameter of 8 mm has been cut.

We investigated direct interaction of studied fungi by their ability to suppress the mycelial growth in dual culture on PDA using a rating scale for the 3 main types of reactions (A, B, C) and 4 sub-types (C_{A1}, C_{B1}, C_{A2}, and C_{B2}) by method described by Badalyan, Innocenti & Garibyan²².

To determinate the inhibition growth, three discs were transferred to 250 ml flasks containing 50 ml of GPY nutrient medium and incubated at 26±1°C at static conditions. After 14 days of cultivation, surface mycelium was sterile removed, and the culture liquid was mixed with previously prepared and cooled to 40 °C GPYA nutrient medium, in a ratio of 1:1. The

²⁰ Методы экспериментальной микологии / Билай В.И. (ред). Киев : Наукова думка, 1982. 550 с.

²¹ Krupodorova T., Rybalko S., Barshteyn V. Antiviral activity of Basidiomycete mycelia against influenza type A (serotype H1N1) and herpes simplex virus type 2 in cell culture. *Virologica Sinica*. 2014. № 29 (5). P. 284–290. DOI: 10.1007/s12250-014-3486-y.

²² Badalyan S.M., Innocenti G., Garibyan N.G. Antagonistic activity of xylophilic mushrooms against pathogenic fungi of cereals in dual culture. *Phytopathologia Mediterranea*. 2002. № 41 (3). P. 220–225.

resulting mixture (a culture liquid with mycelium residues that did not grow on the surface but in the middle of the medium) was poured into Petri dishes with a diameter of 45 mm. After the medium was solidified, one micromycetes disk was introduced into the center of the Petri dish. Antifungal activity was determined at 7 days of cultivation by the percentage of growth inhibition according to the Vincent formula²³. Taking into account the peculiarity of *Penicillium* sp. the strain to formation numerous of small colonies, the degree of inhibition of its growth was assessed qualitatively: “-” no inhibition, “+” weak, “++” strong inhibition of growth (growth of macromycetes on top of pathogen culture).

Statistical analysis. All experiments were carried out in triplicate. The data were analyzed by Excel statistical functions using the Microsoft Office XP software, Statistical Package for Social Sciences, Program 11.5 Version (SPSS, Inc., 2002). Values are presented as means, with a \pm standard error of the mean (SEM). Differences at $P \leq 0.05$ were considered to be significant.

2. Results and discussion

The maintenance of a pure culture mycelium and investigations of its physico-chemical parameters needed for obtaining a high production and are the first critical stage towards the success of spawn production and mushroom cultivation. It is necessary to take into account that for different isolates and strains of the same fungi species optimal growth conditions can varied. The growth of vegetative mycelium and colony morphology are an important criterion of culture characteristics for biotechnological application. The influence of nutrient media on the growth rate (fig. 1) and on the morphology of colonies (table 1, fig. 2) of *P. eryngii* was determined. The average mycelial growth rate of studied media ranged between 7.7 ± 0.3 and 12.6 ± 0.6 mm/day with the highest growth rate obtained on CZA (fig. 1). This medium without agar also was more suitable for biomass production of *P. eryngii* in other study²⁴. However, the tender, spider-like colony morphology due to very thinner mycelium was inconvenient for further work. No statistically significant differences were found for mycelium growth rate on the GPYA and PDA culture media. However, researchers have reported about different media for optimum *P. eryngii* mycelial growth: glucose peptone²⁵, yeast malt extract^{26,27,28,29}, mushroom complete^{30,31},

²³ Vincent J.M. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*. 1947. № 159. 850 p. DOI: 10.1038/159850b0.

²⁴ Janpoor J., Pourianfar H.R., Shahtahmasebi S. Study on effect of culture medium and growth conditions on Liquid Spawn king oyster mushroom (*Pleurotus eryngii*). *The International Journal of Farming and Allied Sciences*. 2017. № 6 (6). P. 154–156.

²⁵ Alam N., Shim M.J., Lee M.W., Shin P.G., Yoo Y.B. and Le T.S. Vegetative Growth and Phylogenetic Relationship of Commercially Cultivated Strains of *Pleurotus eryngii* based on ITS sequence and RAPD. *Mycobiology*. 2009. № 37 (4). P. 258–266.

²⁶ Alavi A., Goltape E.M., Kashy A., Arzani K., Asadi E. Investigation on Cultivation of Wild King Oyster Mushroom (*Pleurotus eryngii* DC: Fr. Quel) of Charmahal va

potato-dextrose^{32,33}, lilly³⁴. Obtained our results are significant better compared to other similarly experiments of *P. eryngii* growth on MEA (4.96–5.79 mm/day), wheat extract agar (1.54–2.75 mm/day), PDA (1.68–3.04 mm/day)³⁵, and on yeast malt extract agar (0.7–4.2 mm/day)³⁶.

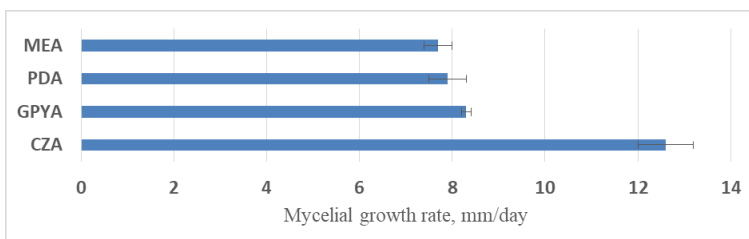


Fig.1. Effect of culture media on *P. eryngii* mycelia growth.
CZA – Czapek Dox Agar, MEA – Malt Extract Agar,
PDA – Potato Dextrose Agar, GPYA – Glucose-Peptone-Yeast Agar

Bakhtiary Province (Iran). *Proceedings of The Fourth International Iran & Russia Conference*. P. 692–696.

²⁷ Zăgrean V., Sbirciog G., Buzatu M.-A., Mândru I. Effect of Nutritive Media and pH on Mycelial Growth of some *Pleurotus eryngii* Strains in vitro. *Bulletin UASVM Horticulture*. 2016. № 73 (2). P. 276–278.

²⁸ Alam N., Shim M.J., Lee M.W., Shin P.G., Yoo Y.B. and Le T.S. Vegetative Growth and Phylogenetic Relationship of Commercially Cultivated Strains of *Pleurotus eryngii* based on ITS sequence and RAPD. *Mycobiology*. 2009. № 37 (4). P. 258–266.

²⁹ Sardar H., Ali M.A., Ayyub C.M., Ahmad R. Effects of different culture media, temperature and pH levels on the growth of wild and exotic *Pleurotus* species. *Pakistan Journal of Phytopathology*. 2015. № 27 (02). P. 139–145.

³⁰ Alam N., Shim M.J., Lee M.W., Shin P.G., Yoo Y.B. and Le T.S. Vegetative Growth and Phylogenetic Relationship of Commercially Cultivated Strains of *Pleurotus eryngii* based on ITS sequence and RAPD. *Mycobiology*. 2009. № 37 (4). P. 258–266.

³¹ Ryu J.-S., Kim M.-K., Kwon J.-H., Cho S.-H., Kim N.-K., Rho C.-W., Lee C.-H., Ro H.-S., Lee H.-S. The Growth Characteristics of *Pleurotus eryngii*. *The Korean Journal of Mycology*. 2007. № 35 (1). P. 47–53.

³² Nguyen T.M. and Ranamukhaarachchi S.L. Effect of different culture media, grain sources and alternate substrates on the mycelial growth of *Pleurotus eryngii* and *Pleurotus ostreatus*. *Pakistan Journal of Biological Sciences*. 2020. № 23. P. 223–230.

³³ Sardar H., Ali M.A., Ayyub C.M., Ahmad R. Effects of different culture media, temperature and pH levels on the growth of wild and exotic *Pleurotus* species. *Pakistan Journal of Phytopathology*. 2015. № 27 (02). P. 139–145.

³⁴ Kim H.-K., Cheong J.-C., Chang H.-Y., Kim G.P., Cha D.-Y., Moon B.-J. The Artificial Cultivation of *Pleurotus eryngii* (I) Investigation of Mycelial Growth Conditions. *The Korean Journal of Mycology*. 1997. № 25 (4). P. 305–310.

³⁵ Zăgrean V., Sbirciog G., Buzatu M.-A., Mândru I. Effect of Nutritive Media and pH on Mycelial Growth of some *Pleurotus eryngii* Strains in vitro. *Bulletin UASVM Horticulture*. 2016. № 73 (2). P. 276–278.

³⁶ Alavi A., Goltape E.M., Kashy A., Arzani K., Asadi E. Investigation on Cultivation of Wild King Oyster Mushroom (*Pleurotus eryngii* DC: Fr. Quel) of Charmahal va Bakhtiary Province (Iran). *Proceedings of The Fourth International Iran & Russia Conference*. P. 692–696.

Table 1

Morphology of *P. eryngii* colonies on different culture media

Culture media	Morphology of colonies				
	Color	Form / Edge	Mycelial growth	Texture	Surface
PDA	white	circular / fimbriate	prostrate – aerial	cottony: rather long, single mycelial hyphae spreading in all directions	flat
GPYA	white and light yellow in the centrum of colony	circular / fimbriate	prostrate – aerial	coriaceous in the centrum and velvety: a dense mat of erect, straight hyphae, usually short	flat
CZA	white	circular / smooth	prostrate	spider-like with cotton in the centrum of colony	flat
MEA	light yellow	circular / fimbriate	prostrate – aerial	woolly: fairly long interwoven hyphae or groups of hyphae, somewhat matted, resembling woolen cloth	flat



Fig. 2. Illustration of *P. eryngii* colonies on different culture media: a – PDA, b – GPYA, c – CZA, d – MEA

Of particular interest is the study of the patterns of mushroom growth in submerged cultivation. The production of mushroom biomass on standard and natural media are quite actual and promising trends in modern biotechnology. The influence of the nutrient medium on biomass and exopolysaccharides production of *P. eryngii* in submerged cultivation has been studied. The obtained data of the dynamics of biomass accumulation on two used liquid medium were subordinate to the general regularities of microorganism's development in submerged cultivation conditions (fig. 3). The results of the study showed that maximum amount of biomass 15.3 ± 0.2 g/l and 14.5 ± 0.8 g/l was obtained on the 8th day of cultivation on GPY medium and 10th day of cultivation on amaranth flour medium, respectively. But crude exopolysaccharides production was better established on natural media. The obtained biomass amount are slightly better than the results of the positive effect of 10-oxo-trans-8-decenoic acid on the growth of *P. eryngii* ($10.2\text{--}12.6$ g/l) on 12 days of cultivation¹⁴ and

significantly exceed of the results (from 1.5 mg/ml to 2.7 mg/ml) obtained on different media by submerged cultivation with and without shaking³⁷.

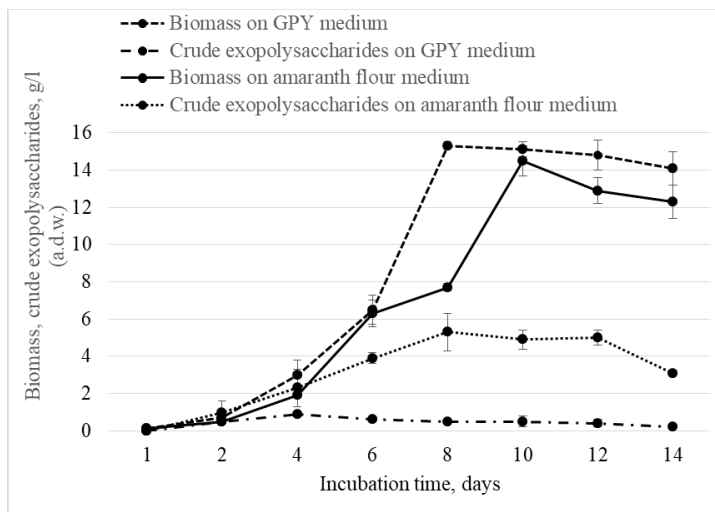


Fig. 3. Dynamics of *P. eryngii* biomass and crude exopolysaccharides production in shake flasks using different media

It's well known that temperature has significant effect on metabolic reactions of fungi. The optimum temperature for *P. eryngii* growing is $20 \pm 1^\circ\text{C}$ (fig. 4). Our results are in line with those that shown optimum temperature 20°C for vegetative growth rate of *P. eryngii*³⁸. In other studies, the most appropriate temperature for mycelial growth were 25°C ^{39,40}, $25\text{--}30^\circ\text{C}$ ⁴¹, 30°C ⁴². It has been reported that different *P. eryngii*

³⁷ Mau J.-L., Ma J.-T. Effect of 10-oxo-trans-8-decenoic acid on Mycelial growth of *Pleurotus eryngii*. *Fungal Science*. 2002. № 17 (1, 2). P. 1–9.

³⁸ Janpoor J., Pourianfar H.R., Shahtahmasebi S. Study on effect of culture medium and growth conditions on Liquid Spawn king oyster mushroom (*Pleurotus eryngii*). *The International Journal of Farming and Allied Sciences*. 2017. № 6 (6). P. 154–156.

³⁹ Alavi A., Goltape E.M., Kashy A., Arzani K., Asadi E. Investigation on Cultivation of Wild King Oyster Mushroom (*Pleurotus eryngii* DC: Fr. Quel) of Charmahal va Bakhtiary Province (Iran). *Proceedings of The Fourth International Iran & Russia Conference*. P. 692–696.

⁴⁰ Soylu M.K., Boztok K., Esiyok D. Mycelial growth performance of the *Pleurotus eryngii* species complex strains on different temperatures. *ISHS Acta Horticulturae 1123: XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): International Symposium on High Value Vegetables, Root and Tuber Crops, and Edible Fungi Production, Supply and Demands*. DOI: 10.17660/ActaHortic.2016.1123.29.

strains growth at broad range of temperature (°C): 10, 15, 20, 25, 30, where the optimum temperature were found to be 20–30°C depending on the strain of the fungus and cultivation medium⁴³.

Mycelium structure and function (in particular cell morphology, cell membrane function, the solubility of salts, the uptake of necessary nutrients) of fungi significant depends of the pH of initial medium. It was established that mycelium of *P. eryngii* could grow over a studied range of pH values with quite smaller differences. The best growth was obtained at 5.0–5.5 pH values since we did not observe a statistically significant difference in mycelial growth at these pH (fig. 5). This observation is in line with Alam et al.⁴⁴ which established that *P. eryngii* growth within pH ranging from 5~9 without any significant variation. Some researches^{45,46,47,48} found that for *P. eryngii* strains maximum mycelial growth was determined at pH 6.0.

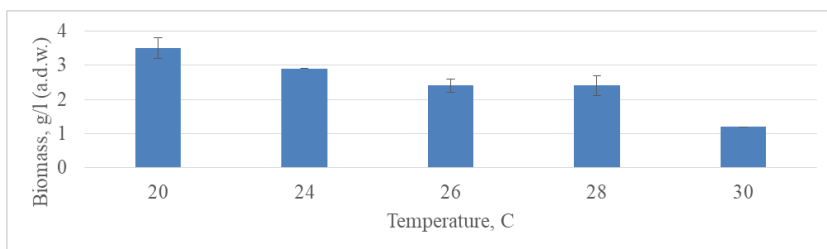


Fig. 4. Effect of temperature on *P. eryngii* biomass production

⁴¹ Sardar H., Ali M.A., Ayyub C.M., Ahmad R. Effects of different culture media, temperature and pH levels on the growth of wild and exotic *Pleurotus* species. *Pakistan Journal of Phytopathology*. 2015. № 27 (02). P. 139–145.

⁴² Kim H.-K., Cheong J.-C., Chang H.-Y., Kim G.P., Cha D.-Y., Moon B.-J. The Artificial Cultivation of *Pleurotus eryngii* (I) Investigation of Mycelial Growth Conditions. *The Korean Journal of Mycology*. 1997. № 25(4). P. 305–310.

⁴³ Miyauchi, S., Kon, K., Yamachi, T. & Shimomura, M. Cultural characteristics of mycelial growth of *Pleurotus eryngii*. 1998. Nipon. Kingakakai, Kaiho. № 39 (3). P. 83–87.

⁴⁴ Ryu J.-S., Kim M.-K., Kwon J.-H., Cho S.-H., Kim N.-K., Rho C.-W., Lee C.-H., Ro H.-S., Lee H.-S. The Growth Characteristics of *Pleurotus eryngii*. *The Korean Journal of Mycology*. 2007. № 35(1). P. 47–53.

⁴⁵ Alam N., Shim M.J., Lee M.W., Shin P.G., Yoo Y.B. and Le T.S. Vegetative Growth and Phylogenetic Relationship of Commercially Cultivated Strains of *Pleurotus eryngii* based on ITS sequence and RAPD. *Mycobiology*. 2009. № 37 (4). P. 258–266.

⁴⁶ Zăgrean V., Sbirciog G., Buzatu M.-A., Mândru I. Effect of Nutritive Media and pH on Mycelial Growth of some *Pleurotus eryngii* Strains in vitro. *Bulletin UASVM Horticulture*. 2016. № 73 (2). P. 276–278.

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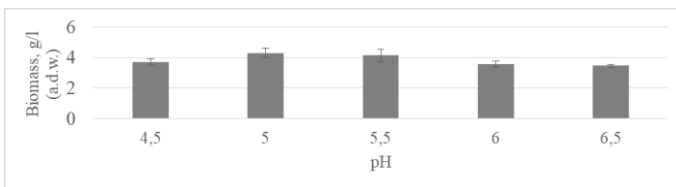


Fig. 5. Effect of initial pH on *P. eryngii* biomass production

The studying of the trophic needs is important for understanding the physiology of mushroom growth. Carbon and nitrogen sources are of paramount importance since they play an important role in growth and development processes. Carbon is needed for synthesis of living cell substances and is involved in oxidation processes in which it is the only one source of energy. Our work indicates that *P. eryngii* can utilize a broad range of carbon sources, such as monosaccharides, disaccharides, and polysaccharides (fig. 6). But arabinose in concentration 20g/l was the most suitable carbon source for *P. eryngii* biomass production (fig. 7). This finding is in line with the ecology of this fungi, which is a weak parasite since arabinose is one of the widespread reserve substances of Apiaceae plants (*Eryngium* spp. and *Ferula* spp.) on the roots naturally grow *P. eryngii*. In the previous reports *P. eryngii* needed for the growth various sources of carbon: dextrin, followed by fructose, mannose and maltose⁴⁹, glucose (3–4%) and dextrin (5%)⁵⁰, glucose, starch, and sucrose⁵¹.

Nitrogen-containing compounds play a significant role in metabolism of fungi. Among the nitrogen sources used in this study, asparagine was found to be the best nitrogen source for biomass production of *P.eryngii* (fig. 8). It was established that asparagine in concentration 0.8–1.6 g/l supported biomass synthesis at the same level (fig. 9). In other study⁵², casamino acid (0.12%) was the most appropriated nitrogen source for *P. eryngii* mycelial growth. However good growth was obtained in medium with serine,

⁴⁹ Alam N., Shim M.J., Lee M.W., Shin P.G., Yoo Y.B. and Le T.S. Vegetative Growth and Phylogenetic Relationship of Commercially Cultivated Strains of *Pleurotus eryngii* based on ITS sequence and RAPD. *Mycobiology*. 2009. № 37 (4). P. 258–266.

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⁵¹ Altaf S.A., Umar D.M., Muhammad M.S. Production of Xylanase Enzyme by *Pleurotus eryngii* and *Flamulina velutipes* Grown on Different Carbon Sources under Submerged Fermentation. *World Applied Sciences Journal* 8 (Special Issue of *Biotechnology & Genetic Engineering*). 2010. P. 47–49.

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glutamic acid, asparagine, alanine, and urea. Ammonium acetate was found to be the best mycelial growth of *P. eryngii*, followed by arginine and urea⁵³.

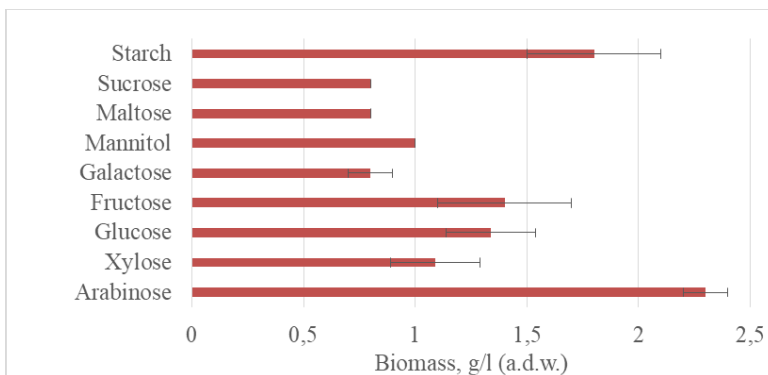


Fig. 6. Effect of carbon sources on *P. eryngii* biomass production



Fig. 7. Effect of arabinose concentration on *P. eryngii* biomass production

Thus, taking into account the results of the conducted research and analysis of literature data, basic needs (temperature, pH, carbon and nitrogen sources) of fungi growth is quite specifically for every strains or isolates. The understanding of the physiological needs of fungi and observing their optimal cultivation conditions will contribute to obtaining their high productivity.

⁵³ Alam N., Shim M.J., Lee M.W., Shin P.G., Yoo Y.B. and Le T.S. Vegetative Growth and Phylogenetic Relationship of Commercially Cultivated Strains of *Pleurotus eryngii* based on ITS sequence and RAPD. *Mycobiology*. 2009. № 37 (4). P. 258–266.

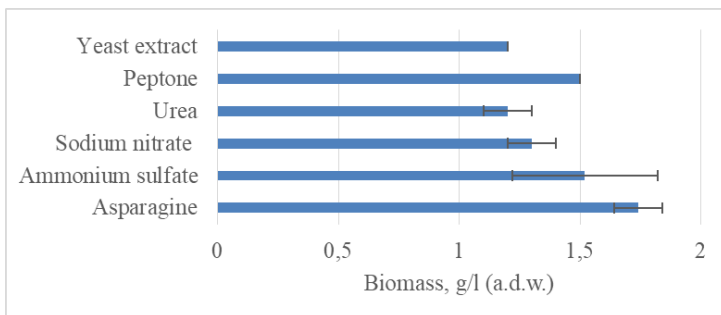


Fig. 8. Effect of nitrogen sources on *P. eryngii* biomass production

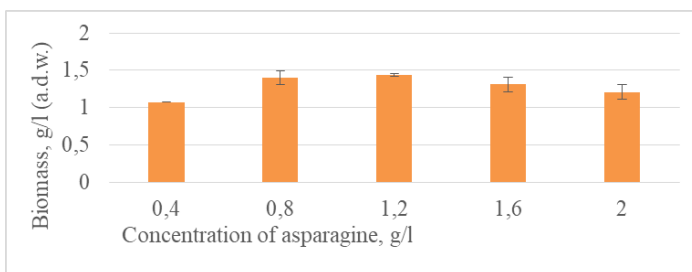


Fig. 9. Effect of asparagine concentration on *P. eryngii* biomass production

The growing of basidiomycete's biomass in controlled conditions is the important direction in the development of new drugs. Therefore, investigations aimed at studying the therapeutic properties of fungi are of particular importance. And it opens up new prospects for the use of biomass of fungi and their metabolites obtained in culture. It was established that *P. eryngii* possessed different pharmacological activities: anticancer, antioxidant, antimicrobial, anti-allergic, antihypercholesterolic, antiosteoporotic, immunomodulating^{54,55}. Research on antiviral, antibacterial, and antagonistic activity is gaining significant attention.

The prevention and treatment of viral diseases remains an urgent problem in modern medicine. Virus infection is one of the challenging

⁵⁴ Zhiming F., Liu Y., Zhang Q. A Potent Pharmacological Mushroom: *Pleurotus eryngii*. *Fungal Genomics and Biology*. 2016. № 6. P. 139. DOI: 10.4172/2165-8056.1000139.

⁵⁵ Staji M., Vukojevi J. and Duleti-Lauševi S. Biology of *Pleurotus eryngii* and role in biotechnological processes: a review. *Critical Reviews in Biotechnology*. 2009. № 29 (1). P. 55–66. DOI: 10.1080/07388550802688821.

global issues with regular widespread epidemics or pandemics resulting in high mortality worldwide⁵⁶. We investigated *in vitro* the antiviral activity of *P. eryngii* mycelia obtained on culture medium with amaranth flour against influenza virus A/FM/1/47 (H1N1) and against herpes virus type 2 (HSV). In general, it should be noted slight antiviral activity of *P. eryngii* mycelia. Extracts from *P. eryngii* mycelia inhibited the reproduction of influenza virus strain A/FM/1/47(H1N1) in MDCK cells reducing the infectious titre by 2.0 lg ID₅₀⁵⁷. However, mycelial extract of this fungus was inactivity against HSV-2. Previously, it was established, that the protease from *P. eryngii* designated pleureryn was active against HIV-1 growth with an IC₅₀ of 2.2 μM by inhibiting HIV-1 reverse transcriptase. A protease was isolated from fresh fruiting bodies of *P. eryngii*, caused (23.1±0.6)% and (91.4±3.2)% inhibition of HIV-1 reverse transcriptase at 3 and 30 mM, respectively⁵⁸.

At the same time, the expediency of finding new highly effective antimicrobial drugs and raw materials for medical purposes is undeniable. The feasibility of such studies is also due to the prevalence of resistance to antibacterial and antifungal drugs. It's known that fungi need antibacterial and antifungal compounds to survive in their natural environment⁵⁹. We studied mycelum and culture liquid of *P. eryngii* cultivated on two liquid media: glucose-peptone-yeast agar culture medium and natural liquid medium with amaranth flour⁶⁰. It should be noted that the use of the natural medium for the cultivation of the fungus allowed us to establish the presence of antibacterial activity. *P. eryngii* mycelium cultivated on amaranth flour medium showed moderate antibacterial activity only against *Bacillus subtilis* (zone of inhibition – 10.0±0.0 mm). Previously, the influence of cultivation substrates (various agrowastes) on the antimicrobial activity of fruiting bodies *P. eryngii* var. *ferulae* was also demonstrated⁶¹. Studied samples of *P. eryngii* (mycelium and culture liquid) were inactive against *Staphylococcus aureus* and *Escherichia coli*. Similar results – moderate

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⁵⁷ Krupodorova T., Rybalko S., Barshteyn V. Antiviral activity of Basidiomycete mycelia against influenza type A (serotype H1N1) and herpes simplex virus type 2 in cell culture. *Virologica Sinica*. 2014. № 29 (5). P. 284–290. DOI: 10.1007/s12250-014-3486-y.

⁵⁸ Wang H, Ng T.B. Pleureryn, a novel protease from fresh fruiting bodies of the edible mushroom *Pleurotus eryngii*. *Biochemical and Biophysical Research Communications*. 2001. № 289. P. 750–755.

⁵⁹ Yamaç M., Bilgili F. Antimicrobial Activities of Fruit Bodies and/or Mycelial Cultures of Some Mushroom Isolates. *Pharmaceutical Biology*. 2006. № 44 (9). P. 660–667. DOI: 10.1080/13880200601006897.

⁶⁰ Zhiming F., Liu Y., Zhang Q. A Potent Pharmacological Mushroom: *Pleurotus eryngii*. *Fungal Genomics and Biology*. 2016. № 6. P. 139. DOI: 10.4172/2165-8056.1000139.

⁶¹ Akyuz M., Kirbag S. Antimicrobial activity of *Pleurotus eryngii* var. *ferulae* grown on various agro-wastes. *EurAsian Journal of BioSciences*. 2009. № 3. P. 58–63.

antibacterial activity against *B. subtilis* and *E. coli* were demonstrated by other authors in submerged culture⁶². Some studies reported that *P. eryngii* extracts performed inhibitory property against a group of bacterial reference strains of medical relevance⁶³.

Continuing a series of studies of the therapeutic activity of *P. eryngii*, we investigated the antifungal activity of its mycelium by direct interactions with micromycetes and of cultural liquid with mycelium fragments by evaluation the growth inhibition of micromycetes in percentage in dual culture. It is known that antibacterial and antifungal activities often correlate with each other. But in our case, the antifungal activity of *P. eryngii* was more pronounced than the antibacterial. The direct interactions between all tested fungi shown different results. The best result (replacement overgrowth) was obtained in the case of *P. eryngii* and *Saccharomycetaceae* fungi cultivation: complete, replacement after initial deadlock with mycelial contact (subtype C_{A2}) was observed with *I. orientalis* and *C. albicans* 17/138; partial replacement (subtype C_{A1}) – with clinical isolates *C. albicans* 311 and 319; replacement overgrowth without initial deadlock (type C) – with *C. albicans* 315. It was established mutual inhibition when neither organism was able to overgrow the other at mycelial contact of *P. eryngii* and *A. niger*. In addition, subtype C_{A1} was characteristic during cultivation *P. eryngii* and *Penicillium* sp. However, a partial growth of *Mucor* sp. on colony *P. eryngii* was observed. The calculation of the antagonistic index (AI), calculated using the well-known formula⁶⁴, indicates significant antifungal activity of *P. eryngii*. AI for *P. eryngii* was 23.5 (13–14 result from 30 studied macromycetes).

We obtained a similar antifungal activity of mycelium fragments and culture liquid of *P. eryngii* obtained by cultivation in a liquid potato-dextrose medium. High antifungal activity of *P. eryngii* was found in our study also against *Saccharomycetaceae* fungi, which had 100% growth inhibition of *I. orientalis* and all *C. albicans* strains. The inhibition of *A. niger* growth was established at 57.6%. *P. eryngii* was less effective against *Penicillium* sp. A weak effect of *P. eryngii* was observed on the ability of *Penicillium* sp. to the formation of numerous small colonies. Wang and Ng⁶⁵ isolated from

⁶² Краснополяская Л.М., Тренин А.С., Бычкова О.П., Цвигун Е.А., Джавахян Б.Р. Погруженная культура *Pleurotus eryngii*: антибиотические свойства и способность к образованию ингибиторов биосинтеза стеролов. *Universum: Химия и биология*. 2015. № 11 (18). URL: <http://7universum.com/ru/nature/archive/item/2703>.

⁶³ Zhiming F., Liu Y., Zhang Q. A Potent Pharmacological Mushroom: *Pleurotus eryngii*. *Fungal Genomics and Biology*. 2016. № 6. P. 139. DOI:10.4172/2165-8056.1000139.

⁶⁴ Badalyan S.M., Innocenti G., Garibyan N.G. Antagonistic activity of xylophilic mushrooms against pathogenic fungi of cereals in dual culture. *Phytopathologia Mediterranea*. 2002. № 41 (3). P. 220–225.

⁶⁵ Wang H., Ng T.B. Eryngin, a novel antifungal peptide from fruiting bodies of the edible mushroom *Pleurotus eryngii* isolated from fruiting bodies of the mushroom *Pleurotus eryngii*. *Peptides*. 2004. № 25 (1). P. 1–5.

fruiting bodies of *P. eryngii* an antifungal peptide designated as eryngin inhibited mycelial growth of *Fusarium oxysporum* and *Mycosphaerella arachidicola*.

Thus, the mycelium and culture liquid of *P. eryngii* obtained on different media have insignificant antiviral, antibacterial activity and possess relative strong antifungal action.

CONCLUSIONS

The understanding of the impact of nutritional requirements of mushrooms is a key component toward designing efficient biotechnology strategies. Obtaining the high yield of fungi biomass depends on many factors, first of all, on satisfaction the needs of mushrooms for nutrients, which is directly dependent on the nutrient medium. Among these factors also: medium acidity, temperature, incubation period, cultivation method. Moreover, these factors are unique for each strain of fungi. The average mycelial growth rate on Czapek Dox Agar, Malt Extract Agar, Potato Dextrose Agar, Glucose-Peptone-Yeast Agar composed media ranged between 7.7 ± 0.3 and 12.6 ± 0.6 mm/day with the highest obtained on Czapek Dox Agar. Temperature 20°C and pH values between 5.0–5.5, presence of arabinose (concentration – 20g/l) and asparagine (concentration 0.8–1.6 g/l) gave the best results for *P. eryngii* biomass synthesis. The biomass and exopolysaccharides synthesis of *P. eryngii* varied depending on the media employed. Considering the insignificant difference of biomass formation on the studied media and the best indicators of the synthesis of exopolysaccharides on the natural medium, amaranth flour medium is more appropriated medium for these purposes.

The growing of fungi biomass in controlled conditions is an actually direction in the development of new drugs with low toxicity. *P. eryngii* mycelium and its culture liquid obtained on different media have some therapeutically activities: slight antiviral against influenza virus strain A/FM/1/47(H1N1) with the reducing of the infectious titre by 2.0 lg ID₅₀, insignificant antibacterial activity against *Bacillus subtilis* (zone of inhibition – 10.0±0.0 mm) and strong antifungal action against *Issatchenkia orientalis*, *Candida albicans* strains including clinical isolates, good effect against *A. niger* and less effective against *Penicillium* sp.

In general, the biomass production of the fungus largely depends on most of the studied factors, such as temperature, duration and culture medium, sources of nitrogen and carbon. Only the acidity of the medium had a negligible effect of biomass accumulation. The influence of the medium cultivation on mycelial growth, morphology of colonies, crude exopolysaccharides production and on the manifestation of antibacterial activity was also established.

Obtained results serve as a base or provide information of nutritional requirements for *P. eryngii* growth and of its some therapeutically potential.

The strategies used in this study can help increasing vegetative growth and can be useful by substrates choosing for *P. eryngii* cultivation.

SUMMARY

Last time of considerable interest is one of the important edible mushrooms *Pleurotus eryngii*, which fruit bodies possesses numerous medicinal properties. The main factors that affected on *P. eryngii* mycelium growth and its therapeutic effect have been studied fragmentary. Thus, the optimizing the growth conditions and synthesis of therapeutically active metabolites is relevant.

The highest mycelial growth rate has been obtained in this work on Czapek Dox Agar. Temperature 20°C and pH values between 5.0–5.5, presence of arabinose and asparagine gave the best results for *P. eryngii* biomass synthesis.

P. eryngii mycelium and its culture liquid demonstrated some therapeutic activities: slight antiviral against influenza virus strain A/FM/1/47(H1N1), insignificant antibacterial activity against *Bacillus subtilis* and strong antifungal action against *Issatchenkia orientalis*, *Candida albicans* strains including clinical isolates, good effect against *Aspergillus niger* and was less effective against *Penicillium* sp.

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Information about authors:

Krupodorova T. A.,

Candidate of Biological Sciences,
Senior Researcher of the Laboratory for Extraction of Vegetable Raw
Materials and Bioconversion
Institute of Food Biotechnology and Genomics
of the National Academy of Sciences of Ukraine
2a, Osipovskogo Str., Kyiv, 04123, Ukraine;

Barshteyn V. Yu.

Candidate of Technical Sciences,
Head of the Laboratory for Extraction
of Vegetable Raw Materials and Bioconversion
Institute of Food Biotechnology and Genomics
of the National Academy of Sciences of Ukraine
2a, Osipovskogo Str., Kyiv, 04123, Ukraine