# **PHOTOREGULATION OF THE BIOSYNTHETIC ACTIVITY OF** *Laricifomes officinalis* **USING COLLOIDAL SOLUTIONS OF METAL NANOPARTICLES AND LASER IRRADIATION**

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The *aim* of the work was to study the influence of biogenic metal nanoparticles on the growth characteristics and biosynthetic activity of the fungus *Laricifomes officinalis*, as well as the effects of photocatalytic activity of NPs after exposure to low-intensity laser radiation under deep cultivation conditions.

*Material and Methods.* Traditional mycological methods, colloidal solutions of nanoparticles biogenic metals, and unique photobiological methods were used. The effect of light on the biosynthetic activity of *L. officinalis* was studied using low-intensity coherent monochromatic laser light with specified spectral and intensity characteristics. The experiment used water-based colloidal solutions of biogenic metals such as Fe, Mg and Ag, obtained by a patented method.

*Results*. Treatment of the inoculum with colloidal solutions of nanoparticles of all used metals increased the growth of *L. officinalis* by 31–54%, while irradiation of the fungal inoculum with laser light in a medium with nanoparticles reduced the growth activity of the *L. officinalis* mycelium by 14.4– 22.6%. All nanoparticles suppressed the biosynthesis of extracellular polysaccharides, whereas treatment of the inoculum with colloidal solutions of FeNPs and MgNPs stimulated the synthesis of endopolysaccharides. At the same time, laser light irradiation in the presence of AgNPs increased the amount of endopolysaccharides, while FeNPs and MgNPs slightly inhibited their synthesis. Treatment of the inoculum with colloidal metal solutions and laser light affected the total phenolic content (TPC) in the mycelial mass. The highest TPC values in ethanol extracts with AgNPs and laser light irradiation were  $97.31 \pm 3.7$  mg of GAEs/g of dry mass.

*Conclusions***.** The research results gave ground to consider nanoparticles of biogenic metals (AgNPs, FeNPs, MgNPs) and low-intensity laser light as a promising regulators of the biosynthetic activity of *L. officinalis* in the biotechnology of its cultivation.

*Key words:* laser; mycelial biomass; polysaccharides; total phenol compounds; antioxidant activity.

 Modern advances in nanotechnology open up many opportunities for their application in biology, medicine, genetic engineering, agricultural technologies, food, and chemical industries [1–6]. In recent years, the application of nanotechnology in biological systems has led to the emergence of new fields such as nanomedicine and nanobiotechnology,

which focus on the development of new therapeutics approaches ranging from targeted drug delivery to manipulation of individual biomolecules and new bioimaging agents [5, 7]. It is the small size of nanoparticles (NPs) that determines a number of features of their interaction within the cells of living organisms, including fungi. Understanding

the interactions between nanoparticles and biological objects is essential for the further safe application of nanotechnology in modern biology. The influence of various materials as biological agents in the form of nanoparticles on the development of living organisms has been actively studied in recent years, including in mycology at the stages from the germination of spores to the formation of fruiting bodies [3, 8, 9]. However, given the wide range of biochemical processes in fungal organisms, there remains a wide range of insufficiently studied issues of the influence of nanoparticles on the development of fungi, requiring additional detailed studies. One of these understudied phenomena is the combined effect on processes in the fungal organisms of metal nanoparticles and artificial irradiation. Both of these factors (nanoparticles and artificial lighting) independently demonstrate the ability to both stimulate and inhibit the development of living organisms, including plants and fungi [10–12]. The synergistic action of two independent factors — metal nanoparticles and a laser — may open up new effective mechanisms for controlling processes in biological systems, in particular through photocatalysis mechanisms and enhanced photostimulation in the presence of nanoparticles.

Previous data shown the effect of colloidal solutions of metal nanoparticles AgNPs, FeNPs, and MgNPs on the biosynthetic activity of the medicinal fungus *Inonotus obliquus* (Ach.: Pers.) Pilát [13]. Moreover, numerous studies have proven that low-intensity laser radiation and LEDs can be effectively used as an environmentally friendly growth stimulator and biosynthetic activity of biotechnologically important species of edible and medicinal mushrooms [14–19].

It is known that under the influence of light, under certain conditions, the catalytic properties of materials can change, in particular, intensify [20]. The phenomenon of photocatalysis has also been established for nanoparticles of biogenic metals, which act as catalysts for various biochemical reactions [10, 21]. Today, photocatalysis using nanoparticles is widely employed in agriculture and wastewater treatment [22–24].

The medicinal fungus *Laricifomes officinalis* (Vill.) Kotl. and Pouzar, also known as agarikon, has a wide range of pharmacological properties due to the various compounds it contains. Over 150 bioactive compounds, including polysaccharides (such  $as \beta$ -glucans and heteroglucans), triterpenoids, sterols, coumarins, and phenolic compounds,

have been identified in the fruiting bodies and mycelium of *L. officinalis* [25–29]. Due to the combined action of bioactive compounds that mushroom raw materials and agarikon extracts demonstrated a wide range of pharmacological activity [27, 28, 30–33]. Considering the pharmacological activity of *L. officinalis*, intensifying the processes for obtaining mycelial mass and metabolites of this species using environmentally safe factors may be of practical and scientific interest.

 Taking into account the above, the purpose of our work was to study the effect of biogenic metal nanoparticles on the growth characteristics and biosynthetic activity of the medicinal macromycete *L. officinalis*, as well as the effects of photocatalytic activity of NPs following exposure to low-intensity laser radiation under deep cultivation conditions.

## **Materials and Methods**

*Mushroom sample.* The macromycete *Laricifomes officinalis* was chosen as a model object. A pure strain of *L. officinalis* IBK 5004 was supplied by the Mushrooms Collection of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (*IBK*).

*Preparation of Colloidal Solutions of Metal Nanoparticles*. Colloidal solutions of biogenic metals, water-based, such as Fe, Mg, and Ag, produced by a patented method of bittern natural colloidal solutions of the above metals were used [34]. The maximum size of NPs is no more than 100 nm. Colloidal solutions of nanometals were used in a concentration of  $10^{-10}$  M. When choosing the concentration, we were guided by our data and the results obtained by other researchers who studied the possibilities of using them to increase the productivity of agricultural plants [13, 35].

*Inoculum preparation and culture conditions*. Cultivation of the seed mycelium of *L. officinalis* was carried out on a basic liquid nutrient medium and its modification with the addition of a colloidal solution of metal nanoparticles. Basic medium (control) glucose-peptone-yeast (GPY),  $g/l$ : glucose — 30.0; peptone  $-3.0$ ; yeast extract  $-2.0$ ;  $KH_{2}PO_{4} - 1.0$ ;  $K_{2}HPO_{4} - 1.0$ ;  $MgSO_{4}$   $7H_{2}O -$ 0.25; pH 5.5. Modification with the addition of a colloidal solution of metal nanoparticles:

medium A: GPY with silver nanoparticles (AgNPs);

medium B: GPY with iron nanoparticles (FeNPs);

medium C: GPY with magnesium nanoparticles (MgNPs).

100 ml of liquid medium was placed in Erlenmeyer flasks with a capacity of 0.5 L, then sterilized in an autoclave for 0.5 h at a temperature of  $120^{\circ}$ C.

After inoculation of the mycelium *L. officinalis*, it was cultivated in dark at  $25^{\circ}$ C for 12 days under vibration at a speed of 120 rpm.

*Effect of biogenic metal nanoparticles and laser light on growth characteristics and biosynthetic activity*. The study of the effects of metal nanoparticles and low-intensity laser irradiation on the growth characteristics and biosynthetic activity of *L. officinalis in vitro* was conducted using a methodology developed by the authors. The pre-grown physiologically active inoculum of *L. officinalis* was added in an amount of 10% of the total volume into flatbottomed Erlenmeyer flasks with a capacity of 0.5 L containing 100 ml of nutrient medium. After inoculation, part of the flasks was used as a control (without irradiation), and the other part was irradiated with blue laser light  $(\lambda = 488 \text{ nm}).$ 

*Irradiation*. Аrgon ion laser (modified model LGN-106M1 manufactured by NPO "Plasma") with wavelengths of 488.0 nm was used as the source of coherent visible light [19]. The laser irradiation system was developed at the Institute of Physics of the National Academy of Sciences of Ukraine. Irradiation of flasks containing inoculum mycelial was carried out in flasks where the thickness of the layer of nutrient medium with mycelial did not exceed 1 cm). After irradiation, all experimental variants, both irradiated and non-irradiated, were cultured under the conditions described previously.

*Measurement of mycelia growth*. The mycelial biomass produced in each treatment was harvested by vacuum filtration to separate the culture broth. The fungal biomass was washed several times with distilled water and oven dried at  $60^{\circ}$ C until constant weight.

*Quantification of polysaccharides*. The content of exopolysaccharides in the culture liquid was determined by the weight method [18]. Intracellular polysaccharides quantification followed the procedure outlined by our research team [18].

*Preparation of Aqueous-Alcoholic Extracts of Mycelial Mass*. Extracts (70% ethanol and methanol) were prepared at the rate of 20 mg of dry mycelial mass per 1 ml of solvent according to a previously described method [36].

*Determination of Total Phenols Content*. Total phenols of *L. officinalis* extracts were determined according to the method [37].

The absorbance was measured at 750 nm using a spectrophotometer (U-1800, Hitachi Hightechnologies Co., Tokyo, Japan), with gallic acid used as a standard. The content of total phenols was calculated based on the calibration curve of gallic acid [the equation of standard curve: absorbance at 750 nm =  $0.0025C_{\text{gallic acid}} (\mu g/mL) + 0.0982$ ,  $R^2 = 0.985$ ]. Results were expressed as milligrams of gallic acid equivalents (GAEs) per gram of mushroom extract. The studies were carried out in triplicate.

*Determination of Radical scavenging activity (RSA*). The free radical scavenging activity of *L. officinalis* extracts was measured using 1-diphenyl-2-picrylhydrazyl (DPPH) according to a standard procedure [36].

*Statistical Analysis*. Experiments were conducted in five replicates. The Statistica 6.0 software was used for results processing. The significance of the differences between the results was assessed using the Student's t-test, and significance was accepted for *P* values  $< 0.05.$ 

# **Results and Discussion**

*Effect of photoactivated colloidal solutions of metal nanoparticles on the growth activity of L. officinalis.* 

The influence of nanoparticles on biophysical and biochemical processes in living organisms was the sad determined by the chemical composition of nanoparticles, as well as the characteristics of their interaction with the environment, caused by their small size.

The results obtained confirmed our previously established photostimulating effect of low-intensity laser radiation on the growth activity of various types of macromycetes, including *L. officinalis* [14, 18, 36].

The accumulation of mycelial mass by photoactivated *L. officinalis* mycelial without nanoparticles (NPs) was 67.6% higher than in the control (without irradiation and without nanoparticles) (Fig. 1). The addition of a colloidal solution of all nanoparticles used in the work to the inoculum mycelial led to stimulation of growth activity by 31% for AgNPs, for FeNPs and MgNPs by  $50 - 54\%$ compared to the control (mycelium without NPs), respectively. Irradiation of mycelial in a medium with NPs reduced the growth activity of mycelium induced by NPs in all experimental variants. The greatest effect of inhibition of growth activity was recorded for the medium with AgNPs — 23.2%, the least for FeNPs  $-10.5\%$  (Fig. 1).



*Fig. 1.* **Growth activity of** *Laricifomes officinalis* **after exposure to low-intensity laser irradiation in a nutrition medium with nanoparticles** 

The data are presented as mean ± standard error of the mean. \**P* < 0.05 as compared to control (without irradiation and without NPs)

Compared to conventional materials, nanoparticles have unique catalytic, magnetic, chemical, photoelectrochemical, and optical properties [38].

Depending on the reaction mechanism, nanoparticles can be divided into two groups: 1) nanoparticles, which quickly undergo chemical reactions and serve as delivery systems for essential substances to the body: microelements, vitamins, enzymes, drugs; 2) nanoparticles that are relatively stable, maintaining their chemical composition, size, and shape for a long time [39, 40]. Nanoparticles of the second group can remain in the body for a relatively long time, causing a catalytic effect, entering interacting physically with cell membranes or other components, and influencing biochemical processes in the body [41]. Their presence in a biological environment can alter its optical properties, affecting light interactions within it. When light excites surface plasmon resonances in nanoparticles (plasmons), it changes the way nanoparticles interact with biological systems, such as cell membranes and the intercellular environment. This interaction can influence the stimulation of biochemical processes and photodynamic effects in living systems [42, 43].

There is a limited number of scientific literature regarding the biological activity of photoactivated nanoparticles. The high surface-to-volume ratio, especially in metal nanostructures, results in completely different and sometimes unknown electrical, magnetic, and optical properties. Under the conditions of our experiment, biogenic metal nanoparticles — silver, magnesium, and iron — were used. Compounds of these metals served the basis of several important drugs widely used in biology [22, 44–46]. The effectiveness of NPs depends on their ability to interact with cells. The size of NPs plays a crucial role in their cellular interactions and also determines their biodistribution [47].

Currently, colloidal solutions of nanosized biogenic metals (Zn, Fe, Cu, Ag, Mg, Mn, Mo) are used in agriculture at very low concentrations to produce environmentally friendly crops. Due to their nanosize, they easily penetrate cells and therefore can regulate plant growth and development, have antibacterial and antioxidant properties, since they induce endogenous protective mechanisms, optimize the development of metabolic processes under varying weather and climatic conditions that develop during ontogenesis. They contribute to the realization of adaptive and productive potentials inplants [35, 48]. Furthermore, colloidal solutions of biologically active metals obtained through nanotechnology exhibit anti-stress properties and enhance the resistance of cells in animal, plant, bacterial and fungi to various adverse factors [35, 48].

#### **Effect of photoactivated colloidal solutions of metal nanoparticles on polysaccharide synthesis**

Among the biologically active compounds of *L. officinalis*, polysaccharides are of interest due to their wide range of pharmacological properties, such as antimicrobial antioxidant, anti-inflammatory effects [27, 28].

Analysis of the obtained data indicates that laser irradiation induced an increase in the synthesis of both exopolysaccharides and endopolysaccharides in a medium without NPs (Fig. 2). Adding all nanoparticles used in the experiment to the nutrient medium containing the *L. officinalis* inoculum suppressed the biosynthesis of exopolysaccharides (Fig. 2). Laser irradiation in the presence of AgNPs and FeNPs further reduced the synthesis of exopolysaccharides, with a slight increase in their amount observed only in the presence of magnesium nanoparticles.

At the same time, the addition of FeNPs and MgNPs contributed to an increase in the synthesis of endopolysaccharides by more than 2 times. While silver nanoparticles reduced the amount of polysaccharides in the mycelial mass (Fig. 3). Training with laser light in the presence of silver nanoparticles stimulated the synthesis of endopolysaccharides, while MgNPs and FeNPs in the nutrient medium inhibited their formation (Fig. 3).

Polysaccharides and polysaccharideprotein complexes extracted from fruit bodies, mycelial mass and cultural liquid





The data are presented as mean  $\pm$  standard error of the mean.  $\dot{P}$  < 0.05 as compared to control (without irradiation and without NPs)



*Fig. 3.* **Synthesis of endopolysaccharides from** *Laricifomes officinalis* **before and after exposure to laser radiation in a medium with nanoparticles**

The data are presented as mean  $\pm$  standard error of the mean.  $*P < 0.05$  as compared to control (without irradiation and without NPs)

of edible and medicinal macromycetes are recognized as the main components and one of the promising biologically active substances. They are non-toxic, easily isolated and purified, and have a complex effect on the immune system. According to literature data, high molecular weight  $\beta$ -(1,3)-(1,6)glucans are responsible for physiological and pharmacological characteristics of fungal polysaccharides [49–52]. The manifestation of various biological effects of fungal polysaccharides are primarily determined by the uniqueness of their molecule, which is influenced by the characteristics of their primary and secondary structure, molecular weight, monosaccharide composition, type and degree of branching, length of side chains, type of bond in the molecule and its conformation [51–54].

Investigations into the mechanism of the immunomodulatory action of fungal polysaccharides has led to the assumption that there is a complex interaction between immunological, metabolic, and epigenetic changes [51, 55]. It has been experimentally confirmed that  $\beta$ -glucan molecules are responsible for the most biological manifestations [49–52, 55]. It has been established that  $\beta$ -glucans are pathogenassociated molecular structures, and contain fragments that are complementary to the parts that bind the surface receptors of cells

of the innate immune system of animal cells. The central mechanism of action of  $\beta$ -glucans involves interaction with pattern recognition receptors (PRRs) on innate immune cells, such as macrophages and dendritic cells [51,56]. Cellular responses induced by fungal  $\beta$ -glucans depend on their specific interactions with one or more cellular surface receptors (PRRs), such as the Lactosylceramide receptor (LacCer), CR3 receptor, and the Dectin-1 receptor, which can cause further effects in the cell when different signal paths are activated and engaged [51, 56].

The antioxidant, anti-inflammatory, and anticancer activity of *L. officinalis* polysaccharides has been experimentally established [57, 58]. Therefore, the development of technologies to intensify the synthesis of these valuable biologically active substances is relevant. Our results suggest that low-intensity laser light can be used in the biotechnology of deep cultivation of *L. officinalis* as a stimulator of biosynthetic activity, including mycelial mass and polysaccharides (Fig. 1). To intensify the process of intracellular polysaccharide synthesis, the inoculum can be treated with colloidal solutions of FeNPs and MgNPs. At the same time, irradiation with laser light in the presence of nanoparticles of all the biogenic metals we used did not lead to stimulation of the synthesis of polysaccharides.

*Table 1*

**Values of Total Phenolic Content (TPC) of the extracts mycelial Laricifomes officinalis IBK 5004**   $(n = 3, X \pm SD)$ 

<b>Irradiation modes</b>	TPC mg of GAE/g DM	
	Ethanol extract	Methanol extract
Before exposure to low-intensity laser radiation		
control	$56.57 \pm 2.7$	$42.11 \pm 2.2$
AgNPs	$88.31 \pm 3.4$	$81.12 \pm 2.4$
FeNPs	$64.42 \pm 2.1$	$60.81 \pm 2.1$
MgNPs	$61.11 \pm 3.2$ <sup>*</sup>	$58.12 \pm 3.2^*$
After exposure to low-intensity laser irradiation (488 nm)		
control	$84.72 \pm 2.1$	$80.73 \pm 2.1$
AgNPs	$97.31 \pm 3.7$ *	$88.02 \pm 3.4$ *
FeNPs	$72.15 \pm 2.1^*$	$65.31 \pm 2.1$
MgNPs	$73.18 \pm 3.3$	$68.74 \pm 3.1$

*Note.* The data are presented as mean  $\pm$  standard error of the mean.\**P* < 0.05 as compared to control (without nanoparticals and without irradiation).

## **Effect of photoactivated colloidal solutions of metal nanoparticles on the total content of phenolic compounds and antioxidant activity**

The results of spectrophotometric studies showed a high content of phenolic compounds (TPC) in ethanol extracts of the mycelial mass (Table 1). Depending on the treatment of the inoculum with colloidal solutions of NPs and the irradiation mode, a wide range of TPC values was observed from  $42.11 \pm 2.2$  to  $97.31 \pm 3.7$  mg of GAEs/g of dry mass. The highest TPC values were found in ethanol extracts of *L. officinalis* treated with AgNPs and irradiated with laser light —  $97.31 \pm 3.7$  mg of GAEs/g of dry mass. The lowest values were recorded in methanol solutions of mycelial mass with MgNPs without irradiation:  $58.12 \pm 3.2$  mg of GAEs/g of dry mass (Table 1).

AgNPs exhibit enhanced catalytic activity due to their small size and large surface area. Currently, nanoparticles of biogenic metals receive the greatest attention in biomedicine for treating various acute infectious diseases [59]. In addition, among noble metal nanoparticles, AgNPs, and AuNPs are the most chemically biocompatible. Despite their inertness, AgNPs effectively interact with antimicrobial compounds as they induce the production of reactive oxygen species such as hydrogen peroxide, thereby enhancing their antimicrobial activity. Recently, AgNPs were also found to have anti-angiogenic, anti-permeability, and anti-inflammatory potential, making them an effective tool in healthcare field [60].

As per the literature, the composition of *L. officinalis'* fruiting bodies and mycelial mass contains various phenolic compounds. Among the 21 phenolic compounds analyzed, the presence of two (catechin and gallic acid) was confirmed. Additionally, exogenous amino acid phenylalanine was identified in the extract from *in vitro L. officinalis* cultures. However, p-hydroxybenzoic acid was observed in the fruiting body extract [26, 30, 32, 58]. The biological activity of fungal phenolic compounds may be related to their ability to chelate metals, inhibit lipoxygenases, and scavenge free radicals [30, 61, 62]. Many degenerative diseases are associated with the negative effects of free radicals, as they cause oxidative damage to DNA, proteins and other macromolecules. These damages accumulate with age and are considered the main type of endogenous damage to the body leading to aging [61]. Free radicals are neutralized by cellular defense systems, as well as phenolic compounds that protect cells from oxidative damage. However, this is not enough to completely prevent damage caused by oxidative stress. That is why exogenous dietary antioxidants or natural products based on medicinal mushrooms are promising for use as nutraceuticals for chronic diseases [62, 63]. Only a few studies directly link the bioactive compounds of *L. officinalis* with brain functions to date. It was found that administering phenol compounds (flavonoids) daily for 6 weeks in doses ranging from 100 to 400 mg/kg can help counteract oxidative stress in the aging brain of mice. Nervous tissue, due to its high energy and oxygen demands



*Fig. 4.* **Radical scavenging activity (RSA) of mycelial mass extracts of the** *Laricifomes officinalis* **IBK 5004 in an environment with nanoparticles**

The data are presented as mean ± standard error of the mean. \**P* < 0.05 as compared to control (without nanoparticals and without irradiation)





The data are presented as mean  $\pm$  standard error of the mean.  $\dot{P}$  < 0.05 as compared to control (without nanoparticals and without irradiation)

(approximately 20% of the oxygen available in the human body is consumed by the brain), is particularly vulnerable to overproduction of reactive oxygen species. This overproduction can lead to neurodegenerative disorders such as Alzheimer's disease, multiple sclerosis, Huntington's disease, or Parkinson's disease [26, 64].

The results of our study of ethanol and methanol extracts of mycelial mass from *L. officinalis* showed high values of RSA relative to DPPH (Fig.4; Fig. 5). The highest RSA values (96.06%) were observed for samples obtained as a result of treatment with colloidal solutions of AgNPs and laser irradiation (Fig. 5). The smallest values of methanol solutions were treated with MgNPs without irradiation (Fig. 4). The correlation between high RSA and high TPC was observed in all *L. оfficinalis* extracts. Other antioxidants present in further extracts can also be added to RSA.

Our results regarding the correlation between the amount of phenolic compounds and antioxidant activity are consistent with the data of other researchers [37].

The differences in the influence of the biogenic metal nanoparticles and laser irradiation we studied on the biosynthetic activity of *L. officinalis* can be explained by the difference in their ability to penetrate the fungal cell, different mechanisms of biochemical action, and optical properties. Thus, the results of our research suggest

the possibility of implementing effective biotechnologies using colloidal solutions of biogenic metal nanoparticles and low-intensity laser irradiation.

## **Conclusion**

The photocatalytic activity of biogenic metal nanoparticles (AgNP, FeNP and MgNP) as growth regulators and activators of biosynthetic activity of the fungus *L. officinalis* was studied. Environmentally friendly methods and modes of targeted regulation and intensification of the stages of cultivation of *L. officinalis* using colloidal solutions of Fe, Ag and Mg nanoparticles and low-intensity laser irradiation have been proposed.

The new scientifically substantiated experimental results obtained complement and expand our understanding of the processes of photoinduction of macromycetes and can be used to create an environmentally friendly and effective biotechnology for cultivating *L. officinalis in vitro*.

#### *Author Contributions*

Author Contribution O. M. & N. P. planned the work, contributed to the article's conception, manuscript article writing, editing, and conducted a study under submerged cultivation. A. N. defined the light sources design and its modes of operations, calculated the doses of irradiation inoculum,

carried out its irradiation, discussed the irradiation effects, and participated in the paper preparation. K. L. provided colloidal solutions of biogenic metal nanoparticles, discussed the nanoparticles effects, and participated in the paper preparation. N.S. provided the new literature data for review. All authors contributed to the manuscript's revision and read and approved the submitted version.

# **REFERENCES**

- 1. *Bayda S., Adeel M., Tuccinardi T., Cordani M., Rizzolio, F.* The History of Nanoscience and Nanotechnology: From Chemical–Physical Applications to Nanomedicine. *Molecules*. (2019), 25(1): 112. https://doi.org/10.3390/ molecules25010112
- 2. *Kah M., Tufenkji N., White J.C*. Nano-enabled strategies to enhance crop nutrition and protection. *Nature Nanotechnology*. 2019, 14(6): 532–540. https://doi.org/10.1038/s41565- 019-0439-5
- 3. *Lateef A., Darwesh O.M., Matter I.A*. Microbial Nanobiotechnology: The Melting Pot of Microbiology, Microbial Technology and Nanotechnology. 2021: 1-19. https://doi. org/10.1007/978-981-33-4777-9\_1
- 4. *Lowry G.V., Avellan A., Gilbertson L.M*. Opportunities and challenges for nanotechnology in the agri-tech revolution. *Nature Nanotechnology*. 2019, 14(6): 517–522. https://doi. org/10.1038/s41565-019-0461-7
- 5. *Zhang B., Xie M., Bruschweiler-Li L., Brüschweiler R*. Nanoparticle-Assisted Metabolomics. *Metabolites*. 2018, 8(1): 21. https://doi. org/10.3390/metabo8010021
- 6. *Yadav S., Chander S., Gupta A. Kataria N., Khoo K.S.* Biogenic engineered zinc oxide nanoparticle for sulfur black dye removal from contaminated wastewater: comparative optimization, simulation modeling, and isotherms. *Bioengineered.* 2024, 15(1). https:// doi.org/10.1080/21655979.2024.2325721
- 7. *Mitragotri S., Anderson D.G., Chen X., Chow E.K., Ho D., Kabanov A.V., Xu C.* Accelerating the Translation of Nanomaterials in Biomedicine. *ACS Nano*. 2015, 9(7): 6644–6654. https://doi.org/10.1021/acsnano.5b03569
- 8. *Adebayo E.A., Azeez M.A., Alao M.B., Oke M.A., Aina D.A.* Mushroom Nanobiotechnology: Concepts, Developments and Potentials. In: Lateef A., Gueguim-Kana E.B., Dasgupta N., Ranjan S. (eds) Microbial Nanobiotechnology. Materials Horizons: From Nature to Nanomaterials. Springer, Singapore. 2021, https:// doi.org/10.1007/978-981-33-4777-9\_9

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- 9. *Dhanjal D.S., Mehra P., Bhardwaj S., Singh R., Sharma P., Nepovimova E., Kuca K.* Mycology-Nanotechnology Interface: Applications in Medicine and Cosmetology. *International Journal of Nanomedicine*. 2022, 17: 2505– 2533. https://doi.org/10.2147/IJN.S363282
- 10. *Dziwulska-Hunek A., Kachel M., Gagoś M., Szymanek M.* Influence of Silver Nanoparticles, Laser Light and Electromagnetic Stimulation of Seeds on Germination Rate and Photosynthetic Parameters in Pumpkin (*Cucurbita pepo* (L.) Leaves. *Applied Sciences*. 2021, 11(6): 2780. https://doi.org/10.3390/ app11062780
- 11. *Hassan M., Shaaban S.A., El Ziat R.A., Khaled K.A.* Laser-induced changes in the gene expression, growth and development of *Gladiolus grandiflorus* cv. "White Prosperity." *Scientific Reports*. 2024, 14(1): 6257. https://doi.org/10.1038/s41598-024- 56430-6
- 12. *Swathy P.S., Kiran K.R., Joshi M.B., Mahato K.K., Muthusamy A*. He–Ne laser accelerates seed germination by modulating growth hormones and reprogramming metabolism in brinjal. *Scientific Reports*. 2021, 11(1): 7948. https://doi.org/10.1038/s41598-021- 86984-8
- 13. *Poyedinok N., Mykchaylova O., Sergiichuk N., Tugay T., Tugay A., Lopatko S., Matvieieva N.* Effect of colloidal metal nanoparticles on biomass, polysaccharides, flavonoids, and melanin accumulation in medicinal mushroom *Inonotus obliquus* (Ach.:Pers.) Pilát. *Applied Biochemistry and Biotechnology*. 2020, 191(3): 1315–1325. https://doi. org/10.1007/s12010-020-03281-2
- 14. *Mykchaylova O., Dubova H., Negriyko A., et al*. Photoregulation of the biosynthetic activity of the edible medicinal mushroom *Lentinula edodes in vitro*. *Photochemical & Photobiological Sciences*. 2024, 23(3): 435–449. https:// doi.org/10.1007/s43630-023-00529-8
- 15. *Poyedinok N.L., Tugay T.I., Tugay A.V., Mykchaylova O.B., Sergiichuk N.N., Negriyko A.M.* Influence of nitrogen concentration on pho-

toinduced growth, enzymatic activity and melanine synthesis by *Inonotus obliquus* (Ach.:Pers.) Pilát. *Biotechnologia Acta*. 2019, 12(4): 34–41. https://doi.org/10.15407/biotech12.04.034

- 16. *Poyedinok N., Mykchaylova O.B., Tugay N. Tugay A., Negriyko A., Dudka I.* Effect of light wavelengths and coherence on growth, enzymes activity, and melanin accumulation of liquid-cultured *Inonotus obliquus* (Ach.:Pers.) Pilát. *Applied biochemistry and biotechnology*. 2015, 176(2): 333–343. https://doi.org/10.1007/s12010-015-1577-3
- 17. *Poyedinok N.L., Mykchaylova O.B., Sergiichuk N., Negriyko A.M.* Realization of macromycete photoinduced growth activity: influence of cultivation ways and the concentration of carbon and nitrogen. *Innovative Biosystems Bioengineering*. 2018, 2(3): 196–202. https://doi.org/10.20535/ ibb.2018.2.3.134629
- 18. *Mykchaylova O., Dubova H., Lomberg M., Negriyko A., Poyedinok N.* Influence of lowintensity light on the biosynthetic activity of the edible medicinal mushroom *Hericium erinaceus* (Bull.: Fr.) Pers. *in vitro*. *Archives of Biological Sciences*. 2023, 75(4): 489–501. https://doi.org/10.2298/ABS230821040M
- 19. *Poyedinok N.L., Mykchaylova O.B., Dudka I.A*. Effect of low-intensity laser irradiation on the cultivated macromycetes seed culture growth activity. *Microbiology and biotechnology*. 2015, (1): 77–86. https://doi. org/10.18524/2307-4663.2015.1(29).48037
- 20. *Hakki A., Schneider J., Bahnemann D.* in Photocatalysis: Fundamentals and Perspectives, ed. J. Schneider, D. Bahnemann, J. Ye, G. Li Puma, D. D. Dionysiou, J. Schneider, D. D. Dionysiou, The Royal Society of Chemistry, 2016:. 29–50. https://doi. org/10.1039/9781782622338
- 21. *Liu L., Zhang X., Yang L., Ren L., Wang D., Ye J. Metal* nanoparticles induced photocatalysis. *National Science Review*. 2017, 4(5): 761– 780. https://doi.org/10.1093/nsr/nwx019
- 22. *Aragaw T.A., Bogale F.M., Aragaw B.A*. Iron-based nanoparticles in wastewater treatment: A review on synthesis methods, applications, and removal mechanisms. *Journal of Saudi Chemical Society*. 2021, 25(8): 101280. https://doi.org/10.1016/j. jscs.2021.101280
- 23. *Pugazhendhi A., Prabhu R., Muruganantham K. Shanmuganathan R., Natarajan S.* Anticancer, antimicrobial and photocatalytic activities of green synthesized magnesium oxide nanoparticles (MgONPs) using aqueous extract of *Sargassum wightii*. *Journal of Photochemistry and Photobiology B: Biology*. 2019, 190: 86–97. https://doi. org/10.1016/j.jphotobiol.2018.11.014
- 24. *Rodríguez-González V., Terashima C., Fujishima A*. Applications of photocatalytic titanium dioxide-based nanomaterials in sustainable agriculture. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*. 2019, 40: 49–67. https://doi.org/10.1016/j. jphotochemrev.2019.06.001
- 25. *Naranmandakh S., Murata T., Odonbayar B., Suganuma K., Batkhuu J., & Sasak, K.* Lanostane triterpenoids from *Fomitopsis officinalis* and their trypanocidal activity. *Journal of Natural Medicines*. 2018, 72(2): 523–529. https://doi.org/10.1007/s11418- 018-1182-1
- 26. *Gafforov Y., Muszyńska B., Sułkowska-Ziaja K., Tomšovský M., Yarasheva M., Pecoraro L., Mykchaylova O., Rapior, S. Laricifomes officinalis* (Vill.) Kotl. & Pouzar *—* Fomitopsidaceae. 2023: 1237–1251. https://doi. org/10.1007/978-3-031-23031-8\_116
- 27. *Flores G.A., Cusumano G., Ianni F., Blasi F., Angelini P., Cossignani L., Ferrante C. Fomitopsis officinalis*: Spatial (Pileus and Hymenophore) Metabolomic Variations Affect Functional Components and Biological Activities. *Antibiotics*. 2023, 12(4). https:// doi.org/10.3390/ANTIBIOTICS12040766
- 28. *Girometta C.* Antimicrobial properties of *Fomitopsis officinalis* in the light of its bioactive metabolites: a review. *Mycology.* 2018, 10(1): 32–39. https://doi.org/10.1080/2150 1203.2018.1536680
- 29. *Hwang C.H., Jak B.U., Klei L.L., Lankin D.C., McAlpine J.B., Napolitano J.G., Pauli G.F.* Chlorinated coumarins from the polypore mushroom *Fomitopsis officinalis* and their activity against *Mycobacterium tuberculosis*. *Journal of Natural Products*. 2013, https:// doi.org/10.1021/np400497f
- 30. *Muszyńska B., Fijałkowska A., Sułkowska-Ziaja K., Włodarczyk A., Kaczmarczyk P., Nogaj E., Piętka, J. Fomitopsis officinalis*: a Species of Arboreal Mushroom with Promising Biological and Medicinal Properties. *Chemistry and Biodiversity*. 2020, 17(6). https://doi.org/10.1002/cbdv.202000213
- 31. *Mykchaylova O.B., Poyedinok N.L., Shchetinin V.M*. Screening of strains of the medicinal mushroom *Fomitopsis officinalis* (Vill.) Bondartsev & Singer promising for biotechnological use. *Innovative Biosystems and Bioengineering*. 2022, 6(3–4): 110–118. https://doi.org/10.20535/IBB.2022.6.3- 4.271383
- 32. *Fijałkowska A., Muszyńska B., Sułkowska-Ziaja K., Kała K., Pawlik A., Stefaniuk D., Jaszek M.* Medicinal potential of mycelium and fruiting bodies of an arboreal mushroom *Fomitopsis officinalis* in therapy of lifestyle diseases. *Scientific Reports*. 2020, 10(1). https:// doi.org/10.1038/s41598-020-76899-1
- 33. *Shi Z. T., Bao H. Y., Feng S*. Antitumor activity and structure-activity relationship of seven lanostane-type triterpenes from *Fomitopsis pinicola* and *F. officinalis*. *Zhongguo Zhongyao Zazhi*. 2017, 42(5): 915–922. https://doi.org/10.19540/j.cnki.cjcmm.20170121.017
- 34. *Lopatko K.G., Melnichuk M.D., Aftandilyants Y.G., Gonchar E.N., Boretskij V.F., Veklich A.N., Trach, V.V.* Obtaining of metallic nanoparticles by plasma-erosion electrical discharges in liquid mediums for biological application. Annals of Warsaw University of Life Sciences-SGGW. *Agriculture.* 2013, 61: 105–115.
- 35. *Lopatko S., Chayka V*. The main ways for metal nanoparticles degradation. *Biological Systems: Theory and Innovation* 2022, 13(3–4). https://doi.org/10.31548/ biologiya13(3-4).2022.061
- 36. *Mykchaylova O.B., Negriyko A.M., Poyedinok, N.L*. Influence of low-intensity light on the biosynthetic activity of the medicinal macromycete *Laricifomes officinalis* (Fomitopsidaceae, Polyporales) *in vitro*. *Biotechnologia Acta*. 2024, 17(1): 43–54. https://doi. org/10.15407/biotech17.01.043
- 37. *Rašeta M., Popović M., Knežević P., Šibul F., Kaišarević S., Karaman M.* Bioactive Phenolic Compounds of Two Medicinal Mushroom Species *Trametes versicolor* and *Stereum subtomentosum* as Antioxidant and Antiproliferative Agents. *Chemistry & Biodiversity*. 2020, 17(12): e2000683. https://doi. org/10.1002/CBDV.202000683
- 38. *Li Q., Liu F., Li M., Chen C., Gadd G.M*. Nanoparticle and nanomineral production by fungi. *Fungal Biology Reviews*. 2022, 41: 31–44. https://doi.org/10.1016/j. fbr.2021.07.003
- 39. *Guo H., Barnard A.S*. Naturally occurring iron oxide nanoparticles: morphology, surface chemistry and environmental stability. *J. Mater. Chem. A*. 2013, 1(1): 27–42. https://doi.org/10.1039/C2TA00523A
- 40. *Phan H.T., Haes A.J*. What Does Nanoparticle Stability Mean? *The Journal of Physical Chemistry C*. 2019, 123(27): 16495–16507. https://doi.org/10.1021/acs.jpcc.9b00913
- 41. *Salata O*. Applications of nanoparticles in biology and medicine. *Journal of Nanobiotechnology*. 2004, 2(1): 3. https://doi. org/10.1186/1477-3155-2-3
- 42. *Amendola V., Pilot R., Frasconi M., Maragò O.M., Iatì MA. Surface* plasmon resonance in gold nanoparticles: a review. *Journal of Physics: Condensed Matter*. 2017, 29(20): 203002. https://doi.org/10.1088/1361- 648X/aa60f3
- 43. *de Mol N.J., Fischer M.J.E*. Surface Plasmon Resonance: A General Introduction

2010: 1–14. https://doi.org/10.1007/978-1- 60761-670-2\_1

- 44. *Arias L., Pessan J., Vieira A., Lima TMT, Delbem ACB, Monteiro DR.* Iron Oxide Nanoparticles for Biomedical Applications: A Perspective on Synthesis, Drugs, Antimicrobial Activity, and Toxicity. *Antibiotics*. 2018, 7(2): 46. https://doi.org/10.3390/antibiotics7020046
- 45. *Behra R., Sigg L., Clift M.J.D., Herzog F, Minghetti M, Johnston B, Petri-Fink A, Rothen-Rutishauser B.* Bioavailability of silver nanoparticles and ions: from a chemical and biochemical perspective. *Journal of The Royal Society Interface*. 2013, 10(87): 20130396. https://doi.org/10.1098/rsif.2013.0396
- 46. *Magro M., De Liguoro M., Franzago E., Baratella D., Vianello E*. The surface reactivity of iron oxide nanoparticles as a potential hazard for aquatic environments: A study on Daphnia magna adults and embryos. *Scientific Reports*. 2018, 8(1): 13017. https://doi. org/10.1038/s41598-018-31483-6
- 47. *Hoshyar N., Gray S., Han H., Bao G*. The effect of nanoparticle size on *in vivo* pharmacokinetics and cellular interaction. *Nanomedicine*. 2016, 11(6): 673–692. https://doi. org/10.2217/nnm.16.5
- 48. *Vynarchuk K., Lopatko K.G*. Effect of nanoparticles on morphological parameters of wheat. *Molecular Crystals and Liquid Crystals*. 2024: 1–17. https://doi.org/10.10 80/15421406.2024.2358730
- 49. *Bisen P.S., Baghel R.K., Sanodiya B.S., Thakur G.S., & Prasad G.B.K.S. Lentinus edodes*: A Macrofungus with Pharmacological Activities. *Current Medicinal Chemistry*. 2010, 17(22): 2419–2430. https://doi. org/10.2174/092986710791698495
- 50. *Wang Q., Wang F., Xu Z., Ding, Z.* Bioactive Mushroom Polysaccharides: A Review on Monosaccharide Composition, Biosynthesis and Regulation. *Molecules*. 2017, 22(6): 955. https://doi.org/10.3390/molecules22060955
- 51. *Meng X., Liang H., Luo L.* Antitumor polysaccharides from mushrooms: a review on the structural characteristics, antitumor mechanisms and immunomodulating activities. *Carbohydrate Research*. 2016, 424: 30–41. https://doi.org/10.1016/j. carres.2016.02.008
- 52. *Venturella G., Ferraro V., Cirlincione F., Gargano M.L*. Medicinal mushrooms: Bioactive compounds, use, and clinical trials. *International Journal of Molecular Sciences*. 2021, 22(2): 1–31. https://doi.org/10.3390/ IJMS22020634
- 53. *Xu X., Yan H., Tang J., Zhang, X.* Polysaccharides in *Lentinus edodes*: Isolation, Structure, Immunomodulating Activity and

Future Prospective*. Critical Reviews in Food Science and Nutrition*. 2014, 54(4): 474– 487. https://doi.org/10.1080/10408398.20 11.587616

- 54. *Cateni F., Gargano M.L., Procida G., Venturella G., Cirlincione F., Ferraro V.* Mycochemicals in wild and cultivated mushrooms: nutrition and health. *Phytochemistry Reviews*. 2022, 21(2): 339–383. https://doi.org/10.1007/ s11101-021-09748-2
- 55. *Roszczyk A., Turło J., Zagożdżon R., Kaleta B.* Immunomodulatory Properties of Polysaccharides from *Lentinula edodes*. *International journal of molecular sciences*. 2022, 23(16). https://doi.org/10.3390/IJMS23168980
- 56. *Łysakowska P., Sobota A., Wirkijowska A*. Medicinal Mushrooms: Their Bioactive Components, Nutritional Value and Application in Functional Food Production — A Review. *Molecules*. 2023, 28(14): 5393. https://doi. org/10.3390/molecules28145393
- 57. *Muszyńska B., Grzywacz-Kisielewska A., Kała K., Gdula-Argasińska J.* Anti-inflammatory properties of edible mushrooms: A review. *Food Chemistry*. 2018, 243: 373–381. https:// doi.org/10.1016/j.foodchem.2017.09.149
- 58. Sułkowska-Ziaja K., Fijałkowska A., Muszyńs*ka B*. Selected Species of Medicinal/Arboreal Mushrooms as a Source of Substances with Antioxidant Properties. In Reference Series in Phytochemistry 2022, Springer Science and Business Media B.V.: 95–121. https:// doi.org/10.1007/978-3-030-78160-6\_38
- 59. *Vorobyova V., Vasyliev G., Uschapovskiy D., Khrokalo L., Skiba M*. Green synthesis,

characterization of silver nanoparticals for biomedical application and environmental remediation. *Journal of Microbiological Methods*. 12022, 93: 06384. https://doi. org/10.1016/j.mimet.2021.106384

- 60. *Levy L., Sahoo Y., Kim K-S., Bergey E.J., Prasad P.N*. Nanochemistry: Synthesis and Characterization of Multifunctional Nanoclinics for Biological Applications. *Chemistry of Materials*. 2002, 14: 3715–3721. https://doi.org/10.1021/cm0203013
- 61. *Cheung L.M., Cheung P.C.K., Ooi V.E.C*. Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry*. 2023, 81(2): 249–255. https://doi.org/10.1016/ S0308-8146(02)00419-3
- 62. *Reis F.S., Barros L., Martins A., Ferreira I.C.F.R.* Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: An inter-species comparative study. *Food and Chemical Toxicology*. 2012, 50(2): 191–197. https://doi. org/10.1016/J.FCT.2011.10.056
- 63. *Reis F.S., Martins A., Vasconcelos M.H., Ferreira, I.C.F.R.* Functional foods based on extracts or compounds derived from mushrooms. *Trends in Food Science and Technology*. 2017, 66: 48–62. https://doi. org/10.1016/J.TIFS.2017.05.010
- 64. *Teleanu R., Chircov C., Grumezescu A., Volceanov A, Teleanu DM.* Antioxidant Therapies for Neuroprotection — A Review. *Journal of Clinical Medicine*. 2019, 8(10): 1659. https://doi.org/10.3390/jcm8101659

## **ФОТОРЕГУЛЯЦІЯ БІОСИНТЕТИЧНОЇ АКТИВНОСТІ** *Laricifomes officinalis*  **З ВИКОРИСТАННЯМ КОЛОЇДНИХ РОЗЧИНІВ НАНОЧАСТИНОК МЕТАЛІВ ТА ЛАЗЕРНОГО ОПРОМІНЮВАННЯ**

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*Мета.* Робота спрямована на визначення впливу наночастинок біогенних металів на ростові характеристики та біосинтетичну активність лікарського гриба *Laricifomes officinalis*, та ефекти фотокаталітичної активності наночастинок після впливу низькоінтенсивного лазерного випромінювання за умов глибинного культивування.

*Матеріали та методи*. Використовували традиційні мікологічні методи, колоїдні розчини наночастинок біогенних металів, унікальні фотобіологічні методи. Вплив світла на біосинтетичну

активність *L. officinalis* вивчали за допомогою когерентного монохроматичного лазерного світла низької інтенсивності із заданими спектральними та інтенсивними характеристиками. В експерименті використовувалися водні колоїдні розчини наночастинок біогенних металів, таких як FeNPs, MgNPs і AgNPs, отримані методом об'ємного електроіскрового диспергування металів у рідині.

*Результати.* Оброблення інокулюму *L. officinalis* колоїдними розчинами наночастинок усіх використаних металів посилює ріст на  $31-54\%$ , а опромінення інокулюму гриба лазерним світлом у середовищі з наночастинками знижує ростову активність міцелію на 14,4–22,6%. Усі наночастинки металів пригнічували біосинтез позаклітинних полісахаридів, тоді як оброблення посіву колоїдними розчинами FeNPs та MgNPs стимулювало синтез ендополісахаридів. Водночас опромінення лазерним світлом у присутності AgNPs збільшувало кількість ендополісахаридів, тоді як FeNPs та MgNPs дещо пригнічувало їх синтез. Оброблення посівного матеріалу колоїдними розчинами металів і лазерним випромінюванням впливало на кількість загальних фенольних сполук (TPC) у міцеліальній масі. Найвищі значення TPC зафіксовано у етанольних екстрактах міцеліальної маси з AgNPs та опромінених лазерним світлом становлять  $97,31\pm3,7$  мг еквівалента галової кислоти на 1 г сухої маси (мг ЕГК/г). Найнижчі значення у розчинах метанолу з MgNPs без опромінення становили 58,12±3,2 мг ГКЕ/г сухої маси.

*Висновки.* Результати досліджень дають підстави розглядати наночастинки біогенних металів (AgNPs, FeNPs, MgNPs) та низькоінтенсивне лазерне світло як перспективний регулятор біосинтетичної активності *L. officinalis* у біотехнології його культивування.

Ключові слова: лазер, міцелійна маса, полісахариди, загальні фенольні сполуки, антиоксидантна активність.