

The influence of laser irradiation on the development of vegetative mycelium *Pleurotus ostreatus*

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The article presents the results of the study on the influence of laser irradiation on the development of vegetative mycelium and the period of the occurrence of the corcules of the fruit bodies of *Pleurotus ostreatus*. It has been established the growth processes of *P. ostreatus*, can be best stimulated by applying laser irradiation of mycelium with a green spectrum of light for 10 s. According to this mode of exposure, the best increase in the growth rate of mycelium of 38.3% and the appearance of the largest number of rudiments of the fruiting bodies were recorded. Laser irradiation of mycelium for 10 s with red and blue light spectrum increased the growth rate of mycelium from 7.41 to 20.4%, respectively, and the number of rudiments of the fruiting bodies increased by 1.5 to 2 times. Laser irradiation of the mycelium with 5 s, 15 s, and 20 s with red, blue, and green light spectra did not have a significant effect on the growth processes of *P. ostreatus*. These data open significant prospects for the modification of the existing cultivation technologies, which would increase the economic efficiency of the biotechnological cultivation process of *P. ostreatus*.

Keywords: basidium mushrooms, laser irradiation, photoactivation, *Pleurotus ostreatus*

INTRODUCTION

After *Agaricus bisporus*, *Pleurotus ostreatus* is known as the second most cultivated species of edible fungi in the world. Due to the ability of the *Pleurotus* genus to grow on a variety of ligno-cellulosic wastes, their cultivation can be an effective technology for the processing of organic

waste products in agriculture, which would enable the production of protein-rich products and contribute to the reduction of environmental pollution (Bhattacharjya et al., 2015; Figlas et al., 2016). On the other hand, the use of fungi as food will overcome the deficiency of protein in the developing countries. Relying on standard mushroom cultivation techniques, researchers add new technological components to this process that allow shortening the cultivation time, increasing the yield and the quality of the fruiting

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bodies. However, the solution of these issues is impossible without the use of knowledge on the physiology of fungi, combined with information on the environmental factors that affect the growth and development of macromycetes (Tisch, Schmoll, 2010).

One of these factors is the light that regulates morphogenetic processes in many types of fungi, although they are not phototrophic organisms. The nature of the effect of light depends on its spectral characteristics and the duration of the light (Kamada et al., 2010). Mushrooms can absorb almost ultraviolet, blue, green, red, and distant red light, using up to 11 photoreceptors and signalling cascades to control most of the genome and adapt to environmental conditions (Zhenzhong, Reinhard, 2018). Recently, the mechanisms of the photoreceptor of fungi have attracted intensive research (Kritskiy et al., 2010; Tisch, Schmoll, 2010; Poyedinok, 2015). Several types of photoreceptors in mushrooms were described (Herrera-Estrella, Horwitz, 2007). Thus, in the basidiomycetes *Coprinus cinereus*, *Pleurotus ostreatus*, and *Lentinula edodes* the genes encoding the receptors responsible for the perception of blue light were found. The study into the fungal genome revealed the photoreceptor genes encoding proteins that are sensitive to red light (Kamada et al., 2010). The green light is perceived by opsin systems based on retinal, the biological functions of which still need to be clarified (Zhenzhong, Reinhard, 2018). The use of artificial light to stimulate biological processes in fungi is limited by the methods that require prolonged illumination of crops at different stages of morphogenesis, which leads to additional energy expenditure.

On the other hand, studies conducted by Karu at the cellular level for some biological objects showed that short-term irradiation (a few seconds) with low-intensity light in relatively small doses (102–103 J/m²) contributes to a long-lasting macro effect (Karu, 1986). The positive influence of UV and γ irradiation on the yields of *P. ostreatus* fungus was also investigated, and it was found that laser irradiation at doses of 45–230 mJ/cm² stimulated

sprout growth and mycelium growth in *Hericium erinaceus*. The known influence of low-intensity light on the linear growth and biomass accumulation by different types of macromycetes (*Agaricus bisporus*, *Inonotus obliquus*, *Ganoderma lucidum*, *Hericium erinaceus*, and *Lentinula edodes*) (Poyedinok et al., 2013). According to Poyedinok, at different stages of ontogenesis, macromycetes are sensitive to low-intensity light in the visible wavelength range with different spectral and energy characteristics (Poyedinok, 2015). According to modern mechanisms of photoregulation of living organisms, photochemical reactions occur due to the excitation of electrons in the atoms of the absorbing light of matter. At the molecular level, this is expressed as photoionization of matter, its reduction, photooxidation, photodissociation of molecules, and their reorganisation – photoisomerization (Karu et al., 1994). Analysis of the results of research on photoreceptor mechanisms in mushrooms obtained by local and foreign scientists proves the possibility of using laser radiation in order to increase the biological activity of cultivated macromycetes, to stimulate their growth and intensify the technological stages of cultivation. Nonetheless, the above methods of intensifying growth processes are complex in terms of practical use, since they require the use of stationary laser installations, argon and helium-neon lasers that have significant energy intensity, large dimensions and masses. LED lasers, which have high efficiency (up to 50%), speed (up to 10–11 s), comfort of excitation, the possibility of generating radiation with the required wavelength, small dimensions and technological compatibility with elements of optical integrated circuits are much more effective for the intensification of metabolic processes of macromycetes (Vasiura, 1998). Also, they are low cost and require little power consumption when applied. However, the literature data on the influence of LED laser systems on the parameters of fungal growth are limited, thus this issue requires further study. The purpose of the work was to investigate the effect of laser irradiation on the development of vegetative mycelium

and the period of the appearance of the buds of the fruiting bodies of *P. ostreatus*.

MATERIALS AND METHODS

The P-192 strain of *Pleurotus ostreatus* (Jacq.:Fr.) Kumm mushroom from the collection of cabbage mushrooms of the Department of Plant Physiology and Biochemistry of Vasyly's Stus Donetsk National University was studied. The strain under research was isolated in a pure culture of wild-growing fruiting bodies of basidium mushrooms collected in different areas of the Donetsk region. In order to study the effect of laser irradiation on the development of the vegetative mycelium of the *P. ostreatus* fungus, the strain P-192 was cultivated for seven days in standard wort-agar environment (4° by Baling) in standard Petri dishes (9 cm in diameter), prior to the inoculation of mycelia in Petri dishes separated by a sterile steel tube into pieces 5 × 5 mm in size. Then each inoculum was irradiated with LED lasers: BRP-3010-5 with a 635 nm red wavelength radiation, BBP-3010-5 with the radiation of the blue spectrum at a wavelength of 405 nm, and BGP-3010-5 with the green spectrum of radiation at a wavelength of 532 nm (BOB LASER Co., China). The power of each laser was 100 mW. The density of the energy of laser irradiation was calculated by Vakarchuk (Vakarchuk, 2012). The energy dose of irradiation (the energy of light falling into the unit area) was determined as the product of the energy density and the time of irradiation. In all variants of the experiment, the irradiation energy was within the range of 25–102.5 mJ/cm². This value was selected according to the results of the literature (Poyedinok, 2015). Irradiation of mycelium was carried out in keeping with the scheme presented in Table 1. Unsaturated mycelium was used for control culturing. Surface cultivation of mycelium *P. ostreatus* was carried out in a thermostat at a temperature of 26°C in a standard wort-agar medium (4° Baling). Each day of the cultivation period, the radius of the colonies on the media in Petri dishes was measured in four perpendicular directions. The presence of “buckles” – specific morpholog-

Table 1. The scheme of irradiation of mycelial fungus *P. ostreatus*

| Sample variant | Duration of irradiation, s | | | Energy of irradiation, mJ/cm ² |
|----------------|----------------------------|------|-------|---|
| | Red | Blue | Green | |
| 1 | 0 | 0 | 0 | 0 |
| 2 | 5 | 0 | 0 | 25.05 |
| 3 | 0 | 5 | 0 | 25.05 |
| 4 | 0 | 0 | 5 | 25.05 |
| 5 | 10 | 0 | 0 | 51.1 |
| 6 | 0 | 10 | 0 | 51.1 |
| 7 | 0 | 0 | 10 | 51.1 |
| 8 | 15 | 0 | 0 | 77.3 |
| 9 | 0 | 15 | 0 | 77.3 |
| 10 | 0 | 0 | 15 | 77.3 |
| 11 | 20 | 0 | 0 | 102.5 |
| 12 | 0 | 20 | 0 | 102.5 |
| 13 | 0 | 0 | 20 | 102.5 |

ical formations on the *P. ostreatus* hyphae – was determined by microscopic control. After complete overgrowth of Petri dishes with mycelium, they were transferred to a growth chamber at a temperature of 12–16°C to be tested for the ability to form the buds of the fruiting bodies (primordia).

Based on the obtained data, the average daily gain of mycelium, the average speed of the radial growth (mm/day), the time interval before the formation of primordia (number of days), and the average number of primordia per Petri dish were determined. In order to evaluate the growth of the cultures, a method based on the study and analysis of the dynamics of increasing the radius of colonies from the time of cultivation was applied (Bysko et al., 1983).

All the necessary experiments were replicated three times. The method of dispersion analysis was applied to determine the probability of exposure to laser irradiation. The comparison of the mean values was based on the Dunnett method. The processing was carried out using a package of statistical programs designed at the Department of Plant Physiology and Biochemistry of the Donetsk Vasyly's Stus National University (Prysedskyy, 2005).

RESULTS AND DISCUSSION

Since the photoreceptor system of mushrooms is known to be adapted to light in the range of wavelengths from 350 to 730 nm (Corrochano, 2007), this spectral row is represented by the wavelengths chosen by us (red, blue and green spectra). The results of the research demonstrate the positive effect of laser irradiation on the development of vegetative mycelium of the studied strain *P. ostreatus*. The response to irradiation varied according to the spectral characteristics of light and the duration of exposure (Table 2).

From day 3 of cultivation, a significant probable difference was observed in the growth of micelles irradiated with red (Option 5), blue (Option 6), and green (Option 7) light for 10 s compared to non-irradiated mycelium (Option 1). In particular, the average daily gain of mycelium was 3.27, 2.76, and 3.72 mm respectively, i.e., these figures exceeded the control figures by 32.7%, 27.6%, and 37.2%, respectively. Laser irradiation of mycelium for 5 s in the 2nd and 3rd experimental variants accelerated the average daily gain of the mycelium of

the investigated strain by 1.42 mm (red spectrum of irradiation), 1.91 mm (blue irradiation spectrum), and 1.61 mm (green spectrum of irradiation) compared with the control sample. Laser irradiation of mycelium for 15 s with red, blue and green spectra probably increased mycelium growth by only 14.8% (option 8) and 12.7% (options 9 and 10), respectively. Because of irradiation of mycelium for 20 s, no significant change in average daily sprout was observed. On day 4 of cultivation, a significant increase in mycelium was observed because of laser irradiation for 10 s with the green light: by 38.3% more than the control sample. The reaction in response to the effect of the red and blue light spectra at the same duration of exposure was quite high: the average daily gain of mycelium increased by 28.5% and 21.5%, respectively. Micellar irradiation for 5, 15, and 20 seconds did not cause significant changes in mycelium growth. For the effects of green (variant 7) irradiation with a duration of 10 s already for 8 days of cultivation, the radius of the colonies reached 45.05 mm, which is 19.2% more than the control sample. The Petri dishes were completely overgrown with mycelium. In other

Table 2. Increase of the radius of *P. ostreatus* colonies due to laser irradiation

| Sample variant | Age of the colony, days | | | | |
|------------------------|-------------------------|---------------|---------------|---------------|----------------|
| | 2 | 3 | 4 | 8 | 9 |
| Radius of colonies, mm | | | | | |
| 1 | 5.42 ± 0.13 | 10.05 ± 0.25 | 14.34 ± 0.14 | 37.81 ± 0.34 | 43.02 ± 0.11 |
| 2 | 5.63 ± 0.18* | 11.42 ± 0.26* | 14.83 ± 0.14* | 38.13 ± 0.36* | 44.96 ± 0.28* |
| 3 | 5.82 ± 0.26* | 11.91 ± 0.42* | 14.81 ± 1.06* | 37.86 ± 0.28* | 44.54 ± 0.46* |
| 4 | 5.84 ± 0.13* | 11.62 ± 0.37* | 14.86 ± 0.88* | 38.93 ± 0.43* | 44.06 ± 0.41* |
| 5 | 6.92 ± 0.21* | 13.27 ± 0.45* | 18.44 ± 0.07* | 42.04 ± 0.25* | 45.04 ± 0.53* |
| 6 | 6.44 ± 0.32* | 12.76 ± 0.48* | 17.43 ± 0.41* | 39.66 ± 0.42* | 45.09 ± 0.42 * |
| 7 | 6.02 ± 0.71* | 13.72 ± 0.27* | 19.83 ± 0.21* | 45.05 ± 0.43* | 45.06 ± 0.12* |
| 8 | 6.05 ± 0.24* | 11.54 ± 0.49* | 15.15 ± 0.28* | 38.74 ± 0.27* | 44.34 ± 0.13* |
| 9 | 5.94 ± 0.24* | 11.33 ± 1.03* | 15.93 ± 0.31* | 37.86 ± 0.29* | 44.64 ± 0.25* |
| 10 | 5.82 ± 0.32* | 11.32 ± 0.41* | 15.66 ± 0.31* | 38.04 ± 0.17* | 45.04 ± 0.15* |
| 11 | 5.03 ± 0.14* | 10.34 ± 0.21* | 14.74 ± 0.21* | 36.88 ± 0.46* | 43.08 ± 0.42* |
| 12 | 5.86 ± 0.27* | 10.14 ± 0.41* | 14.93 ± 0.32* | 36.54 ± 0.35* | 42.06 ± 0.21* |
| 13 | 5.66 ± 0.16* | 10.93 ± 0.12* | 14.29 ± 0.24* | 37.86 ± 0.48* | 42.09 ± 0.43* |

Note. * – the difference is statistically significant compared to the control variant ($P < 0.05$), (variants of the experiment according to Table 1).

variants of radiation, the change in the radius of colonies of mycelium was negligible and only for the effects of red and blue laser irradiation this figure increased by 11.2% and 4.5%, respectively.

We found out that laser irradiation positively influenced the average rate of the radial growth of mycelial *P. ostreatus*. Thus, for the effects of red laser irradiation of mycelium for 10 s, the average speed of radial growth was 20.4% higher than the control ones, while the effects of exposure to the blue spectrum increased by 7.4% (Fig. 1).

The best growth was recorded as a result of irradiation with the green light for 10 seconds, the average speed of the radial growth exceeded the control sample by 27.8%. Laser irradiation of micelles for 5 s, 15 s, and 20 s with red, blue, and green light spectra did not have a significant effect on the growth processes of *P. ostreatus*.

The next stage of our research was the study of the effect of laser irradiation of mycelium on the formation of the buds of the fruiting bodies (primordia). The appearance of the buds was recorded as early as day 13 of cultivation in almost all variants of experiment, including the control one. In the dishes with unpecked mycelium, the first primordia appeared on day 15 of cultivation. On day 13 of cultivation, most of the primordia (nine pieces) were formed under the effects of laser irradiation with the green

spectrum of light for 10 s. Irradiation with red and blue light for 10 s contributed to the formation of primordia in six pieces. Under the effect of laser irradiation of mycelium for 5 s and 15 s of red, blue, and green light, there were 5–6 pieces of primordia. For these modes of micellar irradiation for 20 s, the number of rudiments of the foetal bodies was insignificant (1–3 pieces). On day 15 of cultivation, the largest number of primordia was formed under the action of radiation for 10 s with the green light – 180.2% more than the control ones. Irradiation with the red and blue light for 10 s increased the number of primordia from 80.3% to 120.4%, respectively. Laser irradiation of mycelium over 5 s and 15 s with red, blue and green light increased the number of formed primordia from 40.3 to 60.4%, respectively. Under the effects of radiation for 20 s, the number of primordia was almost the same as in the control sample. On day 18 of cultivation, the maximum number of buds of the fruiting bodies was recorded under the irradiation of mycelium for 10 s with the green spectrum of light, which is 137.5% higher than the control sample. Micellar irradiation for 5 s, 15 s, and 20 s with red, blue and green light spectra increased the number of primordia by 1.5–2 times. On day 21 of cultivation, the largest number of primordia (26 pieces) was formed due to laser irradiation of mycelium with the green light for 10 s. The irradiation with blue and red light for 10 s

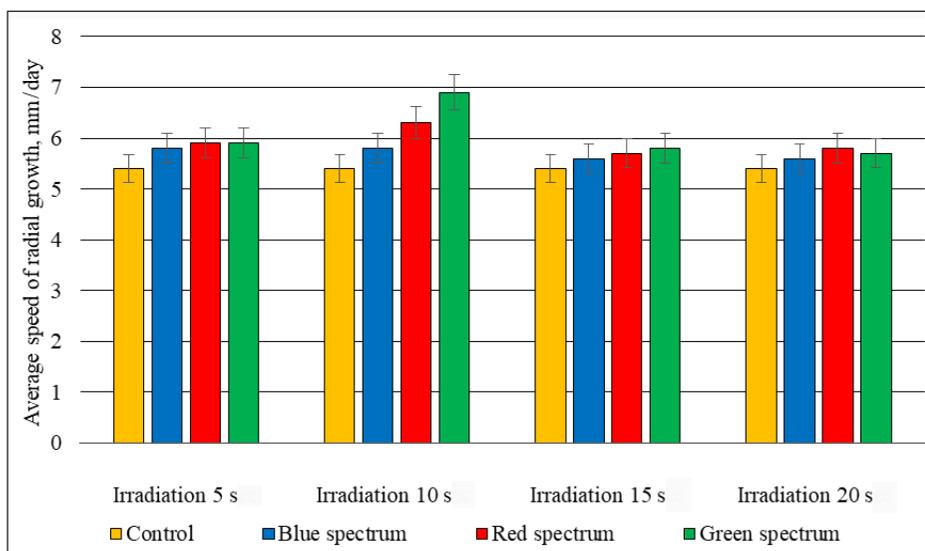


Fig. 1. Influence of laser irradiation on the average rate of the radial growth of *P. ostreatus* mycelium

increased the number of primordia by 46.1% and 38.4%, respectively. In other studied modes of exposure, the number of primordia increased from 23.5 to 30.1% (Fig. 2).

When irradiated for 20 s, the number of primordia almost did not exceed the control sample. On day 24 of cultivation, the most buds of the fruiting bodies formed due to the effects of green laser irradiation that lasted for 10 s; their number was 73.7% higher than the control variant. Laser irradiation with the blue light for 10 s contributed to an increase in the number of primordias by 36.8% compared to the control variant. Irradiation with the red spectrum increased the number of buds of fruiting bodies by 26.3%. The irradiation of mycelium for 5 s and 15 s with red, blue and green spectra increased the number of primordia by 15.8–26.3%. Under irradiation of mycelium for 20 s, a small difference was found between the number of buds of the fruiting bodies compared with the control variant.

It was established that laser irradiation affects the growth processes of *P. ostreatus*. The reaction in response to the laser exposure depends on the duration of exposure and the spectrum of light. The effect of green laser irradiation that lasted for 10 s is the most effective. In this mode

of exposure, the best increase in the growth rate of mycelium was found to be 38.3%, and the greatest number of buds of the fruiting bodies appeared. Laser irradiation with red and blue spectra increased the average radial growth rate from 7.4% to 20.4%, respectively, and the number of buds of the fruiting bodies increased by 1.5–2 times, respectively. The irradiation of mycelium that lasted for 5 s, 15 s, and 20 s did not have a significant effect on the growth processes of *P. ostreatus*. The results of our research coincide with the literature data (Doroshkevich, 2007; Poyedinok, 2015). Responses to the action of laser irradiation are associated with the changes in the parameters of cellular homeostasis, and they fit into the theory of universal mechanisms of photostimulation. According to this theory, the main physical or chemical changes caused by exposure to photoceptor molecules are accompanied by a cascade of biochemical reactions in cells that do not require further activation by light (Karu et al., 1994). The stimulation of growth processes for the production of uterine and sown micelles is crucial in the cultivation of macromycetes, since it reduces the probability of pathogenic microflora contamination with mycelium and improves its adaptive properties.

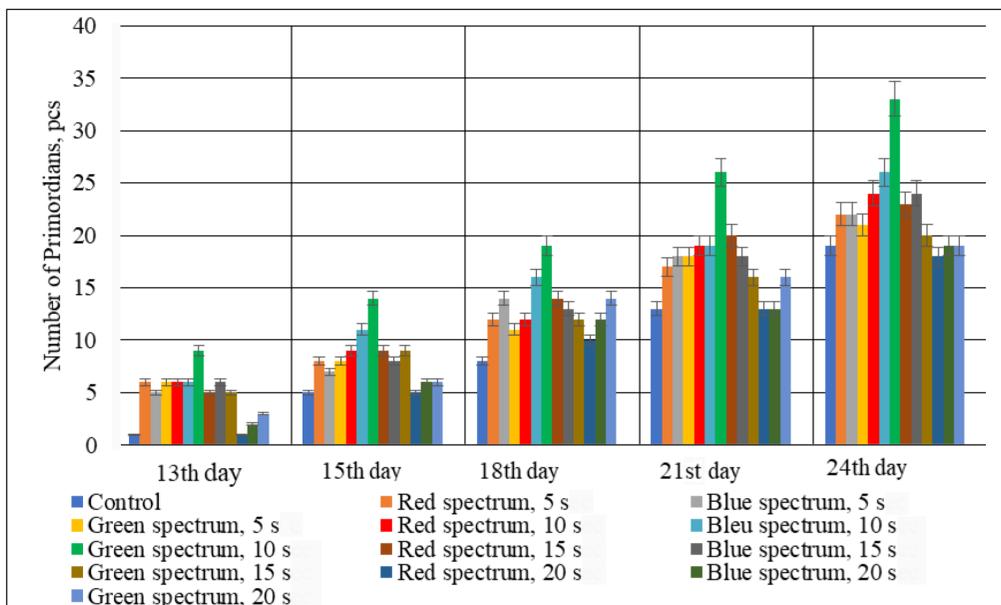


Fig. 2. The effect of laser irradiation on the period of appearance and the number of rhizomes of the fruiting bodies of *P. ostreatus* (variants of the experiment according to Table 1)

CONCLUSIONS

The research validates the fact that the best way to stimulate *P. ostreatus* growth processes, is to apply laser irradiation of mycelium with the green light spectrum for 10 s. The effect of this irradiation regime resulted in a maximum increase in the growth rate of mycelium by 38.3% and the appearance of the largest number of buds of the fruiting bodies. Laser irradiation of mycelium for 10 s with red and blue light spectra increased the growth rate of mycelium from 7.4% to 20.4%, respectively, and the number of buds of the fruiting bodies increased by 1.5 – 2 times. Laser microwave irradiation for 5 s, 15 s, and 20 s with red, blue and green spectra of light did not significantly affect the growth processes of *P. ostreatus*. These data open significant prospects for the modification of existing cultivation technologies, which would increase the economic efficiency of the process of biotechnological cultivation of *P. ostreatus*.

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**LAZERIO SPINDULIUOTĖS POVEIKIS
GLUOSNINĖS KREIVABUDĖS *PLEUROTUS
OSTREATUS* GRYBIENAI**

Santrauka

Straipsnyje pateikiami lazerio spinduliuotės poveikio vegetatyviniam grybienos vystymuisi ir *Pleurotus ostreatus* vaisiakūnių formavimuisi tyrimų rezultatai. Nustatyta, kad norint stimuliuoti *P. ostreatus* augimo procesus, geriausia grybieną 10 sek. veikti žalios šviesos spektro spinduliuote. Taikant šį būdą buvo užfiksuotas ryškus grybienos augimo greičio padidėjimas (38,3 %) ir didžiausias vaisiakūnių užuomazgų skaičius. Paveikus grybieną raudonos ir mėlynos šviesos lazeriu 10 sek., grybienos augimo greitis padidėjo atitinkamai 7,41 ir 20,4 %, o vaisiakūnių užuomazgų skaičius išaugo 1,5–2 kartus. Lazerinė spinduliuotė raudonos, mėlynos ir žalios šviesos spektru 5 sek., 15 sek. ir 20 sek. neturėjo reikšmingo poveikio *P. ostreatus* augimo procesams. Šie duomenys atveria didelę esamų auginimo technologijų modifikavimo perspektyvą, o mūsų tiriamuoju atveju patvirtina *P. ostreatus* biotechnologinio auginimo proceso didesnę ekonominę efektyvumą.

Raktažodžiai: papėdgrybiai, lazerio spinduliuotės poveikis, fotoaktyvinimas, *Pleurotus ostreatus*