DOI: 10.5937/SustFor2592061V
UDK: 581.5:582.284=111
Original scientific paper

THE EFFECT OF TEMPERATURE AND NUTRIENT MEDIUM ON GROWTH OF *Fistulina hepatica*

Aleksandar VEMIĆ[1], Sanja LAZIĆ[1], Katarina MLADENOVIĆ[1], Jelena BOŽOVIĆ[1], Danilo FURTULA[1], Bojan KONATAR[1], Radojica PIŽURICA[1]*

Abstract: *Fungus Fistulina hepatica represents one of the especially important fungi in forestry. Due to better knowledge of Fistulina hepatica bioecology as well as the possibility of usage, effect of temperature and nutrient media on pure cultures was investigated. Temperature of 25 °C was optimal for mycelium growth, while the biggest growth of cultures was recorded on MEA nutrient medium. Somewhat slower growth was recorded on SMA and PDA nutrient medium while the slowest growth was on CMA nutrient medium. On all nutrient media mycelium was white, while there were differences in shape of cultures. Obtained results indicate better possibility of stimulating and growing certain strains of Fistulina hepatica. Medicinal properties and usage of obtained results were discussed.*

Keywords: ecological conditions, heart rot, brown oak, usage

UTICAJ TEMPERATURE I HRANLJIVE PODLOGE NA RAZVOJ *Fistulina hepatica*

Sažetak: *Gljiva Fistulina hepatica predstavlja jednu od posebno značajnih gljiva u šumarstvu. Zbog boljeg poznavanja bioekologije Fistulina hepatica, kao i mogućnosti njenog intenzivnijeg korišćenja, ispitan je uticaj temperature i hranljive podloge na razvoj čistih kultura. Temperatura 25 °C je bila optimalna za razvoj micelije, dok je najveći rast kultura zabeležen na MEA hranljivoj podlozi. Nešto sporiji rast je zabeležen na SMA i PDA hranljivoj podlozi dok je najsporiji rast bio na CMA hranljivoj podlozi. Na svim hranljivim podlogama micelija je bila bele boje, dok su postojale razlike u izgledu kultura. Dobijeni rezultati ukazuju na bolju mogućnost stimulisanja i gajenja pojedinih sojeva Fistulina hepatica. Lekovita svojstva gljive i upotreba dobijenih rezultata su diskutivani.*

Ključne reči: ekološke karakteristike, centralna trulež, smeđi hrast, korišćenje

[1] Institute of Forestry, Kneza Višeslava 3, 11030 Belgrade, Serbia
*Corresponding author. E-mail: aleksandar.vemic2@gmail.com

1. INTRODUCTION

The genus *Fistulina* consists of poroid fungi including edible species (González et al., 2021). *Fistulina hepatica* (Schaeff.) With. causes brown heart rot, most often in oaks (*Quercus* spp.), that in the initial phase leads to change in colour of the wood, and that is why this type of wood is especially valued in industry (Schwarze et al., 2000; Karadžić 2010). In addition to oaks, the occurrence of *F. hepatica* has also been confirmed on sweet chestnut (*Castanea sativa* Mill.) on which it causes similar damages (Piętka & Ciurzycki 2018).

In the case of sweet chestnut (*Castanea sativa*), a correlation was established between certain environmental factors such as altitude, stand density, average annual temperature, or exposure, and increase in rot intensity (Regué et al., 2019). The time of ripening of *F. hepatica* fruiting bodies has a greater significance then the temperature for spreading of spores of this fungus (Marčiulynas & Menkis 2023). Therefore, the objective of the research was to examine the influence of temperature and nutrient medium on the development of tested isolate of *F. hepatica*. The obtained results will have simultaneous importance in the protection of forests as well as in better production, i.e. the use of this fungus.

2. MATERIAL AND METHOD

2.1 The examining of the effect of temperature on the growth of cultures

The isolate of *F. hepatica*, strain HF1 was taken from mycological collection of the Institute of Forestry, Belgrade. Fragments measuring 5×5 mm were placed in the centre of petri dish on 3% MEA (malt extract - Biolab, Hungary; agar - Torlak, Serbia) nutrient medium.

The petri dishes were exposed to temperatures of 12 °C, 17 °C, 25 °C and 30 °C. The experiment was completed after 34 days when the first culture filled the petri dish, and all cultures showed clear growth. Each of the temperatures contained 3 repetitions. The diameter of the cultures was measured in 2 directions at right angles from the centre of the petri dish. The average diameter value was used for comparing the growth.

2.2 The examining of the effect of nutrient medium on the growth of cultures

For examining of the effect of nutrient medium on the growth of cultures, the isolate of *F. hepatica*, identical as in the experiment of examining the effect of temperature was used. Four types of nutrient media were selected: sabouraud maltose agar (SMA; Torlak, Serbia), malt extract agar (MEA; Lab M, UK), corn meal agar (CMA; Himedia, India) and potato dextrose agar (PDA; Lab M, UK). Each of the nutrient media contained 9 to 10 repetitions. Cultures were stored in an incubator at 25 °C in dark regime. The experiment was completed after 25 days when the first culture filled petri dish. The diameter of the cultures was measured in the same way as when examining the effect of temperature.

2.3 Statistical methods

The dimensions of cultures in experiments were tested for normality and homogeneity of variances, as well as normality of residuals. Depending on the statistical significance of the result, parametric or non-parametric tests were used. General linear model (GLM) was used for examining the effect of temperature on the growth of cultures. Tukey's post hoc test was used for determining the differences in growth of cultures between different pairs of temperatures.

Kruskal-Wallis test was used for testing of differences in dimensions of cultures on different nutrient media. Dunn's post hoc test was used for comparison of different pairs of cultures between tested nutrient media.

All statistical analyses were carried out using software packages SPSS 27 (IBM Corp.) and Microsoft Office Excel 2021 (Microsoft Corp.).

3. RESULTS AND DISCUSSION

There was statistically significant difference in the growth of *F. hepatica* cultures at different temperatures ($F = 662.938$; $p < 0.001$). Cultures at 12 °C showed the slowest growth, with an average diameter of cultures of 8.00 mm (Figure 1). Larger dimensions of cultures, with an average value of 51.17 ± 3.75 were at 17 °C (Figure 1). The largest diameter of cultures was recorded at 25 °C, and it amounted to 80.17 ± 1.53 mm (Figure 1). At 30 °C the growth of the cultures was also good, with an average value of 65.17 ± 1.04 mm, slightly smaller then at 25 °C (Figure 1).

The colour of the mycelia was the same at all temperatures. The cultures were white, cottony and raised around inoculum.

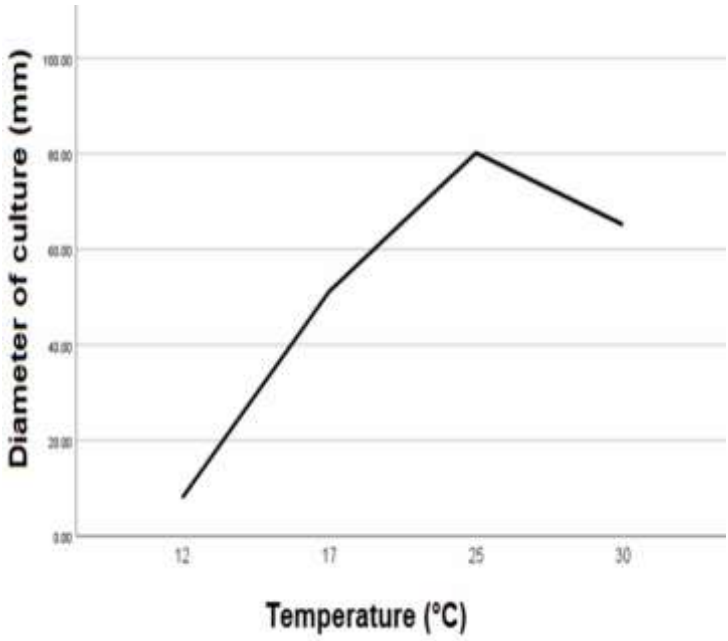

Figure 1. *Growth of Fistulina hepatica cultures at different temperatures*

Nutrient medium showed great influence on growth and appearance of the cultures (Figure 2, Figure 3). There was statistically significant difference in growth of cultures on different nutrient media ($H = 27.737$; $p < 0.001$).

The largest growth of cultures was on MEA nutrient medium (81.22 ± 2.25 mm), and somewhat slower on PDA nutrient medium (78.05 ± 1.99 mm). Nutrient medium SMA showed the same growth as MEA and PDA, i.e. it was on the transition between these two nutrient media (80.80 ± 0.86 mm). The slowest growth of the cultures was on CMA nutrient medium (69.75 ± 2.62 mm).

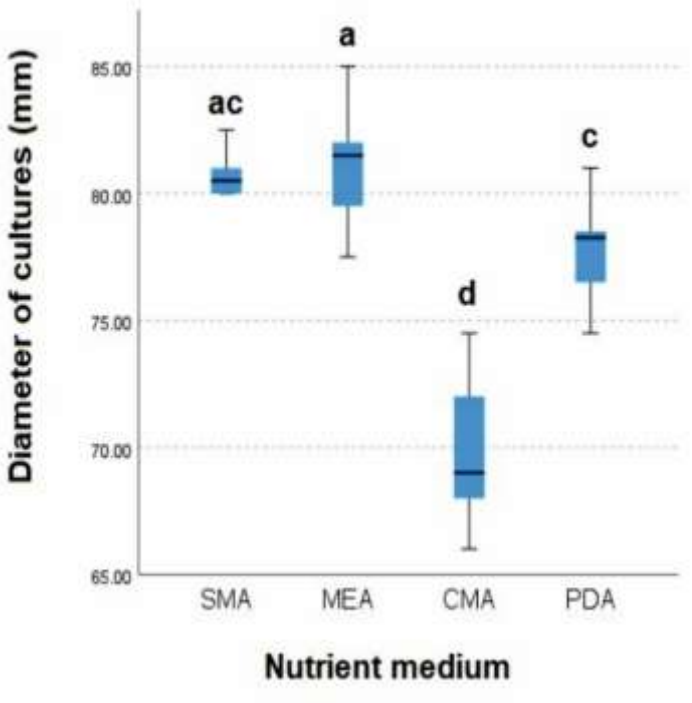

Figure 2. *Growth of Fistulina hepatica cultures on different nutrient media*

The appearance of cultures differed depending on the nutrient medium used (Figure 3). On SMA nutrient medium, the mycelia were white, solid, compact, occasionally grey in central part (Figure 3). On MEA and PDA nutrient media it was white, velvety, cottony, raised around inoculum (Figure 3). On CMA nutrient medium the mycelia were white, raised around inoculum, slightly concentric (Figure 3).

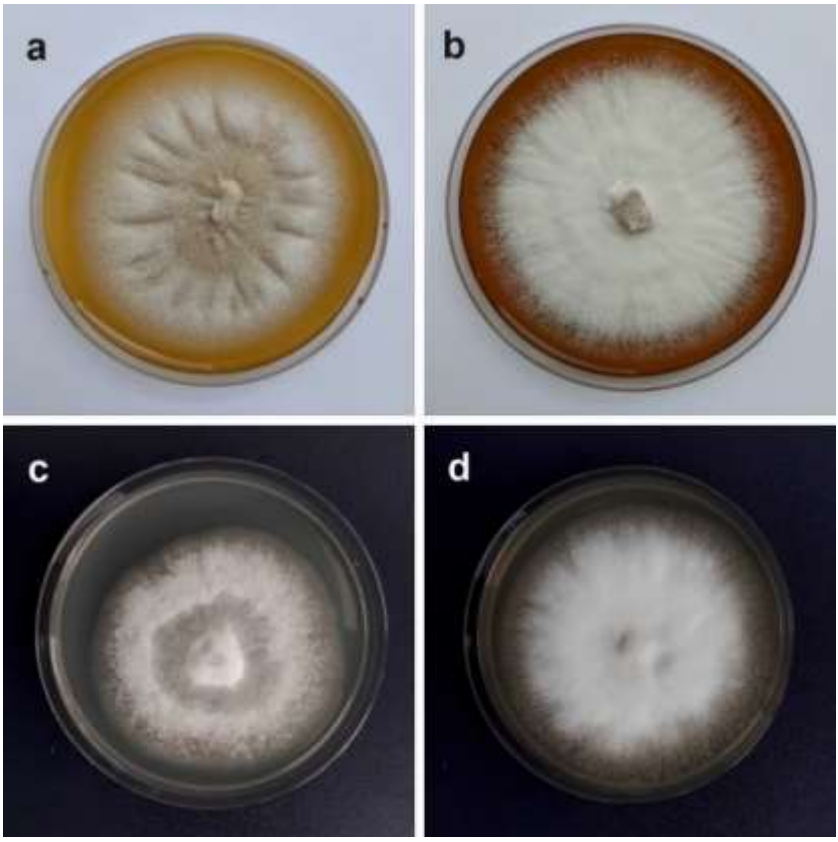

Figure 3. *Representative photographs of Fistulina hepatica cultures on different nutrient media: a) SMA, b) MEA, c) CMA, d) PDA*

The most important medicinal properties of *F. hepatica* are presented below, based on literature review (Table 1). Several categories of the most important medicinal properties were singled out regarding literature sources (Table 1).

Table 1. *Medicinal properties of Fistulina hepatica*

Use value	Component	Reference
Antibacterial properties	Methanolic extracts, polyacetylenic fatty acid derivatives, octadeca-8,11-dienoic acid methylester	Karaman et al. (2009) Aleem et al. (2012) Ivanova et al. (2013) Dubljanin et al. (2018) Whaley et al. (2023)
Antifungal properties	Feldin	Lee et al. (2020)
Antioxidant properties	Alkali extracts	Oke & Aslim (2011) Savino et al. (2016) Dubljanin et al. (2018) Rašeta et al. (2025)
Bioactive components	Proteins, phenols	Yıldız et al. (2017), Fedotov (2020)
Other properties (diabetes treatment, etc.)	Volatile components	Wu et al. (2005) Rašeta et al. (2025)
Traditional use	-	Jorjadze et al. (2023)

Efficient use of *F. hepatica*, as well as preventing damages which occur in woods depend on timely detection of this fungus. In the case of sweet chestnut (*Castanea sativa*) wood loses its value even in the initial stage of rotting due to the change of colour of wood (Meijer et al., 2025). It has been determined that higher temperatures have a more favourable effect on the development of this fungus than lower temperatures. In this sense, an increase of temperature as a consequence of climate change can have a negative effect on the trees, increasing damages. Heart rot has a long period of development, while infection is present on young trees (Vasaitis 2013). Therefore, earlier thinning of the stands significantly reduces occurrence of this fungus (Meijer et al., 2024). Hence, more frequent monitoring of the health and vitality of the trees is recommended.

On the other hand, occurrence of *F. hepatica* fruiting bodies can be positive for easier isolation and use of this fungus. Determined optimal temperature and nutrient medium can significantly accelerate the growth of certain isolates of *F. hepatica*. Cultivation of *F. hepatica* has several advantages. First of all, there are examples that the content of metals, including heavy metals, is higher in wild than in cultivated specimens of *F. hepatica* (Yildiz et al., 2019; Thachunglura et al., 2025).

Taxonomy of the species *F. hepatica* is insufficiently studied. The imperfect stage of *F. hepatica* belongs to the genus *Confistulina* (Stalpers & Vlug 1983). Intensive cultivation of *F. hepatica*, comparing of different isolates, and inoculation of trees can significantly contribute to better knowledge of morphology of this fungus.

5. CONCLUSION

This paper presents the effect of temperature and nutrient medium on the growth of cultures of the tested isolate of *F. hepatica*. The obtained results have a great significance for understanding the intensity of the rot in natural conditions, as well as the possibility of cultivation of the fungus *F. hepatica*. In accordance with the obtained results the stated conclusions can be presented as follows:

- The optimal temperature for growth of the tested isolate was 25 °C. The smallest growth was recorded at 12 °C. Somewhat larger growth was at 17 °C and 30 °C.
- The average growth rate at 25 °C amounted to 2.36 mm/day, while at 12 °C it amounted to 0.24 mm/day. At 17 °C and 30 °C the average growth rate was 1.50 mm/day and 1.92 mm/day.
- The best nutrient medium for growth of *F. hepatica* cultures was malt extract agar (MEA). Similar growth occurred on potato dextrose agar (PDA), while the growth was somewhat smaller on corn meal agar (CMA). The smallest growth was on sabouraud maltose agar (SMA) nutrient medium.
- The cultures of *F. hepatica* were white on all nutrient media. However, the appearance of the mycelia differed depending on the nutrient medium.
- The most important medicinal properties of *F. hepatica* were divided in several groups: antibacterial properties, antifungal properties, antioxidant

properties and other properties such as the treatment of diabetes. In addition, *F. hepatica* has many bioactive components which can be used in different ways.

Acknowledgement: *This research was carried out under the Agreement on funding by the Ministry of Science, Technological Development and Innovation for the year 2025 451-03-136/2025-03/200027.*

REFERENCE

Aleem, A., Anjum, N.F., & Kavitha, A.N. (2012). Comparative In Vitro Antimicrobial Activity of Three different Macro Fungus Extracts Against Clinical Isolates of Gram-Negative Pathogens. *Research Journal of Pharmacognosy and Phytochemistry, 4(1),* 29-32.

Dubljanin, T., Petrović, M., Kozarski, M., Vunduk, J., Nikšić, M. & Klaus, A. (2018). *Antioxidant and antimicrobial activity of mushrooms Daedalea quercina and Fistulina hepatica*. In M. Pojić (Ed.), IV International congress Food technology, quality and safety (pp.110-116). https://fiver.ifvcns.rs/bitstream/id/6766/bitstream_6766.pdf

Fedotov, O.V. (2020). Phenolic substances as regulators of the intensity of lipid peroxidation processes of the strains *Pleurotus eryngii*, *Fistulina hepatica* and *Agrocybe cylindracea*. *Regulatory Mechanisms in Biosystems, 11(2),* 232-236. https://doi.org/10.15421/022034

González, G.C., Barroetaveña, C., Visnovsky, S.B., Rajchenberg, M., & Pildain, M.B. (2021). A new species, phylogeny, and a worldwide key of the edible wood decay *Fistulina* (Agaricales). *Mycological Progress, 20,* 733-746. https://doi.org/10.1007/s11557-021-01696-7

Ivanova, V., Kolarova, M., Aleksieva, K., Schlegel, R., Schumann, P., & Graefe, U. (2013). Octadeca-8,11-dienoic Acid Methylester, a New Fatty Acid Metabolite from *Fistulina hepatica*. *Journal of Modern Medicinal Chemistry, 1(1),* 43-48. https://doi.org/10.12970/2308-8044.2013.01.01.4

Jorjadze, A., Bussmann, R.W., Paniagua Zambrana, N.Y., Batsatsashvili, K., & Svanidze, E. (2023). Traditional use of Polypores in Georgia (the Caucasus). *Ethnobotany Research and Applications, 26,* 1-18. http://dx.doi.org/10.32859/era.26.5.1-18

Karadžić, D. (2010). Šumska fitopatologija, Univerzitet u Beogradu, Šumarski fakultet.

Karaman, M., Mimica-Dukic, N., Knezevic, P., Svircev, Z., & Matavuly, M. (2009). Antibacterial Properties of Selected Lignicolous Mushrooms and Fungi from Northern Serbia. *International Journal of Medicinal Mushrooms, 11(3),* 269-279. https://doi.org/10.1615/IntJMedMushr.v11.i3.60

Lee, J., Shi, Y.-M., Grün, P., Gube, M., Feldbrügge, M., Bode, H., & Hennicke, F. (2020). Identification of Feldin, an Antifungal Polyyne from the Beefsteak Fungus *Fistulina hepatica*. *Biomolecules, 10(11),* 1502. https://doi.org/10.3390/biom10111502

Marčiulynas A, & Menkis A. (2023). The Release and Spread of Basidiospores of Red-Listed Wood-Decay Fungus *Fistulina hepatica* in Oak Stands. *Diversity, 15(11),* 1110. https://doi.org/10.3390/d15111110

Meijer, A., Muñoz-Adalia, E. J., & Colinas, C. (2024). Early Thinning: A Promising Tool to Prevent *Fistulina hepatica* Heart Rot in *Castanea sativa* Coppice Stands. *Forests*, *15*(9), 1639. https://doi.org/10.3390/f15091639

Meijer, A., Muñoz-Adalia, E.J., Correal-Mòdol, E., & Colinas, C. (2025). Early detection of heartwood rot caused by *Fistulina hepatica* in *Castanea sativa* productive coppices through low-invasive resistance drilling. *Wood Science and Technology*, *59*, 10. https://doi.org/10.1007/s00226-024-01616-4

Oke, F., & Aslim, B. (2011). Protective effect of two edible mushrooms against oxidative cell damage and their phenolic composition. *Food Chemistry, 128(3),* 613-619. https://doi.org/10.1016/j.foodchem.2011.03.036

Piętka, J., & Ciurzycki, W. (2018). The first record of *Fistulina hepatica* (Schaeff.) With. on *Castanea sativa* Mill. in Poland. *Acta Mycologica, 53(1),* 1108. https://doi.org/10.5586/am.1108

Rašeta, M., Mišković, J., Kebert, M., Berežni, S., Krstić, S., Gojgić-Cvijović, G., Pirker, T., Bauer, R., & Karaman, M. (2025). Mycochemical profiles and bioactivities of *Fistulina hepatica* and *Volvopluteus gloiocephalus* from Serbia: Antioxidant, enzyme inhibition, and cytotoxic potentials. *Food Bioscience, 66,* 106221. https://doi.org/10.1016/j.fbio.2025.106221

Regué, A., Bassié, L., de-Miguel, S., & Colinas, C. (2019). Environmental and stand conditions related to *Fistulina hepatica* heart rot attack on *Castanea sativa*. *Forest Pathology, 49(3),* e12517. https://doi.org/10.1111/efp.12517

Savino, E., Girometta, C., Miteva-Staleva, J., Kostadinova, A., & Krumova, E. (2016). Wood decay macrofungi: strain collection and studies about antioxidant properties. *Comptes rendus de l'Académie bulgare des Sciences, 69(6)*, pp. 747+.

Schwarze, F.W.M.R., Baum, S., & Fink, S. (2000). Dual modes of degradation by *Fistulina hepatica* in xylem cell walls of *Quercus robur*. *Mycological Research, 104*, 846-852. https://doi.org/10.1017/S0953756299002063

Stalpers, J.A., & Vlug, I. (1983). *Confistulina*, the anamorph of *Fistulina hepatica*. *Canadian Journal of Botany, 61(6),* 1660-1666. https://doi.org/10.1139/b83-178

Thachunglura, V.L., Rai, P.K., Chawngthu, Z., Lalzarzovi, S.T., Zothanzama, J., & Vanlalhluna, P.C. (2025). Heavy metals bioaccumulation in wild edible mushrooms of an Indo Burma biodiversity hotspot: Suitability assessment for safe consumption and food safety/security implications. *International Journal of Environmental Quality, 70*, 66-75. https://doi.org/10.6092/issn.2281-4485/21806

Vasaitis, R. (2013). Hearth Rots, Sap Rots and Canker Rots, In P. Gontier., & G. Nicolotti (Eds.), *Infectious forest diseases*, (pp. 197-229). CAB International, UK. https://doi.org/10.1079/9781780640402.0197

Whaley, A.O., Whaley, A.K., Orlova, A.A., Volobuev, S.V., Shakhova, N.V, Smirnov, S.N., Pustovit, N.V., & Kraeva, L.A. (2023). Polyacetylene Derivatives from the Polypore Fungus

(*Fistulina hepatica*, Agaricomycetes) and Their Antimicrobial Activity. *International Journal of Medicinal Mushrooms, 25(12)*, 43-53. https://doi.org/10.1615/IntJMedMushrooms.2023050525

Wu, S., Krings, U., Zorn, H., & Berger, R.G. (2005). Volatile compounds from the fruiting bodies of beefsteak fungus *Fistulina hepatica* (Schaeffer: Fr.) Fr. *Food Chemistry, 92(2)*, 221-226. https://doi.org/10.1016/j.foodchem.2004.07.013

Yildiz, S., Gürgen, A., & Çevik, U. (2019). Accumulation of metals in some wild and cultivated mushroom species. *Sigma Journal of Engineering and Natural Sciences, 37(4)*, 1375-1384.

Yıldız, S., Yılmaz, A., Can, Z., Adel Tabbouche, S., Kılıç, A.O., & Sesli, E. (2017). Some bioactive properties of wild and comertial mushroom species. *Journal of Food and Health Science, 3(4)*, 161-169. https://doi.org/10.3153/JFHS17019

THE EFFECT OF TEMPERATURE AND NUTRIENT MEDIUM ON GROWTH OF *Fistulina hepatica*

Aleksandar VEMIĆ, Sanja LAZIĆ, Katarina MLADENOVIĆ, Jelena BOŽOVIĆ, Danilo FURTULA, Bojan *KONATAR, Radojica PIŽURICA*

Summary

Knowledge of bioecological characteristics of different fungi has great importance for various aspects in forestry. Importance of fungus *Fistulina hepatica* is reflected in specific influence on wood colour in initial decay stage. Moreover, *F. hepatica* possesses great medicinal properties. In this study, the effect of temperature and nutrient medium on the development of *F. hepatica* cultures were examined.

Results showed effect of temperature and nutrient medium on growth rate and characteristics of cultures. The fastest growth was recorded at 25°C, followed with 30°C, something smaller growth at 17°C while the smallest growth was at 12°C. The best nutrient medium for *F. hepatica* growth was malt extract agar (MEA). Nutrient media sabouraud maltose agar (SMA) and potato dextrose agar (PDA) showed similar and somewhat weaker effect on growth of *F. hepatica* mycelium. Cultures of *F. hepatica* had the slowest growth on corn meal agar (CMA) nutrient medium.

Appearance of cultures was different according to nutrient medium. On SMA nutrient medium mycelium was compact with expressed stretches, while aerial on edges. On MEA and PDA nutrient media mycelia was fallen, cottony, velvety, on place of inoculum raised. On CMA nutrient medium mycelium was concentric, on place of inoculum also raised.

Prevention of greater damages of trees and medicinal properties of *F. hepatica* were discussed.

UTICAJ TEMPERATURE I HRANLJIVE PODLOGE NA RAZVOJ *Fistulina hepatica*

Aleksandar VEMIĆ, Sanja LAZIĆ, Katarina MLADENOVIĆ, Jelena BOŽOVIĆ, Danilo FURTULA, Bojan *KONATAR, Radojica PIŽURICA*

Rezime

Poznavanje bioekoloških karakteristika različitih gljiva ima veliki značaj za različite aspekte šumarstva. Značaj gljive *Fistulina hepatica* se ogleda u specifičnom uticaju na boju drveta u početnoj fazi truleži. Osim toga, *F. hepatica* poseduje velika medicinska svojstva. U ovoj studiji je ispitan uticaj temperature i hranljive podloge na rast kultura *F. hepatica*.

Rezultati su pokazali uticaj temperature i hranljive podloge na brzinu i karakteristike kultura *F. hepatica*. Najbrži rast kultura je zabeležen na 25°C, potom na 30°C, nešto manji rast na 17°C dok je najmanji rast bio na 12°C. Najbolja hranljiva podloga za razvoj *F. hepatica* je bila malc ekstakt agar (MEA). Hranljive podloge sabouraud maltoza agar (SMA) i krompir dekstroza agar (PDA) su pokazale sličan i nešto slabiji efekat na rast micelije *F. hepatica*. Kulture *F. hepatica* na hranljivoj podlozi kukuruzna kaša agar (CMA) su imale najsporiji rast.

Izgled kultura se razlikovao na različitim hranljivim podlogama. Na SMA hranljivoj podlozi micelija je bila kompaktna sa izraženim linijama i po ivicama vazdušna. Na MEA i PDA hranljivoj podlozi micelija je bila polegla, pamučna, baršunasta, na mestu inokuluma podignuta. Na CMA hranljivoj podlozi micelija je bila koncentrična, na mestu inokuluma takođe podignuta.

Sprečavanje većih šteta na stablima i medicinska svojstva *F. hepatica* su diskutovani.