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Стручни рад

EFFECTS OF *Laetiporus sulphureus* (Bull. ex Fr.) Murrill FUNGUS ON THE BENDING STRENGTH OF SESSILE OAK WOOD

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Abstract: Testing samples were collected from the heartwood of healthy oak trees in Eastern Serbia. Over the periods of two, four and six months, the wood samples were exposed to the mycelia of the fungus that causes cubical brown rot on oak - *Laetiporus sulphureus* (Bull. ex Fr.) Murrill (Sulphur Polypore).The contribution of *Laetiporus sulphureus* (Bull. ex Fr.) Murrill to the decrease in the bending strength of *Q. petraea* wood was investigated. After two, four and six months of being under the effect of *L. sulphureus,* the static bending strength of oak wood substantially decreased compared to the initial value (100%) and amounted to 91.73, 75.17 and 63.25%. The regression line obtained through data processing opened up the possibility to predict the changes of wood properties in certain time periods of the fungus effect under the unchanged environmental conditions.

Keywords: bending strength, sessile oak

УТИЦАЈ ГЉИВЕ *Laetiporus sulphureus* (BULL. EX FR.) MURRILL НА ЧВРСТОЋУ НА САВИЈАЊЕ ДРВЕТА ХРАСТА КИТЊАКА

Извод: Узорци за испитивање прикупљени су из срчике здравог стабла храста у Источној Србији. Током периода испитивања од 2, 4 и 6 месеци узорци дрвета били су изложени утицају мицелије гљиве која узрокује мрку призматичну трулеж храста *Laetiporus sulphureus* (Bull. ex Fr.) Murrill (сумпорњача). Испитан је утицај утицај гљиве *Laetiporus sulphureus* (Bull. ex Fr.) Murrill на смањење чврстоће на савијање дрвета *Q. petraea*. После 2, 4 и 6 месеци под дејством гљиве *L. sulphureus* статичка чврстоћа на савијање храстовог дрвета значајно се смањила у поређењу са почетном вредношћу (100%) и износила је 91,73, 75,17 и 63,25%. Регресиона линија добијена обрадом података отворила је могућност прогнозирања промена својстава дрвета у одређеним временским периодима дејства гљиве под непромењеним условима спољне средине.

Кључне речи: чврстоћа на савијање, храст китњак

1. INTRODUCTION

The development of the wood processing industry is causing a growing demand for high-quality wood raw materials (Miric, M., Schmidt, O., 1992; Miric, M. *et al.,* 2012; Popovic, V. *et al.,* 2020). According to several authors

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(Karadzic, D., 2006; Karadzic, D., 2010; Miric, M., 1993; Schmidt, O., 1994, Karadzic, D. *et al.,* 2020), this demand calls for the preservation and improvement of wood durability, which is directly related to the preservation of physical, chemical, mechanical, aesthetical and other properties. The percentage of the main structural constituents of wood (cellulose, hemicellulose and lignin) varies with species and parts of trees. There is thus more cellulose in softwood than in hardwood tree species, in bolewood more than in branchwood, in earlywood more than in latewood, as discussed by Miric, M., Popovic, Z.V. (1993).

As a host, oak is colonized by a large number of microorganisms, with the focus being on the research of the impact of fungi, particularly those attacking the heartwood as the most valuable tree part (Brown, M.A. *et al.,* 2002; Darrel, D.N., 1985; David, O.M. *et al.,* 2012). Through its enzyme system, the epixylous fungi break down the constituents of wood cell walls, change the percentage of their participation, and thus directly modify wood properties (Chu, K.K.W. *et al.,* 2002; Karadzic, D., Andjelic, M., 2002; Lee, S. *et al.,* 2008). The agents of brown rot (to which the researched fungus *L. sulphureus* belongs) disintegrate primarily cellulose, while the disintegration of lignin occurs to a far smaller extent.

This paper presents the course of change (decrease) in the content of lignin and cellulose in the cell wall, reflected in the decreased bending strength of *Q. petraea* under the influence of *L. sulphureus* after two, four and six-month incubation (Vucetic, J., 1998; Rayner A.D.M. & Boddy, L., 1998; Miric, M., 2005).

2. MATERIAL AND METHODS

The sample used in the research was taken from a 110-year-old healthy sessile oak *Q. petraea* agg. tree, 19 m in height and 35 cm in diameter at breast height. The tree had been cut in Eastern Serbia, in the *Quercetum montanum* association (Tomic, D., 1992) at the altitude of 550 m, with a southern aspect. The analyses were conducted on a log 3.5 m in length (from the stem base to the first live branch). It was cut into tubes following the relevant pattern of the standard prescribed dimensions 2x2x32 cm. Given the small dimensions of the sample used for practical purposes, the cut surface of the tubes was treated with an antiseptic coating to prevent penetration of hyphae from that direction. Since hyphae achieve the most rapid growth through the cross-section, if we don`t prevent their penetration through the cross-section, small samples would quickly rot and the relevant results would not be obtained.

The tubes were dried in a classic wood drying chamber at the temperature of 103±1 ^{0}C and measured with an accuracy of 0.01 g. The control tubes (sound wood) had the bending strength measured using a universal machine for testing wood properties (SRPS ISO 3129:2020; SRPS D.A1.058). The tubes exposed to mycelia were conditioned at approximately 12% moisture content. Mycelia of *L. sulphureus* were resown into plastic Petri dishes containing malt-agar growing medium of the standard concentration. The experiment used sterilized plastic containers with lids to store Petri dishes with fully developed mycelia of *L. sulphureus*. Petri dishes served as glass carriers (dimensions 9x22x35cm) to prevent excessive soaking of moisture from the growing medium, onto which sessile oak wood specimens were placed. In order to ensure high relative air humidity, Petri dishes with 5% boric acid solution were placed on top of the stack.

Bending strength is defined as the resistance of wood to the effect of concentrated, evenly distributed or combined forces that tend to bend or distort it. Therefore, the bending stress is complex stress consisting of the compressive stress in the part of the girder closer to the point of attack and the tensile stress on the opposite side. Between these two zones, there is a neutral axis that, under a load, moves to the side of the tensile stress. (Soskic, B., 1994). Since the calculation of the bending strength involves the cross-section at the point of force, before measuring the strength, all test tubes were cross-measured using a micrometer, with an accuracy of 0.01 mm. The distance between the calipers was 280 mm, and the tubes were exposed to a concentrated force that acted at half the distance between the calipers. All obtained data were processed using standard statistical methods; the results of destruction were compared using a one-way analysis of variance and the test of the least significant differences for the control group, at the time of fungal action (2, 4 and 6 months). Statistical data processing used absolute amounts – N/mm^2, and correlation analysis was performed to determine the correlation between the time of fungal action as an independent variable and the change in the bending strength as a dependent variable.

3. RESULTS AND DISCUSSION

While growing, epixylous fungi, which feed on the basic constituents of wood, change not only its chemical composition but also its whole inner structure. They change or reduce its mechanical, physical, aesthetic and other properties. The agents of brown rot cause chemical changes similar to the changes that occur during the hydrolysis of wood with mild acids (Rayner, A.D.M., Boddy, L., 1998). Through their metabolic processes, fungi modify nutrients into molecules that support their life processes.

The study of the changes in wood properties caused by epixylous fungi should include a brief description of the nutritional requirements of the fungus, chemical composition of wood, and the changes that occur in the chemical composition and structure of the affected wood. All wood-rotting fungi can use final products such as free sugars, lipids, peptides and other primary metabolites. These substances have a crucial impact in the initial stage of wood infestation. Carbohydrates are the most important source of carbon in the nutrition of epixylous fungi. A rich source of carbon is found in the basic structural elements of the wood cell walls (hemicellulose, cellulose and lignin). Cellulose, as the most abundant element on earth, builds the skeletal substance of the cell wall and represents the most important constituent of wood (Knezevic, M., 1975; Markovic, M. *et al.,* 2011a). The disintegration of cellulose does not occur evenly throughout the affected wood because hyphae are not organized and act individually. At first, only a few cells are attacked, but their number gradually rises (Markovic, M. *et al.,* 2011b). As a matter of fact, the disintegration of cellulose is rapid in the initial stages, but then it slows down. The fungus first destroys the free cellulose in the middle layer of the secondary wall because it is the least lignified layer. As soon as the fungus enters the parts of the cellular membrane with a higher content of lignin, disintegration slows down. The disintegration of cellulose in the primary wall is the slowest as this part contains the largest portion of the total lignin (Jankovský, L., 2002). Changes in the wood structure are manifested primarily in the bending strength, with the process being roughly two to three times faster when initiated by brown rot agents. Basic parameters of the bending strength of *Q. petraea* samples that were exposed to the effect of *L. sulphureus* for two, four and six months compared to the control group are presented in Table 1 and expressed in absolute values.

Table 1 Bending strength reduction (%) under the influence of the *L. sulphureus*
Табела 1. Смањење чврстоће на савијање (%) под утицајем гљиве *L. sulphureus*

	0 months / 0 месеци	2 months / 2 месеца	4 months / 4 месеца	6 months / 6 месеци
Number of measurements / Број мерења	30	30	30	30
Minimum value / Минимални износ	109.74	75.87	34.24	16.42
Maximum value / Максимални износ	205.98	206.97	179.49	156.79
Arithmetic mean / Аритметичка средина	156.12	143.21	117.35	98.75
Стандардна девијација / Standard deviation	28.65	29.34	40.62	36.32
Coefficient of variation / Варијациони коефицијент	18.35	20.49	34.62	36.78

Table 1 shows that the data are least scattered, i.e., the coefficient of variation is the lowest in the control group of samples (18.35), while it is the highest after two and six months of exposure to *L. sulphureus* (20.49 and 36.78), which results from the inhomogeneous wood structure and uneven colonization of wood by the fungus. The average bending strength is 156.12 in the control group of samples, 143.21 after two months of exposure to *L. sulphureus*, 117.35 after four months, and 98.75 N/mm^2 after six months of exposure.

Thus, the greatest reduction in the bending strength of *Q. petraea agg*. wood exposed to *L. sulphureus* occurs in the first two months, after which the process slows down. According to Rayner, A.D.M. & Boddy, L. (1998), changes in the properties of wood under the influence of most brown rot agents are reflected primarily in the changes in bending strength. They occur immediately after the first signs of decay have occurred, which is particularly evident in this case. Based on the results of the T-test, shown in Table 2, it is clear that significant differences occur in the first two months of exposure to *L. sulphureus* and apply to all examined groups of samples, except in the period between two and four months of action.

Table 2 Bending strength reduction under the influence of *L. sulphureus* (T test)
Табела 2. Смањење чврстоће на савијање под утицајем гљиве *L. sulphureus* (T тест)

	0 months / 0 месеци	2 months / 2 месеца	4 months / 4 месеца	6 months / 6 месеци
0 months / 0 месеци	-	12.9138	38.7752	57.3717
2 months / 2 месеца		-	25.8614	44.4579
4 months / 4 месеца			-	18.5965
6 months / 6 месеци				-

- Significant difference at the level of 0.05/ Значајна разлика на нивоу 0,05

This means that during this period there is no significant reduction in the bending strength. The differences stem from the large variability of data, not from the exposure to the action of the fungus. The analysis of fractures of the sessile oak tubes exposed to *L. sulphureus* conducted during the bending strength measurement determined that, in the first two months, many specimens have long-fiber fractures besides the smooth ones. Krzusik, F. (1974) states that the wood with high bending strength has long-fiber fractures, while the wood with the medium bending strength has short-fiber fractures and the wood with low bending strength smooth fractures.

Table 3 Correlation analysis of exposure time to fungus *L. sulphureus* and wood properties
Табела 3. Корелациона анализа дужине дејства гљиве *L. sulphureus* и својства дрвета

Tested property / Испитивано својство	Model type / Тип модела	Correlation coefficient / Корелациони коефицијент (r)	Regression equation / Регресиона једначина
Bending strength / Чврстоћа на савијање (σ_s)	Square function / Линеарни модел (x)	±0.992802	$\sigma_s = 151.514 \pm 30.657 \sqrt{T}$

The obtained results lead to the conclusion that after six months of exposure to the fungus the process of wood destruction, although highly advanced, is probably not completed, meaning that there is a possibility that the cell membrane layers may still contain a sufficient quantity of cellulose that provides bending strength.

Correlation analysis was performed in order to establish a correlation between the tested wood properties depending on the time of exposure to the fungus (Table 3).

This is of practical importance in the implementation of protection measures and usability of wood. According to literature sources (Miric, M. *et al*., 2012; Rayner, A.D.M., Boddy, L., 1998; Petrovic, M., 1980), the examined property represents the fastest and clearest indicator of destruction under the influence of epixylous fungi. In that sense, future chemical analyzes of the wood exposed to fungi could give a clearer definition from both qualitative and quantitative aspects, enabling a comprehensive insight into the course and effects of the growth of fungi on wood.

4. CONCLUSIONS

After two, four and six months of *L. sulphureus* action, the bending strength of oak wood decreased significantly compared to the initial value (100%) and amounted to 91.73%, 75.71% and 63.25% of the initial value. In the period between two and four months, the destruction process slowed down and the loss was only 16.02%. In the period between four and six months, the destruction increased slightly, and the bending strength decreased by another 39.46%.

Correlation analysis showed a strong correlation between changes (reductions) in the examined property of *Q. petraea* agg. wood relative to the time of action of *L. sulphureus*. This finding opens up the possibility to use the regression equation to predict changes in wood properties, depending on the time of their exposure to fungi, under unchanged environmental conditions.

If similar experiments are performed in future research dealing with the most important tree species against the most abundant and dangerous wood-destroying fungi in a larger number of monitoring periods, the obtained results could serve as a basis for the development of relevant tables (standards). By crossing the obtained data and analyzing them statistically, we would come to the closest approximate values that could be entered into the relevant tables and applied in practice.

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УТИЦАЈ ГЉИВЕ *Laetiporus sulphureus* (BULL. EX FR.) MURRILL НА ЧВРСТОЋУ НА САВИЈАЊЕ ДРВЕТА ХРАСТА КИТЊАКА

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Резиме

Узорци за испитивање прикупљени су из срчике здравог стабла храста у источној Србији. Током периода испитивања од 2, 4 и 6 месеци, узорци дрвета били су изложени утицају мицелије гљиве која узрокује мрку призматичну трулеж храста *Laetiporus sulphureus* (Bull. ex Fr.) Murrill (сумпорњача). Анализа је вршена на првом трупцу од приданка до прве живе гране (3,4 m дужине). Из трупца су изрезане 4 централне даске, које су потом сечене на секције. Једна даска је представљала контролу, а остале су излагане дејству гљиве у контролисаним условима. Испитан је утицај гљиве *Laetiporus sulphureus* (Bull. ex Fr.) Murrill на смањење чврстоће на савијање дрвета *Q. petraea*. Пошто се савојна чврстоћа израчунава у односу на попречни пресек дрвета на месту дејства силе, пре мерења чврстоће, епрувете су на средини унакрст измерене микрометром, са тачношћу 0,01mm. Размак ослонаца био је 280mm, а епрувете су биле изложене дејству једне концентрисане силе која је дејствовала на половини размака ослонаца. Мерење савојне чврстоће извршено је на универзалној машини за испитивање механичких својстава дрвета код које је потискивач за пренос силе заобљен, са радијусом 15mm. Брзина дејства силе била је равномерна и њено укупно трајање износило је око 2min. Сви добијени подаци су обрађени стандардним статистичким методама и израчуната је аритметичка средина, грешка аритметичке средине, стандардна девијација и варијациони коефицијент. Такође је урађено поређење резултата деструкције помоћу једнофакторијалне анализе варијансе (F-теста), на основу кога је урађен тест најмањих сигнификантних разлика (LSD - Т-тест) између контроле и 2 месеца, контроле и 4 месеца, контроле и 6 месеци, 2 и 4 месеца као и 4 и 6 месеци дејства гљиве. Ради доказивања постојања везе између времена дејства гљиве као независно променљиве величине и промене механичког својства дрвета као зависно променљиве величине, извршена је корелациона анализа. На основу обраде основних параметара, констатовано је да је код савојне чврстоће дошло до великог расипања података. Расипање је најмање у контролној групи узорака - коефицијент варијације износи 18,35, а повећава се у групама узорака који су 2, 4 и 6 месеци били изложени дејству гљиве

L. sulphureus. Тако у групи узорака који су 2 месеца били изложени дејству гљиве *L. sulphureus*, коефицијент варијације износи 20,49, у групи изложеној 4 месеца - 34,62, а у групи изложеној 6 месеци - 36,78. То значи да се расипање података повећава са дужином дејства гљиве, што је вероватно последица неравномерног колонизирања дрвета од стране гљиве. Истраживања су вршена на апсолутно сувом дрвету, а савојна чврстоћа директно зависи од количине влаге у дрвету и највећа је при влази од 4-6%, а на мањој и већој влажности опада. Резултати истраживања су показали да је савојна чврстоћа дрвета китњака просечно износила 143,1N/mm^2 после 2 месеца, 117,35N/mm^2 после 4 месеца и 98,75N/mm^2 након 6 месеци дејства гљиве, што у процентима износи 91,73; 75,17 и 63,25%. Констатовано је да је највеће смањење савојне чврстоће наступило у периоду између 2 и 4 месеца - савојна чврстоћа опада за 16,56%, што је двоструко више од смањења у прва 2 месеца. Између 4 и 6 месеци - савојна чврстоћа опада за 11,92%. Испитивања су показала да након 6 месеци дејства гљиве процес деструкције није завршен и да највероватније у слојевима ћелијске мембране постоји довољна количина целулозе која му обезбеђује постојање савојне чврстоће. Регресиона линија добијена обрадом података отворила је могућност прогнозирања промене својстава дрвета у одређеним временским периодима дејства гљиве, под непромењеним условима спољне средине.