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IMPACT OF THE EDIBLE MUSHROOM ON DESTRUCTION OF OAK WOOD

Miroslava MARKOVIC^{1*}, Snezana RAJKOVIC¹,
Radovan NEVENIC¹, Aleksandar LUCIC¹

Abstract: *The main aim of this investigations has been directed on discovering of optimal conditions for developing of medicinal mushroom Trametes versicolor [Fr.] Pil. in order to compare it with the conditions for developing of the other competitive microorganisms, to discover the possibilities of performing a spontaneous infection in natural condition. The optimum for its growth T. versicolor is at temperature values close to 29⁰ C, and the lowest growth was reported on the base substrate (3.81). In this fungus the initial pH 2.2 was not changed after 21 days of exposure, but the metabolic processes of the fungus at the beginning of exposure caused the mild changes of pH. The results shows that the conditions of temperature and pH, which are convenient for the fungi T. versicolor, are normally present in nature, and that from that point of view these fungi could easily perform spontaneous infection. Species T. versicolor is neither favoured nor inhibited by the environmental factors in comparison with the rival rot fungus.*

Key words: Mushroom, Wood Destruction, Oak, Infection

UTICAJ JESTIVE GLJIVE NA DESTRUKCIJU HRASTOVOG DRVETA

Izvod: *Osnovni cilj ovog istraživanja je bio usmeren na otkrivanje optimalnih uslova za razvoj lekovite gljive Trametes versicolor [Fr.] Pil. u poređenju sa uslovima za razvoj ostalih konkurentnih mikroorganizama, kao i istraživanje mogućnosti spontanih infekcija ovom gljivom u prirodnim uslovima. Konstatovano je da je optimum za rast T. versicolor je na temperaturnim vrednostima bliskim 29⁰C. Najslabiji porast micelije zabeležen je na baznoj podlozi (3,81). Inicijalni pH 2,2 nije se promenio posle 21 dana izlaganja dejstvu gljive, ali su metabolički procesi gljive na početku izlaganja izazvali*

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blage promene inicijalnih pH vrednosti. Rezultati pokazuju da su uslovi temperature i pH koje su pogodni za gljivu *T. versicolor* normalno prisutni u prirodi, pa sa te tačke gledišta ova gljiva može lako izvršiti spontanu infekciju. Vrsta *T. versicolor* nije ni favrizovana, niti inhibirana činiocima životne sredine u odnosu na suprotstavljene gljive truležnice.

Ključne reči: gljiva, destrukcija drveta, hrast, infekcija

1. INTRODUCTION

Mushrooms are the earliest known fungal organisms used as food for their taste and aroma. They are very good sources of protein, vitamins, lipids and mineral elements. (David *et al* 2012). More than 270 mushroom species that exhibit medicinal properties. Although mushrooms vary widely in biology, it is likely that many of the medicinal compounds are shared among different species (Brown *et al*, 2002). *Trametes versicolor* [Fr.] Pil. is famous for its medicinal properties, and is very common in forests. *Trametes versicolor* [Fr.] Pil. is the dried fruiting body or mycelia of *Trametes versicolor* [Fr.] Pil. (alternative names: *Coriolus versicolor* (L. ex Fr.) Quel., *Polyporus versicolor*, *Polystictus versicolor* Fr.) belonging to the Basidiomycetes class and Polyporaceae family. In China, is named *Yun Zhi* (i.e., cloud-like mushroom), probably due to the fact that its wavy surface is covered with fluff. *T. versicolor* is called “turkeytail” in some European countries because it is fan shaped; is zoned with shades of brown, white, and grayin color on the upper surface; and grows in overlapping clusters on dead logs. In Japan, *T. versicolor* is known as *kawaratake*, which means “mushroom by the river bank” (David *et al* 2012).

In the aim of the better disclosure of the nature of fungus *T. versicolor* as the wood-decaying fungus, the investigations of the basic conditions which determine and enable the fungus to perform the infection were done. Under natural conditions the basic determinants of this process are temperature, pH of substrate, status of nutrients in the substrate, the level of inoculum, virulence and the competition of other microorganisms (Darrel, 1985; Chu, 2002; Markovic *et al*, 2003). In this sense, the influence of the temperature and concentration of hydrogen ions in the substrate was studied, since these are the basic parametres of the environment in which all process of the infection and the rot development are performed during the certain period of time (Karadzic and Andjelic, 2002; Lee *et al*, 2008; Markovic *et al*, 2012).

2. MATERIALS AND METHODS

2.1. Investigation of the influence of temperature on the growth of mycelium

The investigation of the speed of the growth of mycelium have been performed by the standard method, in the plastic petri dishes (D = 90 mm), on the base of malt (5 Bé) and agar (2%), at pH=6.0, and in the complete darkness. The growth of the mycelium at temperature 5, 8, 12, 16, 21, 25, 29 and 34⁰C in three replications was monitored. The inoculums were set, because of the speed of the

growth of mycelium, by the wall of petri dish, and the growth of the mycelium was marked every 24 hours, in the direction of the radius of the dish and to the left and right under the angles of 22.5° (three directions) and expressed as the mean value in all replications in millimeters a day.

2.2. Investigation of the influence of pH substrate on the growth and production of mass mycelium

For the investigation of the different observed pH values on the development of mycelium of *T. versicolor* species, the buffered media was prepared. In order to equalize the quantity of the nutrients in the certain parts of buffer, the buffered system was prepared according to the illustrated recipe, by Wolpert method, which was used by several authors (Miric, 1993a; Jankovský *et al*, 2002). By mixing the different volumes 0.3 molar suspensions of phosphate – phosphoric acid (H_3PO_4), potassium-dihydrogen-phosphate (KH_2PO_4) and potassium-hydrogen-phosphate (K_2HPO_4) the bases with the different pH values were obtained, but with the same quantity of phosphate, so that their different quantity would not influence the results. In this way five series of phosphate of 187.5 ml which were poured into 5 erlenmeyers, the volume of which was 300 ml.

A thousand milliliters of the double concentrated melt base was separated and prepared on melt (10 Bé sugar) and agar (4%), which was poured in 5 erlenmeyers of 300 ml volume (of 187.5 ml). This base was autoclaved independently of 0.3 molar suspensions of phosphate and upon the check of pH they were mixed under the sterile conditions. The pH was controlled after the sterilization in order to determine the stability of buffered systems. In this way the base of the standard concentration (5Bé sugar and 2% agar) was obtained, with the physiologically equal percentage of buffer (0.15M). The buffered base which prepared in this way was poured (20 ml each) into the plastic petri dishes ((D=90 mm). For each studied pH value 3 replications were used.

The inoculation was performed in the laminar chamber, by mycelium circle-shaped fragments (D=11 mm), which were set by the rub of the petri dishes. The cultures developed in the thermostat, at $21^{\circ}C$. The growth of mycelium was marked every 24 hours, in three directions – in the direction of the radius, and at the both sides of it under the 22.5° angle. The average daily growth has been determined as the mean value of the growth in these three directions, and the number of days of measurement depended upon the speed of fungus growth at certain (constant) pH values of the nutritive substrate. In order to check the stability of the buffered system (check of pH substrate at the end of the investigation), the liquid base was simultaneously prepared, which was inoculated and incubated under the same conditions. At the end of the experiment pH values of substrate were measured, by digital pH meter Iskra MA 5735.

2.3. Investigation of the influence of mycelium on the change of pH nutrient substrate

For the study of the influence of fungus *T. versicolor* on the change of the pH value of the nutritive substrate, the unbuffered (liquid) media was prepared, according to Schmidt and Liese method (Schmidt, 1994). The quantity of 2,600 ml of the double concentrated melt base (10 Bé sugar) was prepared with the distilled water. Of this quantity 408 ml were poured into 6 Erlenmayer bottles, 500 ml volume (for 6 series) and according to the illustrated recipe in the defined quantity the suspension of distilled water and 1M HCl, or 1M NaOH was added. In this way the needed quantity of the liquid nutritive melt base of the standard concentration (5 Bé sugar) was obtained. Before the sterilization pH values of each series were measured. From each series 120 ml of base were poured into 3 erlenmayers 300 ml volume (for one fungus, in 3 replications), so from 6 series the base was poured into 18 erlenmayers, which were sterilized in the autovlave 20 minutes at $120\pm 1^{\circ}\text{C}$ temperature and 1.4 pressure. After the sterilization pH values were re-measured and they were treated as initial.

The inoculation by *T. versicolor* fungus was performed in the laminar chamber, by circle-shaped mycelium fragments ($D=11$ mm). For each series (initial value pH) the bases were inoculated in 3 erlenmayers.

The incubation lasted for 21 days at 21°C . During the incubation the alternation in pH was measured at every 7 days. For each measurement by sterile sprinkles with needles 10 ml of base was extracted under the aseptic conditions, poured into the cuvettes and pH values were measured by the digital pH meter Iskra MA 5735.

3. RESULTS AND DISCUSSION

The basic precondition for the understanding of the conditions which make it possible for the fungus to colonize the wood is the knowledge of its main physiological characteristics (Miric, 1993b). The fungi isolated from the natural habitats, and then moved and grown in the laboratory conditions, are found in the unusual conditions of existence, which causes their rather different physiological activity (Vucetic, 1998; Markovic *et al*, 2005).

It happens because it is hard to make conditions in the laboratory which adequately reflect the environmental conditions and variation in only one factor without the mutual influence (Rayner and Boddy, 1998; Markovic *et al*, 2011). Therefore, the results obtained by the most accurate laboratory methods cannot be directly applied for the natural conditions as well, and they should be accepted only as the probable indicators of the possible phenomena.

The organisms with the same temperature requirements are rarely found in nature. According to the literature data, the temperature interval of the development of epixyloous fungi range between 0 and 40 degrees. Each fungus have the limit temperatures (minimal and maximal at which the development stops) and the optimal temperatures of development, which are closer to maximal than

minimal ones. The absolute maximal and minimal temperatures are lethal and do not allow the reverse process.

3.1. The influence of temperature on the growth of mycelium

Petrovic (1980) is of the opinion that the real temperature values are always two or three degrees lower than the values obtained in the laboratory conditions. The results of the laboratory study of the influence of different temperatures on the growth of mycelium *T. versicolor* are presented in the Table 1.

Table 1. *The average daily growth of mycelium at different temperatures*

Temperature °C	Average growth of mycelium (mm/day)
5	0.78
8	1.18
12	2.26
16	3.25
21	4.73
25	5.57
29	8.38
34	6.05

At 29⁰ C the growth of mycelium of fungus *T. versicolor* is 8.38 mm/day, so it can be concluded that the optimum for its growth is at temperature values close to 29⁰ C. *T. versicolor* developed at all investigated temperatures, but the growth of it was lower at lower temperatures (at 5⁰ it is only 0.78 mm/day, while at 34⁰ it is as high as 6.05 mm/day).

3.2. Influence of pH values of substrate on the growth and production of the mass of mycelium

The concentration of H ions influences the growth and metabolism of the rot fungi, as well as the development of all plant species (Jankovský *et al*, 2002). Substrate acidity can influence the stimulation or inhibition of the growth of epixyloous fungi, and the change of pH value has the important influence on the velocity of consumption of nutrients and substrate decomposition (Miric, 2005).

3.2.1. Influence of pH substrate on the growth of mycelium

From the Table 2 it can be seen that the growth of the studied species *T. versicolor* was highest (3.81) on the less acid substrate.

Table 2. *The average daily growth of mycelium on the buffered (solid) bases*

Number of series	pH bases		Average growth of mycelium (mm/day)
	Initial pH (after sterilisation)	pH at the end of the experiment	
1	3.2	3.1	1.59
2	3.8	3.6	3.25
3	4.8	4.1	3.81
4	6.0	5.0	2.24
5	7.2	6.5	1.15

The lowest growth (1.15) was reported on the base substrate. According to the control series in the liquid bases, it was concluded that the studied species changed the initial pH by moving it to the optimal values, which is in the limits of tolerance, and, thus, can be considered that all buffered systems were stable during the experiment.

3.2.2. Influence of mycelium on the change of pH nutritive substrate

The results presented in Table 3 show that the greatest change of the initial pH value occurred at the initial pH and is 2.1. In this fungus the initial pH 2.2 was not changed after 21 days of exposure, but the metabolic processes of the fungus at the beginning of exposure caused the mild changes of pH.

Table 3. Change of pH values of base under the influence of mycelium

Number of series	Initial pH (after sterilisation)	The change of pH base after the certain number of days				Weight of the dry mass of mycelium (g)
		7 days	14 days	21 days	Total change pH	
I	2.2	2.4	2.1	2.2	0.0	0.048
II	2.8	2.9	2.9	2.5	-0.3	0.227
III	4.2	4.5	4.1	3.8	-0.4	0.447
IV	4.8	4.8	3.8	3.8	-1.0	0.359
V	5.4	5.5	4.1	3.9	-1.5	0.581
VI	6.2	5.4	4.0	4.1	-2.1	0.488

The weight of the dry mass of mycelium is the greatest in the fifth series, and smallest in the first one, in which there were no changes of pH. It means that this fungus poorly develops on extremely acid substrates and that in this case the change of pH would also happen undoubtedly, but after a longer period of action. It can be said that the less acid substrate close to the pH value 5.4 are more favourable for the development of the fungus *T. versicolor*.

4. CONCLUSION

Temperatures which are optimal for the conclusion of the investigated fungus are at the same time favourable for the growth and development of the numerous species of rot fungi. Therefore, one has to take into account that *T.versicolor* has a great number of rivals in food and environment, which also develop at temperatures which are more favourable for it.

The change of the pH value of substrate on which the cultures *T.versicolor* moved into the direction of the mildly acid reaction, which points to the fact that it, like the majority of rot fungi, prefers the weak acid base. This fact, as well as the former one – regarding the temperature, implies that *T.versicolor* in the competitive relations with other rot fungi conquer the substrate with the equal changes for success, at least regarding the temperature and H⁻ ions concentration.

Given the results of the study of the influence of the temperature and H⁻ ions concentration on the growth and production of mass of mycelium of fungus *T.versicolor*, as well as the change of pH substrate under the influence of this fungus, regarding the possibility for the successful colonisation of the nutritive base

in the nature conditions, it can be concluded that the studied species is neither favoured nor inhibited by the environmental factors in comparison with the rival rot fungus. The phenomenon of the competition of the microorganisms on the same substrate, of the growth inhibition or the occurrence of the antagonism can be the consequence of the metabolism of the opposing fungi species, mycotoxins secretion or antibiotics in front of the increasing mycelial front and sensitivity, i.e. the reaction of the competitive species to it.

Not only the speed, course and consequences of the wood decomposition as substrate and food source do directly depend upon this phenomenon, but also the wood as the very important raw material for procession in the industries of all countries and which is, because of its organic origin, food for a great number of organisms and microorganisms. For this reason it is necessary to investigate the competitive relations of this and other competitive rot fungus in the controlled conditions, in so-called mixed cultures, in the conditions of moisture, temperature and H-ions concentration which are convenient for all species of the competitive fungi.

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IMPACT OF THE EDIBLE MUSHROOM ON DESTRUCTION OF OAK WOOD

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Summary

The investigations of impact of temperature and concentration of H⁺ ions on the growth of fungi *Trametes versicolor* (Fr.) Pil. have been performed *in vitro*. These factors, besides the status of nutrients in substrate, level of inocula and virulence, which undoubtedly have to be treated as necessary in process of colonisation and destruction of the wood, use to be of the crucial significance for performing of infection.

Temperature and pH could be the limiting factor for development of numerous competitive microorganisms colonising the same nutritive substrat.

In order to estimate the impact of these species on succession of microorganisms on the same substrate, it has been investigated the influence of *T. versicolor* on the changing of pH of substrate during its growth by using unbuffered nutritive media. From the other side, impact of pH of substrate on the fungi has been investigated by using buffered nutritive media with stabile pH values during the test. The results shows that the conditions of temperature and pH, which are convenient for the fungi *T. versicolor*, are normally present in nature, and that from that point of view these fungi could easily perform spontaneous infection.

Therefore, at the same time, optimal conditions for the growth of these fungi are also very convenient for the development of other numerous decaying fungi, so that the question of success of infection is emphasized in the light of competitive relations of microorganisms at the same nutritive substrate.

Not only the speed, course and consequences of the wood decomposition as substrate and food source do directly depend upon this phenomenon, but also the wood as the very important raw material for procession in the industries of all countries and which is, because of its organic origin, food for a great number of organisms and microorganisms. For this reason it is necessary to investigate the competitive relations of this and other competitive rot fungus in the controlled conditions, in so-called mixed cultures, in the conditions of moisture, temperature and H-ions concentration which are convenient for all species of the competitive fungi.

UTICAJ JESTIVE GLJIVE NA DESTRUKCIJU HRASTOVOG DRVETA

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Rezime

Istraživanja uticaja temperature i koncentracije H - jona na porast gljive *Trametes versicolor* (Fr.) Pil. su izvršena *in vitro*. Ovi faktori, pored statusa hranljivih materija u supstratu, nivoa inokuluma i virulentnosti, koje nesumnjivo treba da tretirati kao neophodne u procesu kolonizacije i destrukcije od drveta, su od ključnog značaja za uspeh infekcije.

Temperatura i pH mogu biti ograničavajući faktor za razvoj mnogih konkurentskih mikroorganizama pri kolonizaciji istog hranljivog supstrata.

Da bi se procenio uticaj ove vrste na sukcesiju mikroorganizama na istom supstratu, istraživan je uticaj *T. versicolor* na promenu pH podloge u toku njenog razvoja, korišćenjem nepuferovanih hranljivih podloga. Sa druge strane, uticaj pH supstrata na gljivu ispitan je korišćenjem puferovanih hranljivih podloga sa stabilnim vrednostima pH tokom ogleada. Rezultati pokazuju da su uslovi temperature i pH, koji su pogodni za gljivu *T. versicolor*, inače normalno prisutni u prirodi, pa ona može sa jednakim šansama za uspeh izvršiti spontane infekcije.

Dakle, u isto vreme, optimalni uslovi za rast ove gljive su takođe veoma pogodni i za razvoj drugih brojnih konkurentnih mikroorganizama, tako da pitanje uspeha infekcije zavisi od njihovih međusobnih odnosa na istom hranljivom supstratu.

Od ovog fenomena direktno će zavisiti brzina, tok i posledice razlaganja drveta kao supstrata i izvora hrane, ali i drveta kao, za ekonomiju svake zemlje, veoma važne sirovine za preradu koja zbog svog organskog porekla i predstavlja hranu za veliki broj organizama i mikroorganizama. Iz tog razloga potrebno je istražiti kompetitivne odnose ove i drugih konkurentskih vrsta truležnica u kontrolisanim uslovima u tzv. smešanim kulturama, pri uslovima vlage, temperature i koncentracije H – jona koje odgovaraju svim vrstama suprotstavljenih gljiva.

