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INFLUENCE OF FOOD DYES ON THE GROWTH OF *PHLEBIOPSIS GIGANTEA* ISOLATE *IN VITRO*

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Abstract. This study showed the influence of different food dyes on the growth of *Phlebiopsis gigantea* isolate *in vitro*. All used food dyes, blue, green, red and purple did not inhibit growth of mycelium. The only difference in myceliail size was proved, when the diameter of culture growing on medium with green dye was compared with control.

Key words: Phlebiopsis gigantea, dye, growth *in vitro*

INTRODUCTION

Phlebiopsis gigantea (Fr.: Fr.) Jülich is a saprotroph causing white rot of conifer wood [Sierota 1995, Holdenrieder and Greig 1998, Mańka 2005]. This fungus is the only one example of commercial fungus used in biological control of diseases in forestry. Rishbeth [1950, 1951] proposed the idea of using *P. gigantea* in biological control of *Heterobasidion annosum* (Fr.) Bref. He worked out [1961, 1963] the method of preparing inoculum for stump treatment. Since then many isolates of *P. gigantea* have been tested as a biological control agent in many countries: USA, Russia, Canada, Bulgaria, Finland, Austria, France, Germany, Italy, Norway, Great Britain and Poland.

In Poland the first use of biopreparation based on *P. gigantea* (Pg IBL) took place in 1972. This method is very effective in restriction of *H. annosum* spread in pine stands or colonization of a new ecological niche by the pathogen [Sierota 1975, Rykowski and Sierota 1983 a, b, Sierota 1984, 1995, Pratt et al. 2000].

Nowadays in Europe there are available three formulations of biopreparation based on *P. gigantea*. In the Great Britain, the Ecological Laboratory produced small sachets which contain 1-2 ml of *P. gigantea* inoculum (PG Suspension) which should be mixed in 4.5 l water before use. In Finland, it is produced as dry powder. Kemira Agro Oy marked this product as a biological pesticide Rotstop for control of *H. annosum* both on spruce and pine stumps. In Poland, the preparation is produced in bags containing saw-

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dust overgrown by *P. gigantea* mycelium. The treatment of these products in conducted mechanically (Rotstop and PG Suspension) or manually (PG Suspension, Rotstop and Pg IBL). In Poland there are four producers of biopreparation based on *P. gigantea*. In Finland and the Great Britain during the mechanical treatment the pigment is added to the suspension to mark treated stumps. Sachets of Pg Suspension contain besides oidia of *P. gigantea* blue pigment [Korhonen et al. 1994, Pratt et al. 2000, Sierota et al. 2002].

The aim of this study was to check the influence of different food dyes on *P. gigantea* growth *in vitro*.

MATERIAL AND METHODS

In this study four food dyes were used – blue (E-160), purple (E-260), green (E-124) and red (E-132). One gram of each dye was added to 1.5% malt extract agar (Merck, Germany) and diluted with the aid of magnetic mixer. After 10 seconds the medium with dye was poured to Petri dishes. The inoculum of *P. gigantea*, 9 mm in diameter, was taken from 7-day-old culture. Next the inoculum was put in the middle of Petri dish. For control, inoculum was put on 1.5% malt extract agar. Each combination was repeated 10 times. The diameter of mycelium was measured on day 5 after inoculation.

The analysis of variance and the Tukey's test were used to compare the results.

RESULTS AND DISCUSSION

Diameter of *P. gigantea* mycelium differ between 5.6 mm (red dye) to 7.3 mm (green dye). The diameters of *P. gigantea* mycelium shows Table 1.

Table 1. Diameter of *P. gigantea* mycelium growing on the medium with food dyes on day 5 after inoculation
Tabela 1. Średnica grzybni *P. gigantea* rosnącej na pożywce z dodatkiem barwników spożywczych w piątym dniu po inokulacji

Dye Barwnik	Diameter – Średnica		
	average średnia	maximum maksymalna	minimum minimalna
Blue E-160 Niebieski E-160	6.28	6.4	6.0
Green E-124 Zielony E-124	6.9	7.3	6.6
Red E-132 Czerwony E-132	6.42	6.9	5.6
Purple E-260 Fioletowy E-260	6.48	6.7	6.0
Control Kontrola	6.06	6.7	5.6

The diameter of mycelium growing on medium with green dye was biggest among all the samples (6.9 mm) and differed ($p < 0.05$) only from mycelium growing on medium without dyes (Table 2 and 3).

There were no significant influences of red, purple and blue dyes on the growth of *P. gigantea* mycelium ($p > 0.05$).

All food dyes used in this experiment did not inhibit the growth of *P. gigantea in vitro*. Moreover in one case, when the green dye was added, the faster growth of mycelium was noted ($p < 0.05$).

There is lack of available data showing the influence of pigments on *P. gigantea.* In addition there is no information on specific pigments used together with commercial preparation to mark treated stumps.

Sierota [1989] used pigments blue brominate, malachite green and neutral red to investigate their influence on *P. gigantea* growth and on its sporulation *in vitro*. He found that malachite green inhibited mycelium but neutral red pigment increased sporulation of *P. gigantea* in comparison to control. Sierota [1998] showed that pigments could reduce the lost of wood weight decayed by mycelium of *P. gigantea.* Both used method

Table 2. Analysis of variance for diameter of mycelium growing on medium with dyes 5 days after inoculation ($p < 0.05$)
Tabela 2. Analiza wariancji dla średnicy grzybni rosnącej na pożywce z dodatkiem barwników spożywczych pięć dni po inokulacji ($p < 0,05$)

Source of variation Źródło wariancji	SS	df	MS	F	P	Test F
Among groups Pomiędzy grupami	1.9144	4	0.4786	3.305249	0.031144	2.866081
Inside groups W obrębie grup	2.896	20	0.1448			
Total Razem	4.8104	24				

Table 3. The results of the Tukey's HSD post-hoc comparison for for diameter of mycelium growing on medium with dyes five days after inoculation
Tabela 3. Wyniki testu Tukeya HSD dla porównania średnicy grzybni rosnącej na pożywce z dodatkiem barwników spożywczych

Dye Barwnik	Blue E-160 Niebieski E-160	Purple E-260 Fioletowy E-260	Green E-124 Zielony E-124	Red E-132 Czerwony E-132
Purple E-260 Fioletowy E-260	0.95067			
Green E-124 Zielony E-124	0.114458	0.471326		
Red E-132 Czerwony E-132	0.989500	0.999826	0.329185	
Control Kontrola	0.927743	0.471326	0.014513	0.629929

of pigmentation by wood sample submersion in pigment or by painting influenced similarly the decay ability of *P. gigantea.* In the first method malachite green decreased the decay ability of *P. gigantea* about 60% but neutral red pigmnet only about 10% *in vitro*. Loss of wood weight after three-month of incubation was smaller by about 60% in case of painting wood samples with malachite green and in 22.3% in case of neutral red in comparison to control. Sierota [1998] proposed that red pigment might be added to preparation formula. However, as he proved, the red pigment disappeared during incubation of commercial preparation Pg-IBL formula before using.

This study showed that food dye might be used in practice to mark stumps after treatment. All the dyes did not influence the growth of *P. gigantea.* Food dyes are environmentally friendly and are not harmful to people, fungi, animals and plants. These dyes probably disappear very quickly in the environment. The first attempt to spray the dyes on the stumps (Łakomy, unpublish) showed that green and red colours were not visible on fresh pine stumps. The most resistant to environmental influence, especially after stump surface drying, was blue dye, which could pigment wood even 15 days after treatment. In case of changing *P. gigantea* treatment technology from improving formula by brush to spraying stump with oidia the blue food dye could be recommended for using in practice to mark the treated stumps.

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WPŁYW BARWNIKÓW SPOŻYWCZYCH NA WZROST IZOLATU *PHLEBIOPSIS GIGANTEA IN VITRO*

Streszczenie. Celem badań było określenie wpływu barwników spożywczych dodawanych do pożywki na wzrost grzybni *Phlebiopsis gigantea.* Żaden barwnik w sposób istotny nie hamował rozwoju i wzrostu grzybni saprotrofa. Jedyną różnicę stwierdzono pomiędzy rozmiarem grzybni saprotrofa rosnącej na pożywce zawierającej barwnik zielony a grzybnią w kontroli bez dodatku barwnika, po pięciu dniach inkubacji. Barwnik zielony stymulował wzrost grzybni *P. gigantea*.

Słowa kluczowe: *Phlebiopsis gigantea,* barwniki, wzrost *in vitro*

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