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COMPARISON OF GROWTH AND ENZYMATIC ACTIVITY OF MYCELIUM AND YIELDING OF *Pleurotus ostreatus* (FR.) KUMM. ON DIFFERENT SUBSTRATES

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Abstract. Cereal straw is used as the most common substrate for *Pleurotus ostreatus* (Fr.) Kumm. cultivation. However other easily available and cheep materials are being sought. Textile industry wastes, among others, seem to be suitable materials. The effect of the kind of substrate on the mycelium growth, enzymatic activity and yield of *Pleurotus ostreatus* (Fr.) Kumm. was investigated. The subjects of the studies were the 'K22' and 'P80' strains of *P. ostreatus* and four substrates: i.e. alder sawdust, rye straw, hemp and flax shive. The type of substrate was found to affect the examined *P. ostreatus* parameters. The examined strains responded similarly to the applied substrate type. The best mycelium growth was obtained on the substrate from flax shive. The activity of the laccase enzyme turned out to be the highest on the hemp shive and the same substrate also yielded the highest crop of carpophores. The obtained results corroborated high usefulness of hemp and flax shive for the cultivation of *P. ostreatus*.

Key words: oyster mushroom, hemp, flax, laccase

INTRODUCTION

Pleurotus ostreatus (Fr.) Kumm. can be cultivated on a wide range of easily-available waste materials derived from either agricultural or forest production but the most common materials used for their cultivation are cereal straw or sawdust from broad-leaved trees [Shah et al. 2004, Nivedita and Irabanta 2005]. Majority of *P. ostreatus* producers in Poland use wheat or rye straw to manufacture these mushroom types [Ziombra 2005], although numerous attempts are being undertaken in various countries in an attempt to use other locally available and cheap materials. In China, for instance, cotton wastes are successfully employed to cultivate *P. ostreatus* as well as other kinds

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of mushrooms [Oei 2003]. In Poland, manufacturers of natural fibres from hemp and flax have at their disposal post-production wastes which must be utilised [Mańkowska et al. 2007]. Bearing in mind their composition and properties, they could be utilised as substrate for *P. ostreatus* cultivation.

The aim of the performed investigations was to estimate the usefulness of hemp and flax shive in production of *P. ostreatus* and to compare them with other frequently applied substrates. The above objective was to be achieved by comparing mycelium growth and its enzymatic activity as well as the level of yield on substrates selected for investigations.

MATERIAL AND METHODS

Experiments were carried out at the Department of Vegetable Crops of the Poznań University of Life Sciences in 2009 using two strains of *Pleurotus ostreatus* (Fr.) Kumm. designated as 'K22' and 'P80'. Four substrates were tested i.e. alder sawdust, rye straw, hemp and flax shive. Two trials were conducted, namely: laboratory in two series and cultivation in two cycles.

In the laboratory trial, mycelium growth and enzymatic activity on all the above-mentioned substrates was investigated, whereas in the cultivation trial, yields of *P. ostreatus* on rye straw, hemp and flax shive were compared. In the experiments, the alder sawdust was supplemented with wheat bran (10% in relation to the substrate dry matter). No supplementation was employed in the remaining substrates. The substrates were wetted up to 65% moisture content using tap water in both experiments.

The laboratory experiment was conducted in glass 16 cm long test-tubes, 4 cm in diameter. The test-tubes were filled with substrates to the height of 12 cm, closed with lignin corks and sterilised in an autoclave at the temperature of 121ºC for 45 minutes. When the substrates cooled down to the temperature of 25ºC, they were inoculated with mycelia of the examined strains of *P. ostreatus*. Mycelium growth was determined after 10 days of incubation at the temperature of 25ºC. The measure of its growth was the thickness of the substrate layer overgrown with mycelium. When the entire substrate became overgrown with mycelium, measurements of the activity of the laccase enzyme were performed. Laccase activity was determined according to the method given by Niku-Paavola et al. [1990].

In the cultivation experiment, substrates were placed in polypropylene bags of 5 dm^3 volume and subjected to sterilisation in an autoclave at the temperature of 121°C for 1.5 hours. After cooling the substrates to the temperature of about 25°C, they were inoculated with the mycelium in the amount of about 3% in relation to the substrate DM. After inoculation, the substrates were subjected to incubation at the temperature of about 24°C and air relative humidity of 80–85%. Once the substrates were completely covered with the mycelium, plastic bags were transferred to the cultivation room where the temperature was maintained at 16ºC±1 and air relative humidity at 80–85%. The cultivation was additionally lighted using fluorescent light (Day-Light) of 500 lx intensity for 10 hours/day. The cultivation room was aired not to allow CO_2 concentration to exceed 1000 ppm. Carpophores were harvested in clumps at the moment when edges of the oldest caps began to straighten. The yield comprised carpophores together with their stipes cut at the length of 2 cm. They were collected from the first crop in both cycles of the cultivation experiment.

The trial was established in four replications in a random design in both experiments. The obtained research results were subjected to the analysis of variance for two-factorial experiments using Duncan's test at $\alpha = 0.05$. The results were described as average values of the two series in the laboratory experiment and two cycles in the cultivation experiment.

RESULTS AND DISCUSSION

It was found that the kind of the substrate affected the mycelium growth of the examined strains of *P. ostreatus* (fig. 1). The examined mycelium was found to grow fastest on the flax shive substrate and slower on the sawdust substrate. In this case, the response of experimental strains to the type of substrate was similar. The slowest mycelium growth was observed on substrates from hemp shive and straw, although the response of the examined strains to the above-mentioned substrates differed. The mycelium of the P80 strain grew faster on the substrate from hemp shive than on straw, while in the case of the K22 strain the growth of mycelium was faster on the straw substrate. Differences in the growth of the *P. ostreatus* mycelium on various substrates were reported by Obodai et al. [2003], while differences between strains were mentioned by Curvetto et al. [2002].

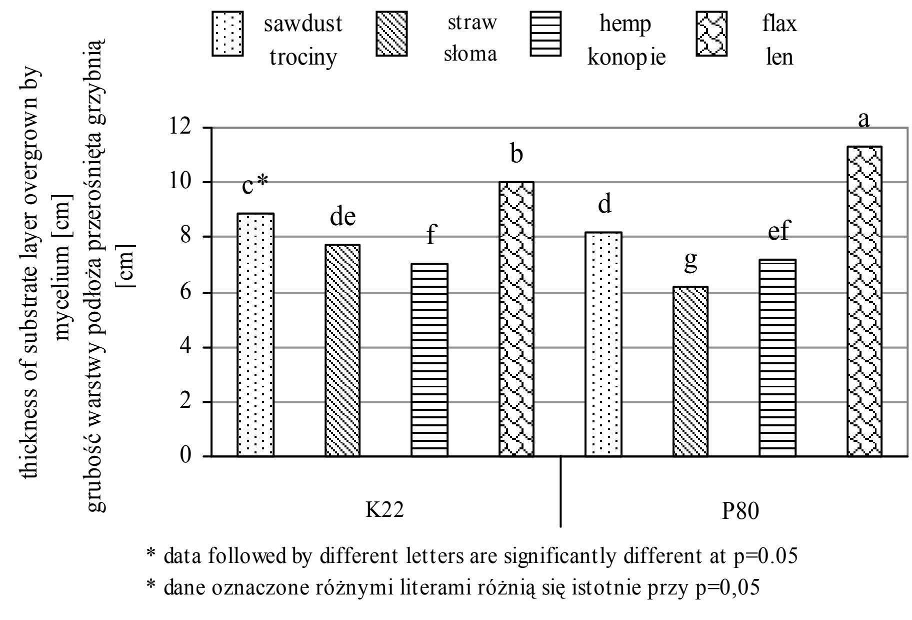

Fig. 1. Effect of substrate type on mycelium growth of two *Pleurotus ostreatus* strains
Ryc. 1. Wpływ rodzaju podłoża na wzrost grzybni dwóch odmian boczniaka ostrygowatego

Laccase activity depended on the substrate type and the strain of *P. ostreatus* (fig. 2). The highest activity of the examined enzyme in both experimental strains was observed on the substrate from hemp shive, while the lowest – on the sawdust substrate. In the case of substrates from flax shive and straw, differences between strains were recorded. In the K22 strain, higher laccase activity was determined on the substrate from flax shive than on the straw substrate, whereas in the case of the P80 strain, the situation was reverse. Different laccase activity depending on the type of substrate and on *P. ostreatus* strain was also demonstrated by Elisashvili et al. [2008]. Mikashvili et al. [2006] found that the activity of this enzyme depended on the type of carbon and nitrogen sources. It is possible that differences in laccase activity determined in our experiments resulted from significant differences in the composition of the applied substrates.

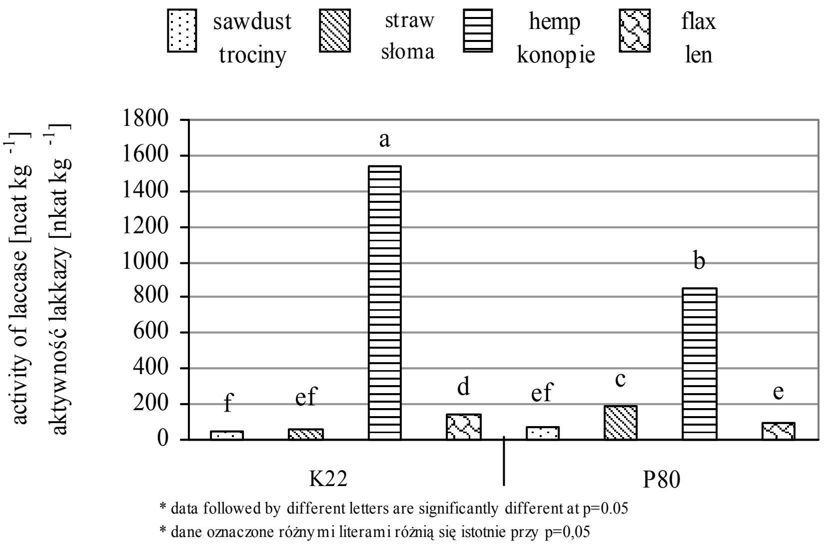

Fig. 2. Effect of substrate type on laccase activity of two *Pleurotus ostreatus* strains
Ryc. 2. Wpływ rodzaju podłoża na aktywność lakkazy u dwóch odmian boczniaka ostrygowatego

The yield of *P. ostreatus* carpophores depended on the kind of substrate. In the case of the K22 strain, the yield obtained from hemp and flax shive substrates was statistically similar but with a tendency towards a greater yield from the hemp shive substrate. A similar response to the type of substrate was found in the case of the P80 strain. Isikhuemhen and Mikiashvili [2009] determined a positive correlation between the loss of the substrate dry matter and biological efficiency. They also reported that laccase activity was higher before *P. ostreatus* harvest than after it. It can be presumed that in our own investigations the activity of laccase and other enzymes could have influenced carpophore yields. Mańkowska et al. [2007] reported that hemp is a rich source of cellulose, lignin and fatty acids. Substances developed in our experiments from the degradation by laccase of lignin as well as other similar compounds could have been a good source of nutrients for mycelium. That explains why the yield obtained on the hemp shive substrate was high.

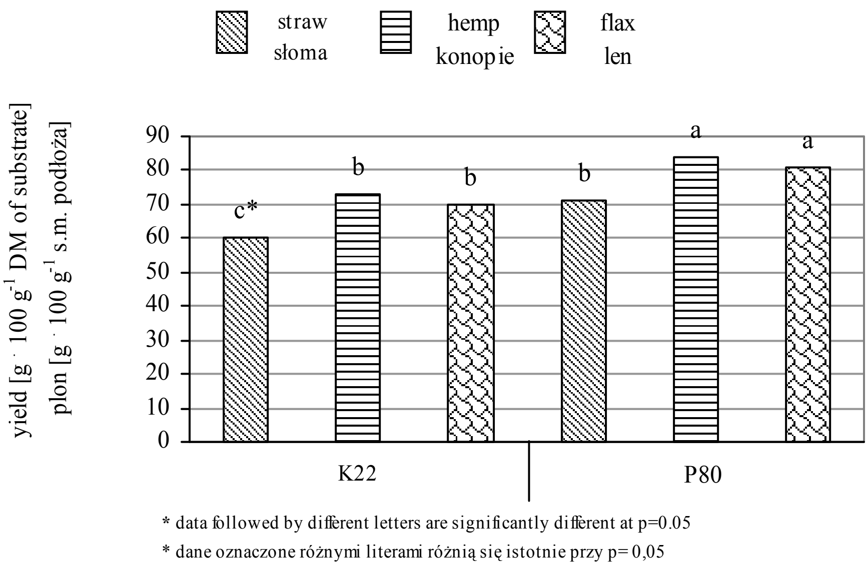

Fig. 3. Effect of substrate type on yield of two *Pleurotus ostreatus* strains
Ryc. 3. Wpływ rodzaju podłoża na plon dwóch odmian boczniaka ostrygowatego

It can be said, on the basis of the obtained research results, that flax and hemp shive can provide a good substrate for *P. ostreatus* cultivation ensuring good yields. Only in the case of hemp shive, poorer mycelium growth can raise certain doubts as it could be associated with a greater risk of substrate infection with competitive microorganisms. However Tasleem-uz-Zaman et al. [1998] demonstrated that hemp extract showed fungistatic action.

CONCLUSIONS

1. The applied type of substrate affected growth and enzymatic activity of mycelium as well as on the yield of *P. ostreatus*. Flax shive turned out to be the best substrate for mycelium growth. The best substrate for *P. ostreatus* cultivation, however, was hemp shive due to high laccase activity and the highest yield.

2. The response of the examined strains of *P. ostreatus* to the substrate type was similar with respect to the investigated traits.

REFERENCES

Curvetto N., Figla D., Devalis R., Delmastro S., 2002. Growth and productivity of different *Pleurotus ostreatus* strains on sunflower seed hulls supplemented with N-NH_4 and/or MN(II). Bioresour. Technol. 84, 171–176.

Elisashvili V., Penninckx M., Kachlishvili E., Tsiklauri N., Metreveli E., Kharziani T., Kvesitadze G., 2008. *Lentinus edodes* and *Pleurotus ostreatus* lignicellulolytic enzymes activity in

submerged and solid state fermentation of lignocellulosic wastes of different composition. Bioresour. Technol. 99, 3, 457–462.
Isikhuemhen O., Mikiashvili N., 2009. Lignocellulolytic enzyme activity, substrate utilization and mushroom yield by *Pleurotus ostreatus* cultivated on substrate containing anaerobic digester solids. J. Ind. Microbiol. Biotechnol. 36, 11, 1353–1362.
Mańkowska G., Menesiak M., Grabowska L., 2007. Ocena zasobów genowych konopi zgromadzonych w Instytucie Włókien Naturalnych w Poznaniu z uwzględnieniem nowych kierunków wykorzystania. Zesz. Prob. Postęp. Nauk Roln. 517, 853–860.
Mikiashvili N., Wasser S.P., Nevo E., Elisashvili V., 2006. Effect of carbon and nitrogen sources of *Pleurotus ostreatus* lignolitic enzyme activity. World J. Microbiol. Biotechnol. 22, 9, 999–1002.
Niku-Paavola M.L., Raaska L., Itavaara M., 1990. Detection of white-rot fungi by a non-toxic stain. Mycolog. Res., 94, 27–31.
Nivedita L., Irabanta S. N., 2005. Recycling of agro-forest wastes trough mushroom cultivation. J. Mycopathol. Res. 43(2), 271–273.
Obodai M., Cleland-Okine J., Vowotor K.A., 2003. Comparative study of the growth and yield of *Pleurotus ostreatus* on different lignocellulosic by-products. J. Ind. Microbiol. Biotechnol. 30, 146–149.
Oei P., 2003. Mushroom cultivation. Backhuys Publishers Leiden, Netherlands.
Shah Z. A., Ashraf M., Ishtiag M., 2004. Comparative study on cultivation and yield performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates (wheat straw, leaves, sawdust). Pakistan J. o Nutrition 3 (3), 158–160.
Tasleem-uz-Zaman K., Nasir M.A., Bokhari S.A.A., 1998. Antifungal properties of some plant extracts. Pakistan J. Phytophatol., 10, 2, 62–65.
Ziombra M., 2005. Boczniak. Hortpress, Warszawa.

PORÓWNANIE WZROSTU I AKTYWNOŚCI ENZYMATYCZNEJ GRZYBNI ORAZ PLONOWANIA BOCZNIAKA OSTRYGOWATEGO *Pleurotus ostreatus* (FR.) KUMM. NA RÓŻNYCH PODŁOŻACH

Streszczenie. W uprawie boczniaka ostrygowatego *Pleurotus ostreatus* (Fr.) Kumm jako podłoże wykorzystuje się przede wszystkim słomę zbóż. Poszukiwane są jednak inne łatwo dostępne i tanie materiały. Materiałem takim mogą być m.in. odpady przemysłu włókienniczego. W doświadczeniach badano wpływ wybranych podłoży na wzrost i aktywność enzymatyczną grzybni oraz wielkość plonu boczniaka ostrygowatego. Przedmiotem badań były dwie odmiany boczniaka ostrygowatego: 'K22' i 'P80'oraz cztery podłoża, tj. trociny olszowe, słoma żytnia, paździerze konopne i lniane. Stwierdzono wpływ rodzaju podłoża na badane cechy boczniaka ostrygowatego. Badane odmiany podobnie reagowały na rodzaj podłoża. Najlepszy wzrost grzybni uzyskano na podłożu z paździerzy lnianych. Aktywność enzymu lakkazy była najwyższa na podłożu z paździerzy konopnych. Na tym samym podłożu uzyskano również największy plon owocników. Uzyskane wyniki potwierdziły dużą przydatność paździerzy konopnych i lnianych do uprawy boczniaka ostrygowatego.

Słowa kluczowe: boczniak, konopie, len, lakkaza

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