MYCELIUM GROWTH AND YIELDING OF *AGROCYBE AEGERITA* (BRIG.) SING. ON DIFFERENT SUBSTRATES.

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ABSTRACT

High yield and good quality of the carpophores is the most important issue that modern mushroom growers focus on during the cultivation work. Crop commodity is usually done on the sawdust of deciduous trees. *Agrocybe ægerita* is an edible mushroom characterized by high content in protein, easily digested by human gastrointestinal. This experiment was set to investigate mycelial growth and yield of two strains of *A. ægerita* on different substrates. In the laboratory experiment, mycelial growth on 8 agar media (PDA, Standard, wheat, MA, CYM, potato-carrot based, 2 sawdust extract: alder, beech and birch (1:1)) and 5 sawdust substrates (birch, beech, oak, maple, alder) was investigated. Petri dishes (Ø 9 cm) for agar media and biological tubes (18 cm long and Ø 2.5 cm) for sawdust substrates were used. In the cultivation studies, two kinds of sawdust substrates were used: birch, beech and mixture of beech and alder (1:1). Each of the sawdust was moisturized up to 70%. After sterilization the substrate was inoculated with mycelium (on grain) of the investigated strains and incubated at 25°C. Later, when mycelium has completely overgrown the substrate the temperature was decreased to 15-17°C to initiate primordia formation. The cultivation was enlightened 10 h/d with Day-Light lamps (500 lx). One crop was harvested after 5 weeks. The carpophores of black poplar mushrooms were picked up in clusters.

The laboratory experiment showed no statistically important difference between the mycelial growths of the investigated strains. The best growing agar media were PDA, MEA and wheat, both strains showed slowest mycelium growth on CYM. The linear mycelial growth was the best on the beech and birch sawdust. The two investigated strains differed significantly within the yield on the beech and birch sawdust. The best substrate for cultivation of *A. ægerita* was birch sawdust. The weight of single carpophores as well as the weight of single cap, as the edible part, was measured. Both investigated strains were characterized by big and heavy carpophores.

INTRODUCTION

The interest of researches all over the world in *Agrocybe aegerita* (Brig) Sing. – black poplar mushroom is continuously growing together with new knowledge obtained on its fabulous properties. *Agrocybe aegerita* is an edible mushroom characterized by high content of protein, easily digested by human gastrointestinal [1, 21]. Its flavor qualities were valued already by the ancient Greeks and Romans and the cultivation of this species reaches almost 2000 years [18]. The taste of the fresh carpophores is mild and makes a very good composition for the poultry and fish dishes, giving them a gentle pork flavor [14]. In nature black poplar mushroom grows in clusters on living and decaying stumps of mostly deciduous trees such as: poplar, willow, black poplar, ash, elderberry, black locust and Brazilian araucaria [20]. The cap of *A. aegerita* is convex, expanding to plane at maturity. Cap diameter is up to 20 cm, yellowish gray to grayish brown, darker towards the center. Gills are at first gray, with spore maturity becoming chocolate brown. Stem is white, adorned with a well developed membranous ring, usually colored brown

from spore fall [16]. *Agrocybe aegerita* gained special attention for its healing properties [3]. It was found to be medically active in several therapeutic effects such as antitumor, antifungal, antioxidant, nerve tonic, hypercholesterolemia and hyperlipidemiê [4, 8, 15, 19]. Extracts from fruiting bodies of *A. aegerita* acquired antimutagenic activities and might play enormous role in cancer prevention [11]. Currently numerous studies are undertaken in order to extend knowledge of those properties as well as wider exploitation of *A. aegerita* in medicine.

First stage of growing mushrooms is mycelium production on agar media. The growth rate of mycelium depends on the type of media and substrate as well as of species and strain of mushroom [2]. The best agar media for maternal mycelium production is the one on which the mycelium growing rate is the quickest and the quality of mycelium hyphae is the best. Choosing the best agar media determinate the shortest time for mycelium production, hence the mushrooms yield production.

High yield and good quality of the carpophores is the most important issue that modern mushroom growers focus on during the cultivation work. Crop commodity, which very often depends on the speed of mycelium growth, is usually done on the sawdust of deciduous trees [12, 17]. The growth speed of mycelium on the growing substrate not always means high and good quality yielding. Therefore after choosing the best substrate for the mycelium growth it has to be verified in cultivation conditions. The aim of this study was to determinate the best substrates for mycelium growth as well as the best substrates for cultivation and fruiting bodies development of *A. aegerita*.

MATERIALS AND METHODS

Materials. Strains of *A. aegerita,* indicated as AE02 and AE05, used in our experiment came from collection of cultivated and medicinal mushrooms of the Department of Vegetable Crops, Poznań University of Life Sciences. The material used for inoculation was granular mycelium prepared according to the recipe recommended by Lemke [7]. Maternal and granular mycelium of examined strains was prepared in the biological laboratory of Department of Vegetable Crops of Poznań University of Life Sciences. Granular mycelium was prepared on wheat grains.

Laboratory experiment. Two separate experiments were established, one for agar media and the other for sawdust substrate. In first experiment mycelium growth on 8 agar media (PDA, Standard, wheat, MA, CYM, potato-carrot based, 2 sawdust extract: alder, beech and birch (1:1)) was investigated (Table 1). Petri dishes (Ø 9 cm) filled with analyzed agar media were inoculated with slices of maternal mycelium (Ø 0.5 cm) placed centered. Mycelium was incubated in 25°C and 80-90% of relative air humidity.

Second experiment was set to asses the mycelium growth on 5 sawdust substrates: birch, beech, oak, maple, and alder. Sawdust was moisturized up to 70%. After sterilization and cooling to the temperature of 21°C the substrate was placed in the biological tubes (18 cm long and Ø 2.5 cm), inoculated with granular mycelium of investigated strains and incubated in 25°C and relative air humidity 85-90%.

Experiments were established in fully randomized design, in 6 replications on Petri dishes; in 4 replications and 2 series in biological tubes. On Petri dishes diameter of media occupied by mycelium was measured after 9 days from inoculation. On biological tubes thickness of substrate mass occupied by mycelium was measured after 18 days from incubation.

When comparing the experimental results, the analysis of variance for factorial experiments was applied using Newman-Keuls test on the level of significance α=0.05. The results of experiments were discussed on the basis of mean values from 1 cultivation cycle.

Table 1: Composition oft he agar media used to study the mycelial growth of *A. aegerita*

Agar Media	Composition (+ fill to 1 liter of distilled H_2O)
Malt Extract Agar (**MEA**) Bioskop/ Lab Empire	- 20.0 g maltose extract - 22.0 agar
Potato Dextrose Agar, Bioskop pH 5.6 +/- 0,2(**PDA**)	- 20.0 g PDA - 22.0 g agar
Potato-carrot agar (**PCA**)	- 100 g carrot - 100 g potato - 1 g glukose - 22 g agar
Wheat	- 200 g wheat grain extract - 3 g glukose - 22 g agar
Sawdust - alder	- 50 g alder sawdust - 3 g glukose - 22 g agar
Sawdust – beech+birch	- 100 g sawdust (1:1) - 3 g glukose - 22 g agar
Complete Yeast Medium **CYM**	- 0.5 $MgSO_4$ $7H_2O$ - 0.46 g KH_2PO_4 - 1.0 g K_2HPO_4 - 2.0 g peptone - 2.0 g yeast extract - 20 g glukose - 22 g agar
Standard	- 1.0 g KH2PO4 - 1.0 g NH4NO3 - 0.5 g MgSO4 - 3.0 g sacharose - 2.0 g glukose - 1.0 g maltose - 20 g agar

Cultivation experiment. The discussed experiment was established in an air conditioned chamber and the cultivation was conducted in plastic bottles of capacity of 600 ml. The substrates used in the experiment were two kinds of sawdust substrates: birch, beech and a mixture of beech and alder (1:1). Each sawdust was moisturized up to 70%. After sterilization and cooling to the temperature of 21°C the substrate was inoculated with granular mycelium of investigated strains and incubated at 25°C and relative air humidity 85-90%. Once the substrate was overgrown by mycelium the temperature was decreased to 15-17°C to initiate primordia formulation. The cultivation was lighted 10 h/d with Day-Light lamps 500 lx.

Experiments were established in fully randomized design, in 4 replications and 2 cultivation cycles. Harvest of *A. aegerita* carpophores was carried out for a period of 6 weeks. The carpophores of black poplar mushrooms were picked up in clusters, no single carpophores were cut out from the sawdust. Yields and dry matter content of carpophores were determined on

the basis of harvested fresh fruiting bodies calculated per 100g of substrates dry matter. For morphological features of carpophores, 10 fruiting bodies were sampled from each repetition and measurements of cap diameter, diameter and length of the stipe together with single cap and carpophore weight were performed.

When comparing the experimental results, the analysis of variance for factorial experiments was applied using Duncan's test on the level of significance α=0.05. The results of experiments were discussed on the basis of mean values from 1 cultivation cycle.

RESULTS AND DISCUSSION

In the first experiments, mycelial growth measurements were performed to investigate the influence of the type of media and substrates on colonization rates. Mycelium growth rate is different between the species and sometimes even within the strains of mushroom and depends on the type of media and substrate [9]. Bilay *et al*. [2] investigated growth rate of 30 species and strains of mushrooms on different agar media. They concluded that different species vary concerning the nutritional requirements. Mycelium of both examined strains presented similar growing rate on all evaluated agar media except from Complete Yeas Media (CYM), where mycelial growth was the slowest (5.2 cm / 9 d), which corresponds with investigation of Kim *et al*. [6] who also obtained the best results on CYM agar media. Other 7 media presented statistically insignificant growth differences, however there was a tendency where mycelium of both strains showed quickest growth on PDA (7.3 cm/ 9 d), followed by MA (7.2 cm/ 9 d) and wheat and birch/beech (7.1 cm/ 9 d) agar media (Fig.1). Comparing both examined strains, regardless agar media the quickest growth was represented by the strain AE05 with 7.0 cm/ 9 d where AE02 was 6.5 cm/ 9 d, the difference being statistically significant. The results of our experiment show the mycelium growth for 9 d depends on the used medium; however the differences between media are not statistically significant. This makes *A. aegerita* mycelium easy to reproduce, which confirm investigation of other authors who used for maternal mycelium productions different agar media obtaining quick growth on each media [2, 6].

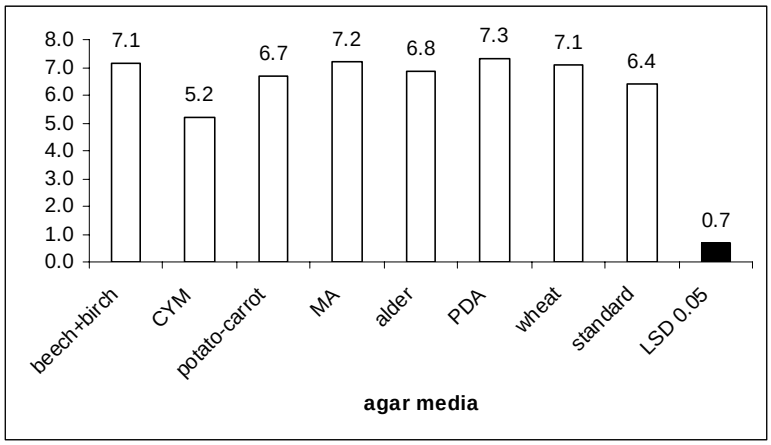

Figure 1: Growth rate of *A. aegerita* strains on different agar media [cm / 9 days].

In the nature carpophores of *A. aegerita* appear commonly on many species of trees, the cultivation substrate is mostly chosen by its availability [14]. In our experiment, both of examined strains showed similar growing rate regardless used sawdust. Mycelium of both strains (AE02 and AE02) showed the quickest growth on beech sawdust (7.1 cm /18 d), little slower growth but statistically significant was on birch sawdust (6.8 cm /18 d), and alder sawdust (6.7 cm /18 d). Definitely the slowest mycelial growth of examined strains was on oak sawdust (4.0

cm /18 d). Growth of mycelium on the maple sawdust was moderately fast (5.6 cm /18 d) (Fig. 2).

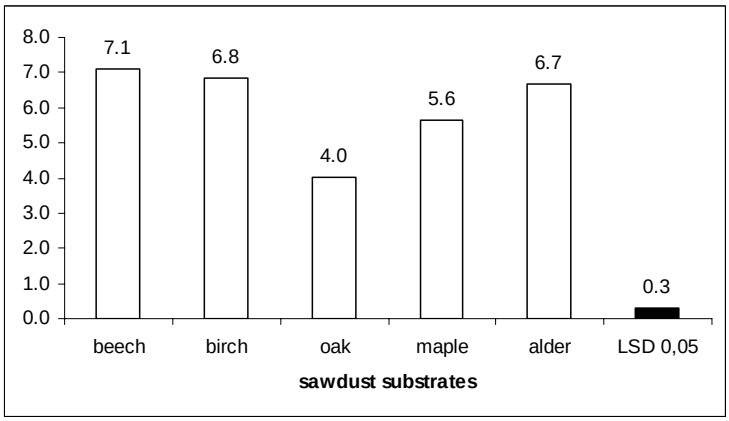

Figure 2: Growth rate of *A. aegerita* strains on different growing substrates [cm / 18 days]

In published works, average yield of *A. aegerita* is 0.5 kg of mushroom on 2.5-3.0 kg of substrate. Yield of carpophores of investigated strains was different and depended on the used cultivation substrate [10, 13]. The highest yield was obtained on substrate composed from beech and alder sawdust (39.5 g/100g of substrate) and birch (36.8 g/100g of substrate; Fig. 3). However mycelium growth rate was statistically quickest on the beech sawdust, yielding on substrate of only beech sawdust was much lower than above mentioned substrates (8.7 g/100g of substrate). Obtained results do not correspond with previous experiments conduced by Sobieralski *et al*. [13], where the highest yield was, on the contrary, obtained from beech sawdust much higher yielding – 49.7 g/100 g of substrate, however yield from birch sawdust was similar (46.1 g/100g of substrate). Zadrazil [22] states the better mycelium growth, as well as higher yield, of *A. aegerita* can be obtained by increasing inorganic nitrogen content or by addition of protein-rich additives in the substrate.

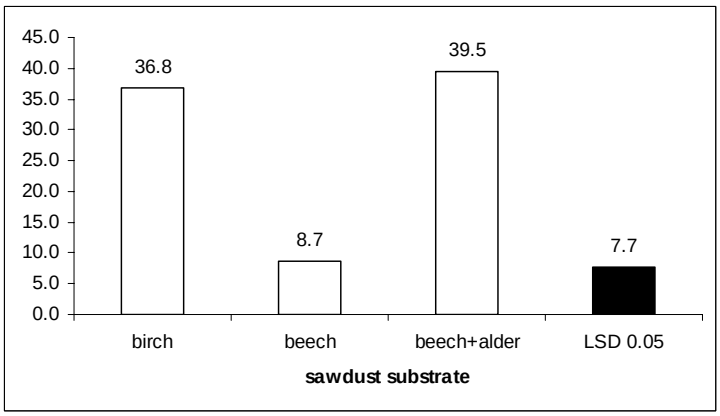

Figure 3: Yield of *A. aegerita* on different substrates [g/100 g substrate DM]

Dry matter (DM) content in the carpophores of cultivated mushrooms is one of the important factors describing the quality of the fruiting bodies. Generally DM content in fruiting bodies of cultivated mushrooms ranges from 7.9 to 11.4% in *Agaricus bisporus* [5], 8.0% in *Pleurotus ostreatus*, 14.3% in *Boletus edulis* [1] and depends on the cultivation substrate used [5]. Content of dry matter in our experiment was estimated after drying the collected carpophores at 105°C for 24 hours. The highest yield based on dry matter content of harvested mushroom was

obtained again on the composition of beech and alder sawdust (3.2 g/100g of substrate DM) and birch (2.9 g/100g of substrate DM), the beech sawdust was only 1.2 g/100g of substrate DM (Fig. 4). However percentage of dry matter within the obtained carpophores compared to the yield harvested was higher in the beech substrate (15.7 %), when on beech/alder and birch it was much more lower (only 8.1 and 8.0 % respectively) (Fig. 5), this corresponds with the results obtained by Bauer Petrovska & Kulevanova [1] where DM content of *A. aegerita c*arpophores was 10.2%.

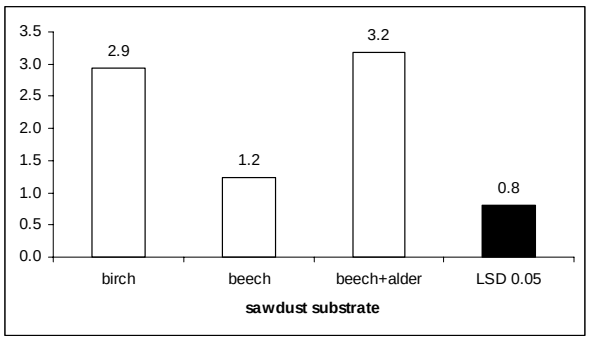

Figure 4 Yield of dried carpophores of *A. aegerita* cultivated on different substrates [g/100g of substrate DM]

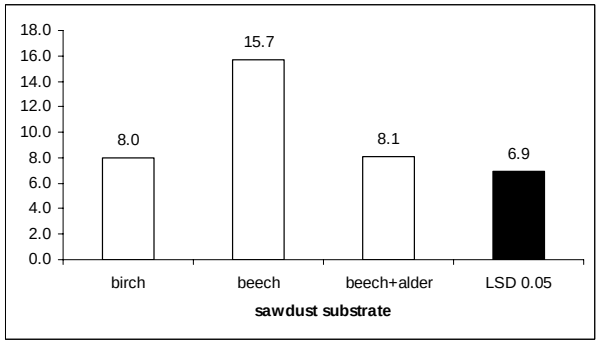

Figure 5: Dry matter content in carpophores of *A. aegerita* cultivated on different substrates [% DM]

Carpophores of *A. aegerita* show differences in their morphology depending mostly on the type of substrate used for cultivation [16]. The morphological features of carpophores in our experiment differed among used cultivation substrate. Carpophores harvested from birch substrate characterized with the heaviest caps (Fig. 6) and carpophores (Fig. 7) (2.2 and 3.7 g respectively), those collected from beech/alder substrate were little bit lighter (1.9 and 3.4 g). However fruiting bodies collected from beech/alder sawdust have bigger caps (3.3 cm) (Fig. 8) and longer stipes (4.8 cm) (Fig. 9) than on birch substrate (3.1 and 4.2 cm respectively). Carpophores harvested from beech sawdust were much lighter (2.0 g), had smaller caps (2.7 cm) and shorter stipes (3.0 cm).

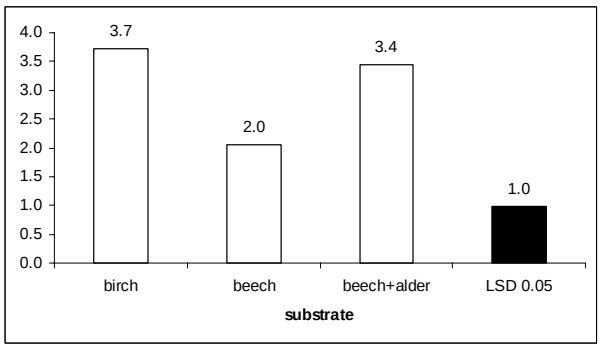

Figure 6: Average weight of carpophores of *A. aegerita* cultivated on different substrates [g/ carpophore]

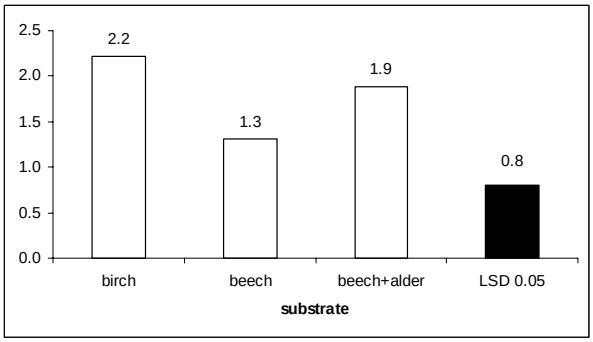

Figure 7: Average weight of caps of *A. aegerita* cultivated on different substrates [g / cap]

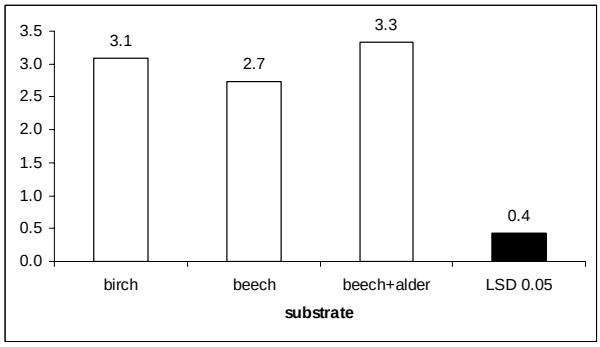

Figure 8: Average cap diameter of *A. aegerita* cultivated on different substrates.
[cm / cap]

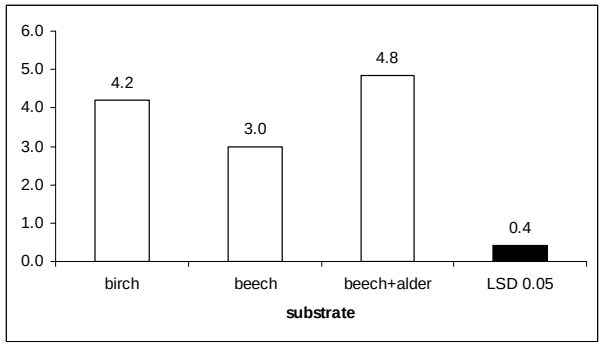

Figure 9: Average stipe length of *A. aegerita* cultivated on different substrates.
[cm / stipe]

CONCLUSION

1- The experiment showed no statistically important difference between the mycelium growth of investigated strains.
2- Suggested agar media for maternal mycelium growth were PDA, MEA and wheat and birch/beech agar media.
3- Both strains showed slowest mycelium growth on CYM comparing to other investigated agar media.
4- The mycelium growth was the best on the beech and birch sawdust, therefore both were used in cultivation experiment.
5- Yield of investigated strains depended on the substrate used for cultivation.
6- The best substrate for cultivation of *Agrocybe aegerita* was birch sawdust and composition of beech and alder sawdust.
7- The carpophores of both investigated strains characterized with the big and heavy carpophores.

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