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Jasińska, Agnieszka
Department of Vegetable Crops, Poznan University of Life Sciences

Siwulski, Marek
Department of Vegetable Crops, Poznan University of Life Sciences

Sobieralski, Krzysztof
Department of Vegetable Crops, Poznan University of Life Sciences

Majtkowski, Włodzimierz
Botanical Garden, The Plant Breeding and Acclimatization Institute (IHAR) Radzików

他

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Comparatve Study of Mycelial Growth and Carpophore Yield of *Agrocybe aegrerita* (Brig.) Sing. on Selected Agricultural and Textile Industry Wastes as a Cultivation Substrate

Agnieszka JASIŃSKA[1], Marek SIWULSKI[1], Krzysztof SOBIERALSKI[1], Włodzimierz MAJTKOWSKI[2], Jerzy ROGALSKI[3] and Shoji OHGA*

Laboratory of Forest Resources Management, Division of Forest Environmental Sciences,
Department of Agro–environmental Sciences, Faculty of Agriculture,
Kyushu University, Sasaguri, Fukuoka 811–2415, Japan
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In modern mushroom cultivation, the most important issue is to obtain possibly highest yields which are consistent with high quality of the carpophores. *Agrocybe aegerita* is an edible mushroom valuable due to medicinal properties. The aim of the performed investigation was to evaluate the mycelium growth and yielding of *A. aegerita* on agricultural and textile industry wastes. The subject of the studies was two strains of black poplar mushroom i.e. AE11 and AE06. The experiment showed that no statistically important differences between the mycelia growth of the investigated strains occurred. The mycelial growth was the best on straw of *Miscanthus gigantheus, Miscanthus sinensis* and *Miscanthus sacchariflorus*. The two investigated strains differed significantly with respect to their yield on the investigated substrates: wheat straw, *M. sacchariflorus* and *P. virgatum* mixture as well as flax and hemp shives mixture. The best substrate for the cultivation of *A. ægerita* AE11strain was mixture of flax and hemp shives while *M. sacchariflorus* and *P. virgatum* mixture for AE06. The content of dry matter was the highest on flax and hemp shives for both investigated strains.

Key words: black poplar mushroom, yielding, shives, energy grass, flax, hemp

INTRODUCTION

Black poplar mushroom – *Agrocybe aegerita* (Brig.) Sing. (=*A. cylindracea* (Brig.) Sing) is an edible mushroom common in the forests of southern Europe, United States and similar climatic zones of the Far East. In nature, *A. aegerita* grows saprophytically on living and decaying stumps of mostly deciduous trees such as: poplar, willow, black poplar, ash, elderberry and black locust (Wright and Alberto 2002). Black poplar mushroom is characterized by high content of protein, easily digested in human gastrointestinal tract (Bauer Petrovska and Kulevanova, 2000; Yildiz *et al*., 2005). Extracts from *Agrocybe aegerita* was found to have antioxidant properties (Cheung *et al*., 2003; Lo and Cheung, 2005; Tsai *et al*., 2007; Mujic *et al*., 2010).

Crop commodity, which very often depends on the speed of mycelium growth, is usually done on the sawdust of deciduous trees (Siwulski and Sobieralski, 2004; Uhart *et al*., 2008). However, experiments made by numerous authors suggest that also many rich in lignocellulosic compounds agricultural and forestry by–products could be used as a cultivation substrate for many edible mushroom (Philippoussis and Diamantopoulou, 2000; Philippoussis *et al*., 2001; Uhart *et al*., 2008; Isikhuemhen *et al*., 2009). White rot fungi, have the ability to decompose lignocellulosic materials to low molecule components, thanks to enzymatic systems which synthesizes hydrolytic enzymes: cellulase and hemicelullase and distinctive oxidative enzymes (Hoff *et al*., 2004) such as: lignin peroxidase, Mn–dependant peroxidase and phenoloxidase containing laccase (Elisashvili *et al*., 2001; Galhaup *et al*., 2002; Moldes *et al*., 2004; Mikiashvili *et al*., 2004; Vikineswary *et al*., 2006). There has been reported that synthesis of specific decomposing enzymes, lignocellulosic compounds decomposition and biological efficiency depends on the cultivation substrate (Valmaseda *et al*., 1991; Baldrian *et al*., 2005; Wang *et al*., 2005). Moreover, enzymatic activity changes throughout all stages (mycelium growth, primordia formation and fructification) of mushroom cultivation (Isikhuemhen *et al*., 2009; Isikhuemhen and Mikiashvili, 2009). The experiments of Sławińska and Kalbarczyk (2011) demonstrated that the activity of the enzymes depends on the stage of mycelium maturity as well as cultivation factors.

Obtaining high yields and good quality of the carpophores is one of the most important issues modern mushroom growers focus on choosing the substrate for cultivation. This is because even well designed cultivation factors such as temperature, relative air humidity, CO_2 concentration and substrate moisture are insufficient when the substrate is either badly chosen or poor in nutrients. The Black poplar mushroom is a species with a wide spectrum of substrates that could be used for its cultivation such as: sawdust from broad–leaf trees, wheat straw, barley, reed, rice husks, sunflower, cotton wastes or peanut shells (Philippoussis and Diamantopoulou, 2000; Philippoussis *et al*., 2001). In recent years attention of the mushroom growers turned also to so called 'energy

[1] Department of Vegetable Crops, Poznan University of Life Sciences, Dąbrowskiego 159, 60–637 Poznań, Poland
[2] Botanical Garden, The Plant Breeding and Acclimatization Institute (IHAR) Radzików, 05–870 Błonie, Bydgoszcz, Poland
[3] Department of Biochemistry, Maria Curie–Skłodowska University, Akademicka 19, 20–033 Lublin, Poland
* Corresponding author: (E–mail: ohga@forest.kyushu–u.ac.jp)

grasses' such as *Miscanthus* or switch grass which are commonly used as a biomass for production of biofuel, mostly because of their high biomass yield (Lewandowski *et al*., 2000; Jones and Walsh, 2001; Boehmel *et al*., 2007; Xie and Peng, 2011). The nutrient content of most energy grasses species is richer than those of broadleaf tree sawdust, and therefore mushrooms cultivated with substrates composed from those grasses have higher nutrition contents than those with sawdust or logs, which makes it perfect substrates for mushroom cultivation (Zhanxi, 2004; Zhanxi, 2005). Energy grasses species are suitable for production of over 45 species of mushroom such as: *Agaricus bisporus, Pleurotus ostreatus, Lentinula edodes, Volvariella volvaceae, Ganoderma lucidum, Pholiota nameko, Flammulina velutipes* or *Hericium erinaceus* including *Agrocybe aegerita* (Zhanxi, 2004; Zhanxi, 2005; Siwulski *et al*., 2010; Shrestha *et al*., 2011; Sobieralski *et al*., 2011). The need for substitutes for fossil fuels is enormous and the production of biomass will increase, because the EU aims at increasing the share of renewable energy of the total primary energy consumption from 6.4% in 2004 to 20% in 2020. Poland is promising bioenergy producing region where the estimated production of the biomass is almost 30 (oven dry tonne) odt ha^{-1} y^{-1} (Smeets *et al*., 2009) and the accessibility of the biomass will be sufficient also for mushroom production. Flax and hemp shives which are post–production wastes from manufacture of natural fibers in Poland which need to be utilized (Mańkowska *et al*., 2007). Textile industry wastes has been used with success for cultivation of *Pleurotus ostreatus* by Siwulski *et al*. (2011) and Sobieralski *et al*. (2011) therefore those were used in the conducted experiment as a cultivation substrates.

However agri–forestry wastes are generally considered to be very good substrates for cultivation of many edible fungi, very often nutrient supplements, high in organic nitrogen, phosphorous, calcium *etc*., such as: rape oil, whey, yeast extract, corn meal, rapeseed meal or flax meal are added to lignocellulosic materials like sawdust and cereals straw, in order to increase substrate degradation and mushroom yield (Kalbarczyk, 2004; Royse *et al*., 2007; Isikhuemhen *et al*., 2009). In the main, yield and quality of carpophores is better on mixed substrate (either it is mixture of different sawdust, straws or other spent substrate) than on uniformed substrate. It might be the result of richer growing environment, more different sources of nitrogen and carbon which are crucial for fruiting body development. Moreover mixed substrates have better water–air conditions which results from its different structure (Philippoussis *et al*., 2001; Uhart *et al*., 2008; Amin *et al*., 2009; Jasińska *et al*., 2012).

The aim of this study was to determine the best substrates for mycelium growth as well as the best substrates for the cultivation of *A. aegerita*.

MATERIALS AND METHODS

Two strains of *A. aegerita*, designated as AE06 and AE11, were used in the described experiments. Both of the strains derived from the collection of cultivated and medicinal mushrooms of the Department of Vegetable Crops, PULS. Investigations were divided into two trials, namely: laboratory in two series and cultivation in two cycles.

In the laboratory trial mycelium growth on six agricultural and two textile industry wastes and their mixtures were investigated: wheat straw (WS), *Miscanthus gigantheus* straw (MG), *Miscanthus sacchariflorus* straw (MS), *Miscanthus sinensis* straw (MSI), *Andropogon gerardii* straw (AG), *Elymus elongatus* ssp. *ponticus* straw (EP) and *Panicum virgatum* straw (PV), hemp shives (HS) and flax shives (LS). The compounds of the above wastes mixtures were as follows:

- straw of *Miscanthus sinensis* mixed at 1:1 ratio (vol.) with straw of:
  - *Miscanthus sacchariflorus* (MSI+MS)
  - *Andropogon gerardii* (MSI+AG)
  - *Elymus elongates* ssp. *ponticus* (MSI+EP)
  - *Panicum virgatum* (MSI+PV)
- straw of *Miscanthus gigantheus* mixed at 1:1 ratio (vol.) with straw of:
  - *Miscanthus sacchariflorus* (MG+MS)
  - *Andropogon gerardii* (MG+AG)
  - *Elymus elongates* ssp. *ponticus* (MG+EP)
  - *Panicum virgatum* (MG+PV)
- flax shives + hemp shives mixed at 1:1 ratio (vol.) (FS1/HS1)
- flax shives + hemp shives mixed at 9:1 ratio (vol.) (FS9/HS1)
- flax shives + hemp shives mixed at 4:1 ratio (vol.) (FS4/HS1)
- flax shives + hemp shives mixed at 7:3 ratio (vol.) (FS7/HS3)
- flax shives + hemp shives mixed at 3:7 ratio (vol.) (FS3/HS7)
- flax shives + hemp shives mixed at 3:2 ratio (vol.) (FS3/HS2)

Substrates were moisturized up to 70% and placed in glass tubes (18 cm long and Ø 2.5 cm). The tubes were field with substrates to the high of 14 cm, closed with cotton corks and sterilised in an autoclave at 121°C for 45 minutes. When the substrates cooled down to the temperature of 25°C, they were inoculated with 1,5 cm of granular spawn of the investigated strains placed on the top layer of substrate inside the biological tubes.

Granular mycelium was prepared on wheat grains according to Lemke (1971). Maternal mycelium and granular spawn of the examined strains was prepared in the laboratory of the Department of Vegetable Crops, PULS.

Incubation was held for 18 days at 25°C and relative air humidity of 85–90%. After 18 days of incubation, the thickness of the substrate layer overgrown by mycelium was measured.

The first trial was established in fully randomized design in 4 replications and 2 series. The results were analyzed using variance for two–factorial experiments using Newman–Keuls test at the level of significance of

α =0,05. The results were discussed on the basis of mean values obtained from two series of experiments.

The second trial was established to compare three different cultivation substrates: wheat straw, mixture of *Miscanthus sacchariflorus* straw with *Panicum virgatum* straw (1:1 vol.) and mixture of flax and hemp shives (1:1 vol.). The agricultural and textile industry wastes and their mixtures were chosen based on the results of first phase of experiment. Each substrate was moisturized up to 70%. Cultivation was established in an air–conditioned chamber in plastic bottles of 600 ml capacity as cultivation containers. Each bottle was filled with the substrate and covered with a plastic lid with 4 ventilation holes with filter. After sterilization at 121°C for 1.5 h and cooling to the temperature of 25°C, the substrate was inoculated with granular spawn of the investigated strains in the amount of #5 in relation to the substrate DM. The incubation was conducted at 25°C and relative air humidity of 85–90%. When the substrates were overgrown by mycelium, the plastic lids were removed and bottles were placed in a cultivation room. The temperature was decreased to 15–17°C to initiate primordial formulation and carpophores development. The air humidity for fruiting bodies development was held at a high level ranging from 85–95%. The cultivation was lighted with fluorescent lamps (Day–Light) with 500 lx intensity for 10 h per day. The cultivation room was aired not to allow CO_2 concentration to exceed 1000 ppm.

Carpophores were picked in clusters; no single carpophores were cut out from the substrate, at the moment when edges of the oldest caps began to straighten. The yield comprised carpophores together with stipes. They were collected from the first crop in both cycles of the cultivation experiment. Yields of carpophores were determined based on harvested fruiting bodies, calculated per 100 g of substrates dry matter.

The dry matter content of carpophores was determined using gravimetric method. Carpophores were first dried for 12 hours at the temperature of 50°C, and later on were dried at 80°C to constant weight.

The experiment was established in fully randomized design, in 4 replications and 2 cultivation cycles. The results were analyzed using variance for two–factorial experiments using Newman–Keuls test at the level of significance of α =0,05. The results were discussed on the basis of mean values obtained from two cultivation cycles.

RESULTS AND DISCUSSION

The first experiment was set up to assess the influence of the type of substrates on colonization rates (Figs. 1 and 2). Lomberth *et al*. (2002) reported that mycelium growth rate differed between species and sometimes even within strains of mushroom and depended on the type of the substrate.

In nature, carpophores of *A. aegerita* appear commonly on many species of trees and bushes and the cultivation is most commonly carried out on sawdust of the above–mentioned trees (Poppe and Höfte, 1995; Siwulski and Sobieralski, 2004; Uhart *et al*., 2008). However, for practical reasons, the cultivation substrate is mostly chosen depending on its availability (Sobieralski *et al*., 2007). In our experiment, both of the examined strains showed a similar mycelium growing rate regardless of the used substrate. From the investigated straws of energy grasses, the best mycelium growth was obtained on the substrate from MG, MSI and MS. The substrates that exhibited the second best mycelium growth were: EP, PV and MSI+MG. Moderate mycelium growth were observed on substrates mixed from straws of *M. sacchariflorus, A. gerardii, E. ponticus, P. virgatum* with *M. sinensis* respectively: MSI+MS, MSI+PV, MSI+AG and MSI+EP. Three substrate mixtures showed significantly slower growth of mycelium than the best and second best substrates. These were: MG+MS, MG+PV and MG+EP. The slowest mycelium growth was recorded on wheat straw (Fig. 1).

The best mycelium growth on textile wastes was recorded on homogenous flax shives (FS). Second best mycelium growth was observed on mixtures: FS7/HS3 and FS1/HS1. Slower mycelium growth was observed on

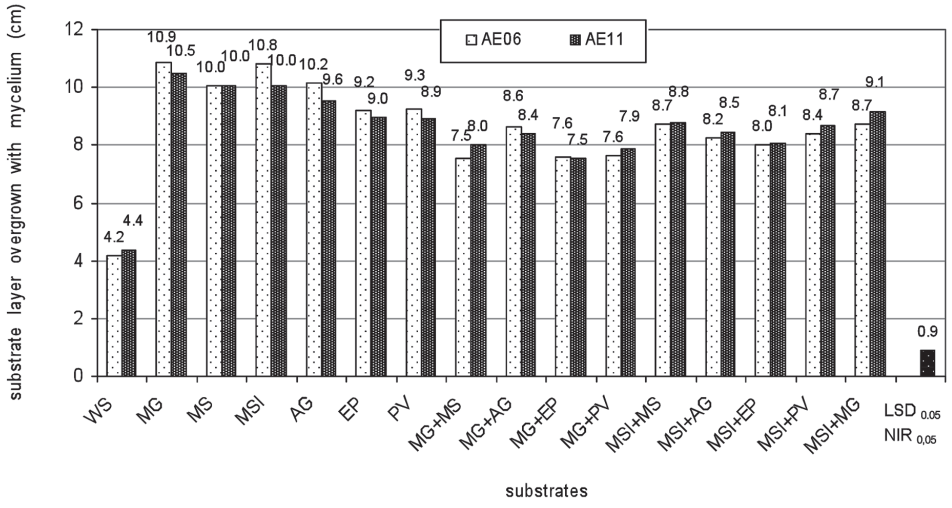

Fig. 1. Mycelium growth of two *A. aegerita* strains on different straw substrates.

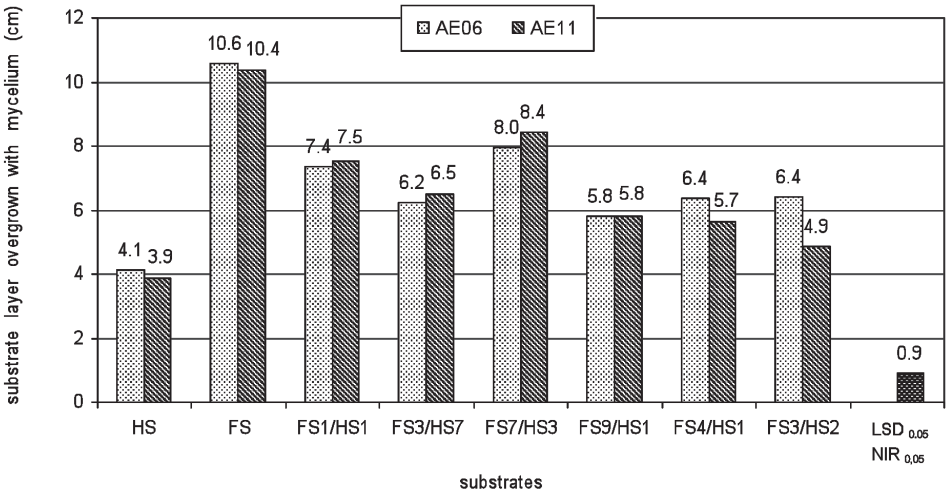

Fig. 2. Mycelium growth of two *A. aegerita* strains on different shives substrates.

mixture FS3/HS7 and FS4/HS1. Mixture FS9/HS1 showed equally slow mycelium growing rate for both investigated strains, while mixture FS3/HS3 showed a slightly faster growth of the AE06 than AE11 mycelium. Slowest growth for both investigated strains was obtained on homogenous hemp shives (HS). Those results correspond with previous experiments carried out by Siwulski *et al.* (2010), where mycelium of *A. aegerita* showed the best growth on homogenous flax shives. The reaction of the strains on the substrate was similar.

In published papers, average yield of *A. aegerita* was reported at 15–20% of fresh mass of the substrate (Philippoussis and Diamantopoulou, 2000; Sobieralski *et al.*, 2007; Uhart *et al.*, 2008; Isikhuemhen *et al.*, 2009; Jasińska *et al.*, 2012). However, there are few specific scientific papers on yielding of *A. aegerita*. Actually, only the wheat straw substrate can be compared with investigations of other authors. No mention of the two other cultivation substrates used in our experiments for the yielding of *A. aegerita* was found in the available literature.

Yield of carpophores of the investigated strains differed and depended on the used cultivation substrate (Siwulski and Sobieralski, 2004; Mujic *et al.*, 2010). The yielding was also different within the investigated strains. The highest yield of strain AE06 was obtained on mixture MSI+PV. Lower yields for AE06 were harvested from two other substrates – WS and FS/HS. Strain AE11 showed significantly highest yield on the substrate mixture from FS/HS. Two other substrates showed exactly the same yield (Fig. 3).

The obtained results differed significantly when compared with those obtained by other authors. Philippoussis and Diamantopoulou (2000) obtained on wheat straw only 14 g/100 g of substrate and Isikhuemhen *et al.* (2009) –10 g/100 g, while in our experiment we harvested, on average, 33 g/100 g of substrate. However, our results were similar to those obtained on the wheat straw by Uhart *et al.* (2008) – the average 28 g/100 g of substrate. Philippoussis *et al.* (2001) obtained little bit higher yields than those reported in our experiment – 47 g/100 g of substrate.

One of the most important factors, which describe the quality of the mushroom fruiting bodies is dry matter

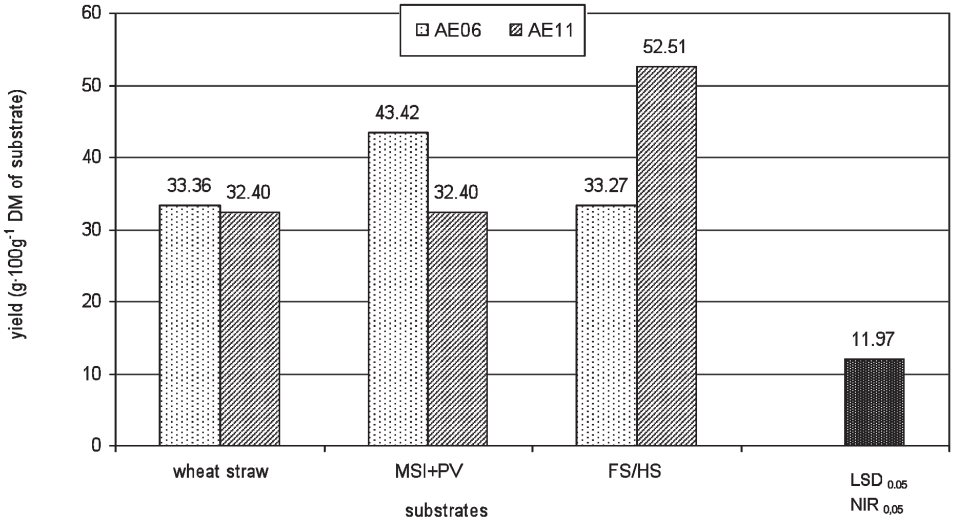

Fig. 3. Yield of two *A. aegerita* strains on different substrates.

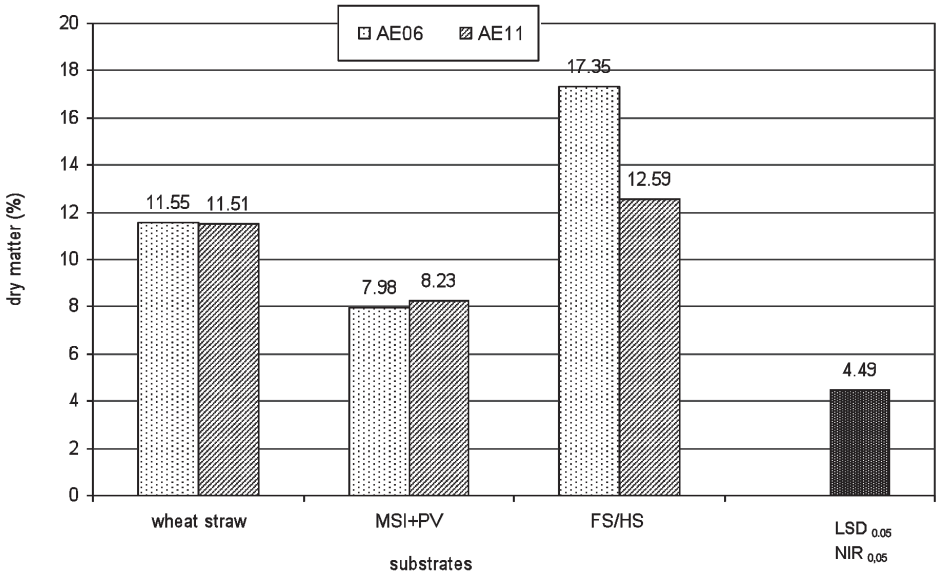

Fig4. Dry matter content of *A. aegerita* carpophores.

(DM) content in the carpophores. Generally, DM content in fruiting bodies of cultivated mushrooms ranges from 7.9 to 11.4% in *Agaricus bisporus* (Colak *et al*., 2007), 8.0% in *Pleurotus ostreatus*, 14.3% in *Boletus edulis* (Bauer Petrovska and Kulevanova, 2000) and depends on the cultivation substrate used (Colak *et al*., 2007). The dry matter content of harvested mushroom was the highest for both strains on the FS/HS substrate mixture (Fig 4). Carpophores harvested from the wheat straw substrate showed lower dry matter content for both strains, with no significant differences between the strains. The lowest dry matter content was obtained on the MSI+PV mixture, also with no significant differences between strains. Percentage of dry matter within the obtained carpophores of strain AE6 compared to the yield harvested was higher in the FS/HS substrate (17.35%), the total yield of AE06 for this substrate was low ($33.27\ g \cdot 100\ g^{-1}$), than for strain AE11, where yield was high ($52.51\ g \cdot 100\ g^{-1}$) and dry matter content was low (12.59%). This results for wheat straw and flax and hemp shives mixture substrates were much higher than those obtained by Bauer Petrovska and Kulevanova (2000), where the DM content of *A. aegerita* carpophores was 10.2%. Probably such big differences can be explained by the fact that the samples of *A. aegerita* in Bauer Petrovska and Kulevanova (2000) study came from natural habitats.

Black poplar mushroom is reported to utilize preferably cellulose before hemicelluloses and lignin, which might suggest that this species is an brown rot fungi (Wang *et al*., 2000; Kempken, 2002). White rot fungi can degrade lignin effectively and than utilize cellulose further, whereas brown rot fungi cannot utilize lignin–cellulose easily. Contrary to the cellulase of the white–rot fungi, that of the brown–rot fungi apparently is constitutive, since activity was abundant in cultures with simple sugars or with non–cellulosic polysaccharides as the sole source of carbon (Highley, 1973). C:N ratio of substrate is crucial to the rate of lignocellulose degradation and mushroom production. The high nitrogen content can exercise a negative effect on mycelial growth, moreover high lignin content proved less efficient, as cellulose may not be readily available as carbon source (Mikashvili *et al*., 2006). Wheat straw substrate was commonly used by many authors as a successful cultivation substrate. However, in our study, it showed significantly slower mycelial growth rate and lower yields than the two other substrates. A possible explanation of the remarkable yield on two other substrates is sensitivity of *A. aegerita* strains to the low cellulose content and relatively high lignin levels together with high C:N ratio of wheat straw (Philippoussis *et al*., 2001). Hemp is rich source of cellulose, lignin and fatty acids (Mańkowska *et al*., 2007), which makes them good source of nutrients for *A. aegerita* mycelium growth and fruit body development. Furthermore, straw of Miscanthus also contain high amount of cellulose and pentosans which are easily accessible and digestible compounds by mycelium of *A. aegerita*, when level of lignin is much lower (Komorowicz *et al*., 2009). Above mentioned data explains why the yield obtained on the hemp/flax shives and miscanthus–panicus mixture was high.

It can be concluded on the basis of the performed investigation that, due to good mycelium growth and yield, flax and hemp shives and their mixtures can be employed as a substrate for the cultivation of *A. aegerita*. In the recent literature there is no reports about growing mushrooms on hemp or flax shives however, some authors mentioned earlier possibilities of utilization of flax wastes for mushroom cultivation of genus: *Pleurotus, Volvariella* and *Auricularia* (Chang, 1976; Chang and Hayes, 1978; Sobieralski *et al*., 2011). There are some doubts if homogenous hemp shives are suitable for commercial cultivation of *A. aegerita*, due to the slow growth rate of mycelium. However, mixtures with flax shives accelerate the growth speed of mycelium. Furthermore, the yield was also higher than on two other investigated substrates (WS and MSI+PV). This may have some connections

with hemp shives antiseptic properties and, therefore, their use as a supplement in cultivation should be considered (Dorna *et al*., 2008). *A. aegerita* is reported to act as an brown rot fungi, decomposing preferably other sugar polymers such as cellulose, with minimal degradation of lignin. Therefore, all agricultural by–products, such as grasses or shives, containing more cellulose than lignin are favorable before forestry wastes, like sawdust. Thus the production of energy grasses is increasing, the availability of biomass will be sufficient to cover biofuel production and mushroom cultivation.

CONCLUSION

1. Mycelium growth, yield and dry matter content of carpophores depended on the substrate and strain used in the experiment.
2. From the energy grasses, the best mycelium growth of both investigated strains was obtained on straw of *Miscanthus gigantheus, Miscanthus sinensis* and *Miscanthus sacchariflorus*.
3. From textile wastes mixtures the best mycelium growth was observed on homogenous substrate from flax shives.
4. The best substrate for cultivation of strain AE06 was a mixture of energy grasses *Miscanthus sacchariflorus* and *Panicum virgatum*, while strain AE11 gave the biggest yield on the mixture of flax shives and hemp shives.
5. The highest dry matter content of carpophores for both strains was obtained on the mixture of flax and hemp shives.

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