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# Morphological and Qualitative Features of *Agrocybe aegerita* (Brig.) Sing. Carpophores Cultivated on Agricultural and Textile Industry Wastes

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The yield and quality of mushrooms depend on the substrate used to cultivation as well as on the cultivation conditions. Morphological features of carpophores are one of the qualitative characteristics of the yield and it highly depends on the substrate used in cultivation. The aim of the conducted experiment was to asses morphological and qualitative features of *Agrocybe aegerita* carpophores cultivated on agricultural wastes: wheat straw, straw of energy grasses (mixture of *Miscanthus sacchariflorus* (MSC) straw with *Panicum virgatum* (*PV*) straw (1:1 vol.) and flax (FS) and hemp shives (HS) mixture (1:1 vol.). The laboratory experiment showed that the heaviest and the biggest carpophores of both investigated strains, were harvested from MSC/PV substrate (7.0 g–AE06 and 5.2 g–AE11). However strain AE06 had heavier caps on FS/HS (4.1 g) but bigger on MSC/PV (39 mm). AE11 harvested from wheat straw showed bigger and heavier caps and carpophores than AE06 (31 mm and 3.1 g and 27 mm and 2.3 g, respectively). The highest dry yield of carpophores was obtained on FS/HS mixture for strain AE11 (6.6 g/100 g substrates DM).

**Key words**: black poplar mushroom, agricultural wastes, energy grasses

## INTRODUCTION

*Agrocybe aegerita* (Brig) Sing. (= *A. cylindracea* (Brig.) Sing) is an edible mushroom commonly known in the countries of Mediterranean See Basin. *A. aegerita* is a saprophytic mushroom, in nature growing on living and decaying stumps of mostly deciduous trees such as: poplar, willow, black poplar, ash (Wright and Alberto, 2002). However numerous ligninocellulosic substrates, such as wheat, barley and maize straw, orange peels, grape stalks, rice husks and sunflower are used for the cultivation of this species (Nicolini *et al*., 1987; Zadrazil, 1993; Philippoussis and Diamantopoulou, 2000). The carpophores of *A. aegerita* grow in clusters appearing from the same basin. When young the cap is convex, later on it is expanding to plane, grow up to 20 cm in diameter. Cap color is predominantly yellowish gray to grayish brown, darker towards the center. Gills are at first gray, with spore maturity becoming chocolate brown. Stem is mostly white, adorned with a well developed membranous ring, usually colored brown from spore fall (Uhart and Alberto, 2007). The carpophores of black poplar mushrooms show large morphological variations, which is expressed in appearing specimens with different color and surface of the cap (Singer, 1986; Watling, 1992). Most commonly those features are coupled with the substrate and the conditions in which the mushroom is growing (Uhart and Alberto, 2007). Furthermore, substrates seem to differentiate the earliness of primordia formulation of *A. aegerita*. In investigation performed by Philippoussis *et al*. (2001) earliest primordia developed on sawdust substrate and cotton husks substrate.

Mushrooms consist mostly of water, in average 90% therefore the best way of preservation is the dried form. The nutrient values of the mushrooms differ between the species: moisture of fruiting bodies can rate from 73 to 91% of fresh matter, carbohydrates from 57 to 83% of dry matter, protein –20 to 40% of dry matter, fat –3.7 to 10.0% of dry matter (Chang and Miles, 1989; Chang and Buswell, 1996; Chang and Mshigeni, 2001). The main component (50%) of dry matter of mushroom are carbohydrates (chitin, cellulose, lignin and fibers – mostly $\beta$–glucans) together with free saccharides (Manzi *et al*., 2001). Content of fat, as mentioned before, is very low. Majority, over 70%, are unsaturated acids, where 15% is ergosterol – a precursor in vitamin D synthesis (Beelman and Royse, 2003). Besides vitamin D, mushrooms are rich in riboflavin, niacin, folic acid and thiamin, the amount of those vitamins are higher than in most of other vegetables (Mattila and Konko, 2001). *Agrocybe aegerita* is also protein rich (34 to 47% of DM) and low fat (3% of DM) mushroom (Bauer Petrovska and Kulevanova, 2000; Yildiz *et al*., 2005; Konuk *et al*., 2006; Tsai *et al*., 2008). Mushroom proteins are digested easier by the human digesting system than many legume sources like soybeans or peanuts and the content of fat is low. Those features make mushrooms desirable component of dietary products. Among above mentioned nutrients, mushroom consist significant amount of active components (polysaccharides, alkaloids, glucans, and lectins) with medicinal and nutritional properties, such as lentinian (antitumor) from *Lentinus edodes*, lovastatin (lowering cholesterol level) from *Pleurotus ostreatus* and ganodermic acids – from *Ganoderma lucidum* responsible

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for immunomodulatory and immunotherapeutic activities. *A. aegerita* is also an interesting species with some remarkable features such as healing properties like: antitumor, antifungal, antioxidant, nerve tonic, hypercholesterolemia and hyperlipidemie (Cheung *et al.*, 2003; Lo and Cheung, 2005; Tsai *et al.*, 2007; Wesser and Weiss, 1999; Mujic *et al.*, 2010); low level of heavy metals accumulation (Cocchi *et al.*, 2006), soil bioremediation ability (Hofrichter and Ullrich, 2006; Gall *et al.*, 2008) and could be used for biological extermination of nematodes (Zhao *et al.*, 2009).

Edible and medicinal mushrooms are commonly cultivated on wide range of organic origin substrates. Mostly sawdust of deciduous trees are used for the cultivation of such popular species as *Lentinula edodes*, *Ganoderma lucidum*, *Auricularia auricula*, *Flammulina velutipes*, *Hericium erinaceus* and *Pholiota nameko* (Stamets, 2000). *Pleurotus* ssp. however in nature occurring on rotten trees and bushes, in commercial production is cultivated on straw of grains, mostly on wheat straw, which is an agricultural waste for this manner. Many researchers show there is wide range of possibilities for utilization of materials which contain cellulose and lignin considered as an spent substrate or by–product by growing edible mushroom such as Black poplar mushroom (Poppe and Hofte, 1995; Poppe, 2000; Zervakis *et al.*, 2001; Rani *et al.*, 2008; Dundar *et al.*, 2009). Most of cultivated species of mushrooms are so called white rot fungi and have ability to decompose lignocellulosic materials to low molecule components, thanks to enzymatic systems (Hoff *et al.*, 2005). Modern literature shows that over 40 species of mushrooms are known to be cultivated on so called 'energy grasses', commonly used as a biomass for biofuel production and textile industry post–production wastes from manufacture of natural fibers (Zhanxi, 2004; Zhanxi, 2005; Siwulski *et al.*, 2011; Sobieralski *et al.*, 2011).

The aim of presented study was to compare morphological and qualitative features of carpophores of *A. aegerita* harvested from selected substrates such as: spent product from textile industry: flax and hemp shives and straw of energy grasses: *Miscanthus sacchariflorus* and *Panicum virgatum*.

## MATERIALS AND METHODS

Two strains of *A. aegerita*, indicated as AE06 and AE11, were used in following experiments. Both of the strains derived from the collection of cultivated and medicinal mushrooms of the Department of Vegetable Crops, PULS.

Experiment was established to compare morphological and qualitative features of Black poplar mushroom carpophores cultivated on three different cultivation substrates: wheat straw (100%), mixture of *Miscanthus sacchariflorus* (MSC) straw with *Panicum virgatum* (PV) straw (1:1 vol.) and mixture of flax (FS) and hemp shives (HS) (1:1 vol.). Substrates were moisturized up to 70%. Cultivation was established in an air–conditioned chamber in plastic bottles of 600 ml capacity as the cultivation containers. Each bottle was filled with the substrate and covered with the plastic lid with 4 ventilation holes with filter. After sterilization in 121°C for 1.5 h and cooling to the temperature of 25°C the substrate was inoculated with granular spawn of investigated strains in the amount of 3% in relation to the substrate DM. The incubation was conducted at 25°C and relative air humidity 85–90%. When the substrates were overgrown by mycelium, the plastic lids were removed and bottles were put in climate chamber. The temperature was decreased to 15–17°C to initiate primordia formulation and carpophore development. The air humidity for fruiting bodies development was held on high level 85–95%. The cultivation was lighted with fluorescent lamps (Day–Light) with 500 lx intensity of 10 h per day. The cultivation room was aired not to allow $CO_2$ concentration to exceed 1000 ppm.

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.

Harvest of *A. aegerita* carpophores was carried out for a period of 6 weeks. The carpophores were picked up in clusters; no single carpophores were cut out from the substrate. Morphological features of carpophores were assessed on 10 fruiting bodies sampled from each repetition. The measurements of cap diameter, diameter and length of the stem together with single cap and carpophores weight were performed. The weight of single carpophores and caps were calculated per 100 g of substrates dry matter. Moreover the dry yield of carpophores was indicated. In addition the content of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) was indicated in the carpophores. Carpophores were dried in 60°C in oven until their weight became stable and powdered. For indication of total forms of P, K, Ca and Mg samples were mineralized in concentrated sulfuric acid. Total nitrogen was indicated after sample mineralization in mixture of sulfuric acid and sulfosalicylic acid with Kjeldahl method in Parnas–Wagner apparatus; phosphorus (P) was indicated with colorimetric method with ammonium molybdate; potassium (K), calcium (Ca) and magnesium (Mg) with Carl Zeiss Jena; Thornwood NY, USA).

Experiment was established in fully randomized design, in 4 replications and 2 cultivation cycles. Differences between mean values of carpophores weight, cap weight and diameter, stem length and diameter were analyzed with two way ANOVA. A value of $P<0.05$ was considered to be significant. Statistic analysis was carried out with STATISTICA. The results were discussed on the basis of mean values obtained from two cultivation cycles.

## RESULTS AND DISCUSSION

Sawdust substrate is of most use for cultivation of *Agrocybe aegerita*, where the use of agricultural wastes as the growing substrate depends mostly on the region of mushroom cultivation and differs significantly (Siwulski and Sobieralski, 2004; Uhart *et al.*, 2008). Authors of this paper did not find the corresponding literature on influence of investigated substrates on the morphological and qualitative features of carpophores. However, carpophores of *A. aegerita* show differences in their morphology depending mostly on the type of substrate used for cultivation (Uhart and Albertó, 2007). For instance on sawdust of deciduous trees carpophores of *A. aegerita* performs heavier and bigger carpophores (Amin *et al.*, 2009; Jasińska *et al.*, 2012). The morphological and qualitative features of carpophores in our experiment differed among used cultivation substrate and investigated strain.

From the investigated cultivation substrates the heavies carpophores were obtained on mixture MSC/PV. Moderate weight of carpophores was observed on mixture FS/HS and the lightest carpophores were obtained from wheat straw. In general strain AE06 performed with heavier carpophores than AE11 on MSC/PV and FS/HS, only on wheat straw strain AE11 had heavier carpophores than strain AE06 (Fig. 1). Carpophores of AE06 harvested from FS/HS substrate characterized with the heaviest caps, those harvested from MSC/PV were lighter, the lightest caps was picked from wheat straw. These results are corresponding with what Philippoussis *et al.* (2001) obtained on wheat straw. Strain AE11 again showed similar dependence: on wheat straw its caps were heavier than strain AE06, however on other two substrates caps were significantly lighter than AE06 (Fig. 2).

The cap diameter showed similar correlation as the cap and carpophores weight. The largest caps strain AE06 presented on MSC/PV, medium sized on FS/HS and the smallest on wheat straw. Caps of AE11 were the largest on FS/HS, medium sized on MSC/PV and the smallest on wheat straw (Fig. 3). Uhart *et al.* (2008) obtained in their experiments on wheat straw cap diameter of maximum 42 mm, which is similar to the diameter obtained by the authors. Amin *et al.* (2009) in their experiment obtained biggest caps on mixed sawdust substrate (gorjan, mango, mahogany, acacia, teak, chamba and rain tree) –58 mm, followed by paddy straw –55 mm. This corresponds with Jasińska *et al.* (2012) experiment where the biggest caps were obtained on mixed sawdust substrate (beech and alder –33 mm). Big and heavy caps are desirable feature in *Agrocybe aegerita* production because they consist the edible part of the carpophore. The stem are basty and hard which makes them not tasty, however edible (Siwulski and Sobieralski, 2004).

The stem length and diameter also depended on the cultivation substrate, however the differences between the strains were of no importance. Both strains AE06 and AE11 grew longest stems from FS/HS substrate, medium sized on wheat straw and MSC/PV (Fig. 4). The stem diameter was again different for cultivation substrates. Differences between the strains were important only on the MSC/PV, where strain AE06 grew thicker stems than strain AE11. The thickest stem were among carpophores harvested from MSC/PV, medium sized on

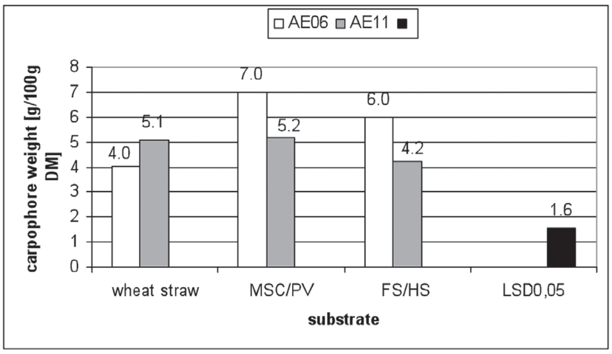


**Fig. 1.** Average weight of carpophores of two investigated strains *A. aegerita* cultivated on different substrates.

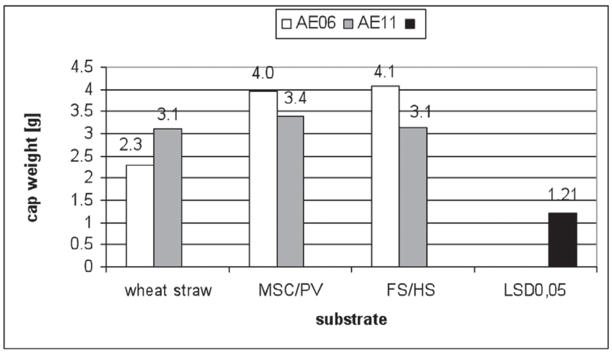


**Fig. 2.** Average weight of caps of two investigated strains *A. aegerita* cultivated on different substrates.

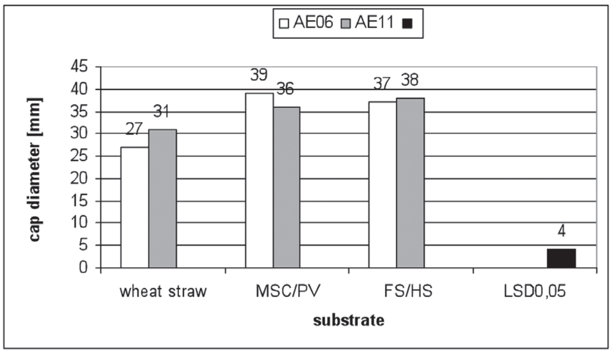


**Fig. 3.** Average cap diameter of two investigated strains *A. aegerita* cultivated on different substrates.

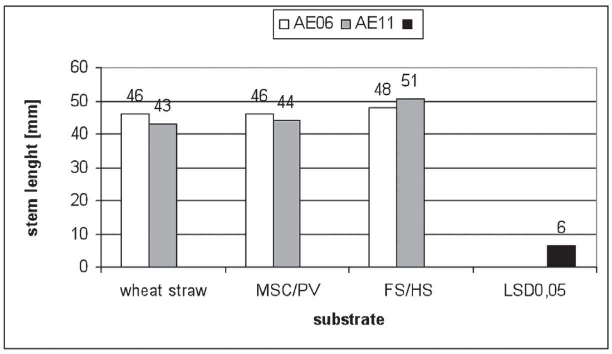


**Fig. 4.** Average stem length of two investigated strains *A. aegerita* cultivated on different substrates.

FS/HS and wheat straw (Fig. 5). In experiment performed by Amin *et al.* (2009) the longest stem was obtained on mixed sawdust substrate (90 mm) and paddy straw (88 mm) and on cotton waste (79 mm) which are much longer than in our experiments, however not desirable feature. Longest stems on sawdust mixture (beech and alder –48 mm) obtained also Jasińska *et al.* (2012). This might be result of different methods of cultivation, in our experiment we used polypropylene bottles while Amin *et al.* (2009) used polypropylene bags, where stems had more space to grow.

The dry yield of carpophores is generally correlated with substrate and the supplementation, substrate moisture and temperature of the cultivation. In our investigation dry yield of carpophores was the highest on mixture of flax/hemp shives for both examined strains and differed significantly between the substrates (Fig. 6). However strain AE11 showed highest dry yield content only on FS/HS, it was much higher than dry carpophore yield of strain AE06 on any other substrate. Second best substrate was wheat straw, for both strains. The lowest dry yield of carpophores was obtained from mixture MSC/PV, for both strains. Comparing obtained results with authors previous experiment (Jasińska *et al.*, 2012), again mixed substrate seems to be in favor for yielding and obtaining the dry yield of carpophores. On mixed substrate from beech and alder sawdust Jasińska *et al.* (2012) obtained 3,2 g/100 g substrates DM, which is much lower than the highest obtained in this experiment on FS/HS mixture.

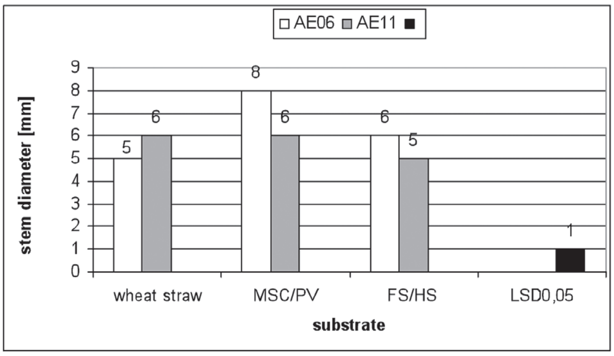


**Fig. 5.** Average stem diameter of two investigated strains *A. aegerita* cultivated on different substrates.

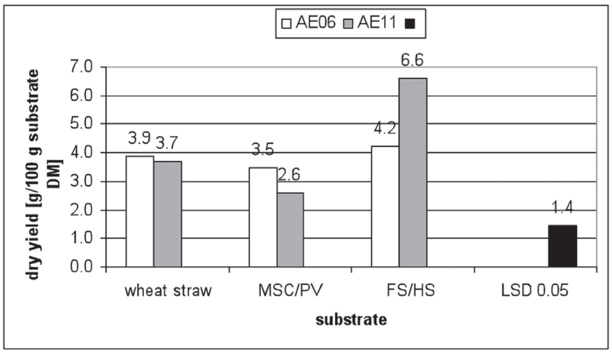


**Fig. 6.** Average dry yield of carpophores of two investigated *A. aegerita* cultivated on different substrates.

Content of N, P, K, Ca and Mg in carpophores of *A. aegerita* cultivated on different substrates is showed in Table 1. Many authors stress the significant differences between chemical composition of carpophores of different species as well as strains of cultivated mushrooms (Crisan and Sands, 1978; Chan *et al.*, 1981; Shin *et al.*, 2007). Furthermore chemical composition of carpophores changes under influence of used cultivation substrate (Chang and Miles, 1989; Cheung, 2008; Siwulski *et al.*, 2011). Konuk *et al.* (2006) indicated levels of macro– and microelements as follows: P –84,5; K – 84,8; Ca –45,7 and Mg –18,8 (ppm/ 1 g of dry matter), those results classified *A. aegerita* within the rich and semi rich in nutrients mushroom among 15 investigated species. Our investigation shows emphatically higher amounts of phosphorous, potassium, calcium and magnesium (Table 1). This could be explained with the subject of study – Konuk *et al.* (2006) investigated wild specimens, whereas in our experiment we examined cultivated strains of *A. aegerita*. The amount of crude nitrogen range between 5.46–7.48% of carpophores DM (Konuk *et al.*, 2006; Bauer Petrovska and Kulevanova, 2000; Yildiz *et al.*, 2005) which corresponds with results of our investigation (5.54 and 7.36%). The highest amount of crude nitrogen was indicated on mixture of FS/HS, medium on MSC+PV and lowest amount on wheat straw. Hemp is rich source of cellulose and lignin making them very good source of nutrient for fruiting body development (Mańkowska *et al.*, 2007).

Overall conclusion on the basis of performed investigation and the data from literature is that carpophores of *A. aegerita* grows bigger and heavier on mixed sub-

**Table 1.** Content of macro- and microelements in carpophores of *A. aegerita* cultivated on different substrates [% D.M.]

| | strain | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AE06 | | | | | AE11 | | | | |
| | Macro– and microelements [% D.M.] | | | | | | | | | |
| substrate | *N* | *P* | *K* | *Ca* | *Mg* | *N* | *P* | *K* | *Ca* | *Mg* |
| wheat straw | 5,54 | 0,69 | 2,6 | 0,012 | 0,13 | 5,22 | 0,72 | 2,91 | 0,036 | 0,16 |
| MSC/PV | 5,73 | 0,69 | 3,27 | 0,047 | 0,17 | 6,07 | 0,76 | 3,42 | 0,052 | 0,18 |
| FS/HS | 7,36 | 0,76 | 2,63 | 0,02 | 0,15 | 6,6 | 0,73 | 2,66 | 0,046 | 0,15 |

strate (either it is mixture of different sawdust, straws or other spent substrate) than on uniformed substrate. This might be result of richer growing environment, more different sources of nitrogen and carbon which are crucial for fruiting body developments. In addition better water–air conditions of mixed substrates resulting from its different structure seam to amend the carpophores performance (Philippoussis *et al.*, 2001; Uhart *et al.*, 2008; Amin *et al.*, 2009; Jasńska *et al.*, 2011).

Comparison of our results with the literature is difficult for the reason that *Agrocybe aegerita* and its cultivation is highly understudied species. There are plenty of works dealing with it's genetics and medicine properties but very few which consider its growth abilities, yield and carpophores morphology in commercial production. Without doubt further experiments on cultivation of *A. aegerita* on different spent substrates are essential to develop appropriate methods of use of agricultural wastes in cultivation of this species.

## CONCLUSION

1. The carpophores morphological features depended on the cultivation substrate.
2. The heaviest and the biggest carpophores were harvested from mixture of *Miscanthus sacchariflorus* straw with *Panicum virgatum* straw.
3. Strain indicated as AE06 showed the biggest and heaviest caps and carpophores on two investigated substrates: *Miscanthus sacchariflorus* straw with *Panicum virgatum* straw and flax and hemp shives mixture than strain AE11.
4. Strain indicated as AE11 harvested from wheat straw showed bigger and heavier caps and carpophores than AE06.
5. The highest dry yield of carpophores was obtained for both strains on flax and hemp shives mixture, however for AE11 it was significantly higher (6.59 g/100 g substrates DM; AE06 –4.23 g/100 g substrates DM) than for both strains on other substrates.
6. *Miscanthus sacchariflorus* straw with *Panicum virgatum straw* and flax and hemp mixture can be successfully used for cultivation of *Agrocybe aegerita* in Poland.

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