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Impact of substrate supplemented with $CaCO_3$ on mycelial growth, yield, morphological features and storability of fruiting bodies of black poplar mushroom *Agrocybe cylindracea* (DC.) Marie.

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Summary: Black poplar mushroom, *Agrocybe cylindracea* deserves special attention, due to its medicinal properties. Water and alcohol extracts from fruiting bodies of the fungus have an anti-oxidant, anti-cancer, anti-fungal, cholesterol and triglycerides blood level lowering abilities. This mushroom is rich in proteins and vitamins, mineral elements and low in fat. The aim of the experiments was to determine effect of a substrate supplementation with $CaCO_3$ on mycelial growth, yield, morphological features and storability of fruiting bodies of four strains of *A. cylindracea* (DC.) Marie. The amount of additive to sawdust substrate affects rate of mycelial growth and yield of investigated strains. *A. cylindracea* mycelial growth was not affected by addition of $CaCO_3$ to substrate, however a significant effect of this additive was found on yield, which was the highest with $CaCO_3$ addition in an amount of 8 g/100 g of substrate. Carpophores characterized with the largest caps diameter, and the largest individual mass obtained of substrate enriched with $CaCO_3$ addition of 8 g/100 g of substrate. In addition, it was found that supplementation with $CaCO_3$ affect storability of *A. cylindracea*. The lowest weight loss of fruiting bodies after 3 and 7 days of storage was found with addition of $CaCO_3$ to substrate in an amount of 4 g/100 g of substrate.

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Key words: additives, black poplar mushroom, calcium carbonate, carpophores, cultivation, fungi, harvest, mycelium, shelf life

Introduction

In nature environment of Poland there are over 4,500 species of macro fungi, with about 1,100 to 1,400 recognized as edible even though there has been considerable research on the taxonomy and phylogeny of these mushrooms, there has been far less research on their domestication (Grzywacz, 2008). However, only less than 10 out of known wild mushroom species around the world have reached large-scale commercial cultivation (Miles & Chang, 2004). One of such species is black poplar mushroom. *Agrocybe cylindracea* (DC.) Maire (= *Agrocybe aegerita*) (Brig.) Sing. *A. parasitica* G. Stev) Cyclocybe cylindracea (DC.) (Uhart & Albertó, 2007; Ryman, 2008; Vizzini & Angelini, 2014; Niveiro et al., 2020) and is found on all continents (Watling, 1992). It usually grows in clusters and most often it can be found on old, dead tree trunks and branches of: poplar, willow, elm, ash, elderberry or black poplar (Poppe & Höfte, 1995).

The knowledge about production and medicinal properties of so called specialty mushrooms in Asia dates back to centuries ago (Oyetayo, 2011; Wachtel-Galor et al., 2011). *A. cylindracea* was already known and valued in ancient Rome for its unique taste (Vessey, 1972). The cultivation of this fungi, however it is well known in China, Japan, Thailand, Korea, but also in Europe: Germany, Greece, Italy and Spain, is still little known in Poland (Nocentini et al., 2000; Jasińska et al., 2014 a,b; Thawthong et al., 2014; Huang & Ohga, 2017; Shahtahmasebi et al., 2018).

The global mushroom cultivation market is evaluated to represent an estimation of USD 16.7 billion in 2021 and it is expected to reach above USD 20.4 billion in 2026 (Market Data Forecast, 2021). Mushrooms can be cultivated with low cost and low usage of land compared to other agricultural products; consequently, many farmers and manufacturers are turning towards the cultivation of mushrooms. In recent years the cultivation of specialty mushrooms increased mainly due to high availability of cheap, often waste material from agricultural and forestry industry production, which may be a potential substrate for growing mushrooms such as *A. cylindracea* (Jasińska et al., 2014 a,b; Thawthong et al., 2014; Huang & Ohga, 2017; Shahtahmasebi et al., 2018).

However, *A. cylindracea* deserves special attention, not only due to its medicinal properties. Water and alcohol extracts from fruiting bodies of fungus have an anti-oxidant, anti-cancer, anti-fungal properties but also cholesterol and triglycerides blood level lowering abilities (Lin et al., 2017; Jing et al., 2018). It is rich in protein, vitamins and mineral elements but low in fat, having a mild flavour and unique taste (Petrovska, 2001; Wojewoda, 2003; Yildiz et al., 2005; Tsai et al., 2008; Krüzselyi et al., 2019). Dry matter content in fruiting

bodies comprise to 9 to 11% (Petrovska, 2001). Detailed investigation concerning the chemical composition as well as antioxidant properties of several species of mushrooms, including black poplar mushroom, was performed by numerous researchers i.e. Petrovska (2001), Konuk et al. (2006); Tsai et al. (2007, 2008); Kumar et al. (2017); Koutrotsios et al. (2020). Zhou et al. (2011) showed high amount of trehalose, a disaccharide in carpophores of *A. cylindracea* which is easily digested by human intestines. Gąsecka et al. (2016) showed that fruiting bodies of this mushroom have superior antioxidant properties after selenium (Se) addition. Young fruiting bodies of black poplar mushrooms have mild and delicate taste (Stamets, 2005) and are valuable addition to various dishes from poultry and fish, soups and sauces. Black poplar mushroom is perfect for drying, during process gaining intensive mushroom like aroma (Siwulski & Sobieralski, 2004). Recent investigations of Silva et al. (2019) showed that the bio-residues after *A. cylindracea* cultivation are source of bioactive extracts of ergosterols, which has been reported to be one of the most important compounds, contributing to the health-promoting benefits, associated with mushrooms' consumption. Which in the COVID-19 pandemic appearance is greatly appreciated discovery.

In the cultivation of saprophytic fungi, to increase level of substrate decomposition and increasing yield of carpophores numerous additives are used. Supplements are rich in organic nitrogen compounds, mineral elements such as phosphorus and calcium (Uhart et al., 2008). The additives are highly locally dependent and geographically oriented. The most frequent are wastes from agricultural industry such as corn cobs, cereal bran, cereal grain, straw etc. (Chang & Miles, 1989; Poppe & Höfte, 1995; Oei, 1996; Jasińska et al., 2014b; Shahtahmasebi et al., 2018). The most common additives are wheat bran and soya flour (Philippoussis & Diamantopoulou, 2000; Siwulski & Sobieralski, 2004) or inorganic nitrogen NH_4NO_3 (Zadrazil, 1999). Ling & Chen, (2000) stated, addition of fish meal extract and spent mushroom substrate from Shiitake production positively affected mycelial growth of black poplar mushroom. Other investigation showed the addition of small amount gypsum or chalk endorse physical properties of substrate, helps to set right pH and results in better mycelial growth and yielding (Oei, 1996; Stamets, 2005). Very important issue which should be considered in substrate composition is the C:N ratio. It has crucial meaning for degradation of lignocellulosic compounds. Well balanced substrate increase mycelial growth and yielding **(**Okhuoya et al., 2000). Various researchers state different amounts for the C:N ratios. Sarker et al. (2008) states, the best C:N ratio for black poplar mushroom cultivation is 36:1, Philippoussis et al. (2001) – 59:1, whereas Isikhuemhen et al. (2009) writes about 72:1 and 81:1. However high amount of nitrogen can also has negative effect on mycelial growth and for fruiting bodies development (Philippoussis et al., 2001; Mikashvili et al., 2006). The average yield 170-200 g/kg (Heo, 2019).

Fruiting bodies of *A. cylindracea* are mostly produced for fresh consumption. Shelf life of the product can be regulated by different methods such as appropriate packaging, low temperature of storage, or storing under modified atmosphere conditions such as: low O_2 levels and high concentrations of CO_2 (Siwulski & Sobieralski, 2004; Stamets, 2005; Li & Zhang, 2008; Jeon et al., 2010). Storability of black poplar mushroom is longer in clusters than when fruiting bodies are stored separately. In temperature of 2-4 ºC fruiting bodies can be stored up to two weeks after harvesting (Siwulski & Sobieralski, 2004; Stamets, 2005; Jeon et al., 2010). Li & Zhang (2008) conducted several investigations on storability of black poplar mushroom, which was packed to plastic containers with different size of filter and stored in different temperatures. The best results were when fruiting bodies were stored in containers with filter sized 1 cm^2 and temperature +3°C. Such packed, fruiting bodies were stored for 21 days without losing quality.

The aim of presented work was to determine the effect of composition of growing substrate and substrate supplementation with calcium carbonate on mycelial growth, yield and morphological features, storage and storability of fruiting bodies of *Agrocybe cylindracea.*

Materials and methods

The study was conducted in laboratory of the Department of Vegetable Crops of Poznań University of Life Sciences and air-conditioned chambers of mushroom farm – Wytwórnia Grzybów Uprawnych in Łobez near Jarocin. The object of the study was four strains of *A. cylindracea*: AE02, AE05, AE06, AE11 originated from the Collection of Edible and Medicinal Mushrooms of Department of Vegetable Crops of Poznań University of Life Sciences. Mother cultures of investigated strains were stored in forms of agar blocks in temperatures 2-5 °C. For all the experiments wheat, *Triticum* (L.), grain spawn was prepared according to method described by Lemke, (1971) using mother cultures of evaluated strains.

The experiment consisted of two parts. Part one – mycelial growth depending on $CaCO_3$ addition was evaluated. Second part focused on yield, fruiting bodies, morphological features and storage capacity depending on $CaCO_3$ addition. Substrate in both parts of experiment was mixture of oak and alder sawdust (4:1 volume; 95% DM – dry matter), supplemented with wheat bran (20%) and maize flour (2.5%). The $CaCO_3$ was added to substrates in three different dosages: 2, 4 and 8 g for 100 g of final substrate. Total yield, which accounted whole fruiting bodies, marketable yield that accounted for caps of fruiting bodies without stipes, cap diameter, length and diameter of stipe, average weight of fresh carpophore and carpophores dry mass were determined.

Part 1 – mycelium growth rate on substrates supplemented with $CaCO_3$ in biological tubes

Sawdused mixed with supplements were moisturised with tap water up to 65% moisture. Biological tubes of 18 cm lenght were filled with supplemented, moisturised sawdust up to 15 cm, corked with ligning corks and sterylised in autoclave in temperature of 121 °C for 1.5 hour. After cooling down to room temperature (c.a 21 °C), substrates were innoculated with spawn of examined strains of *A. cylindracea*. On upper surface of substrate 1-1.5 cm of spawn was placed and again corked with lignin corks. For incubation, biological tubes were than placed in thermostate, at 25 °C with relative humidity (RH) 80-85%, under dark conditions.

When comparing the experimental results, the analysis of variance for factorial experiments was applied (ANOVA, level of significance α=0.05). The results of mycelial growth were established in fully randomized design in four replications in two series. On biological tubes, thickness of substrate mass occupied by mycelium was measured after 18 days from inoculation with accuracy up to 1 mm.

Part 2 – cultivation experiment

Cultivation experiment was conducted in fully randomized design, four replications in two independent growing cycles in the form of two separate cultivations. The growing cycle was considered to be from spawn inoculation until the end of the first flush. Only one flush was harvested in each growing cycle. Sawdust mixed with supplements were moisturized with tap water up to 65% moisture. Standard polipropylene bottles of capacity 1 dm^3 were filled with moisturised, supplemented sawdust in amount of 400 g per bottle. Bottles were closed with lids, which had four ventilation holes on the filter. The substrates were sterilized in an autoclave at 121 °C for 2 hours. After cooling down to room temperature (c.a 21 °C), substrates were inoculated with spawn of *A. cylindracea* at the rate of 3% of dry weight of the substrate. Incubated in dark till the mycelium completely overgrew the substrates. The temperature of incubation was 25 °C with relative humidity 80-85%.

Fructification and yielding: After incubation, bottles were moved to growing chamber. The lids were removed from bottles. The air temperature was maintained at 15-17 °C and relative humidity at 85-95%. Bottles were covered with pergamine paper, which was kept moist. When the pin heads appeared, pergamine paper was removed. Growing light: fluorescent lamps, day – light type (500 lx) were used for 10 hours a day. During yielding, CO_2 concentration of the air was kept not exceeding the limits of 800-1200 ppm. Harvesting of fruiting bodies was carried out within five weeks after inoculation of the substrate with mycelium.

Harvest: The fruiting bodies were harvested as whole clusters, when most of caps was having edges straighten and there was no spore print yet. Clusters were gently removed from surface of substrate so as not to damage fruiting bodies. There was only one flush in each cultivation cycle.

Measuring of morphological features of fruiting bodies, i.e. diameter of the cap, length and diameter of stipe, and average weight of fresh fruiting body was assessed. 10 fruiting bodies were randomly taken from each combination of experiment. All measurements were made with 1 mm accuracy (***Figure 1***).

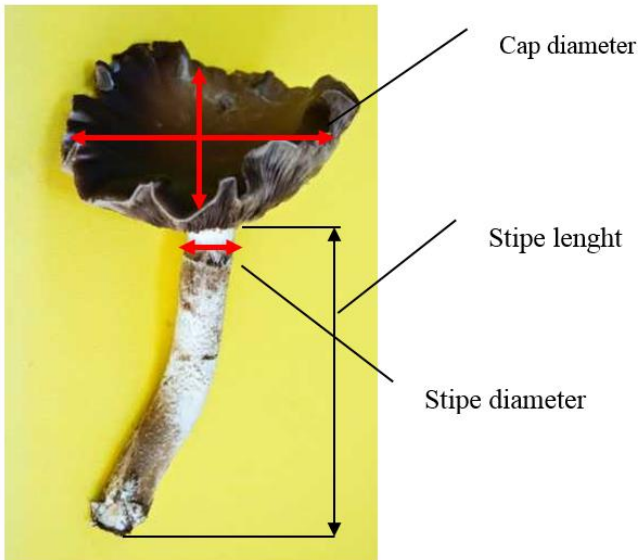

Figure 1. Diagram of biometric morphological features of *A. cylindracea* fruiting body

Qualitative features: Dry matter was evaluated with weight method. The fruiting bodies were dried first in temperature of 60 °C for 12 h, later in the temperature of 80 °C until the constant weight was obtained.

Storage capacity: Storability of fruiting bodies was studied. Collected fruiting bodies were placed in plastic containers with dimensions 10x15 cm, amount of 50 g each, covered by a perforated foil with six vent holes having a diameter of 1 cm and stored in a refrigerator at 2-4 °C. After 3 and 7 days of storage, storability was determined on the basis of quality of fruiting bodies of a 5 grade scale and weight losses during storage. The grading scale was as follows: 5 – very firm – fruiting bodies that look as fresh, with no obvious signs of water loss from twist, curl, without clear bruising, cracks etc.; 4 – firm – fruiting bodies with signs of water loss, but even non-wrinkled, without cracks appear occasionally bruising; 3 – wilted – fruiting bodies with clear signs of water loss, starting to wrinkle shrink, even without cracks, bruises a bit more clear; 2 – very wilted – fruiting bodies of the very clear signs of water loss, wrinkled, shrunken, sometimes cracked caps, slender stipes, visible bruising; 1 – dry – almost dried fruiting bodies, very wrinkled and shrunken.

Chemical analysis: The fruiting bodies were dried first in temperature of 60 °C for 12 h, later in the temperature of 80 °C until the constant weight was obtained and then grounded with laboratory mil. In order to determine general form of phosphorus, potassium, calcium and magnesium material has been subjected to a process of mineralization in concentrated sulfuric acid. In order to determine total nitrogen, mineralization was carried out in a mixture of sulfuric acid and sulfosalicylic acid. After mineralization of the material, the following designations: General N – Kjeldahl distillation method in the camera Parnas-Wagner; P – colorimetric method with ammonium molybdate; K, Ca and Mg – atomic absorption spectrometry (AAS3 camera, Carl Zeiss Jena, Thornwood NY, USA).

Results

Influence of calcium carbonate addition to substrate on mycelial growth, yield, morphological features and storability of carpophores

Based on conduceted experiment significalntly important influence of addition of calcium carbonate ($CaCO_3$) to growing substrate on mycelial growth, yield, fruiting bodies morphological features and storablity was stated.

The fastest growth regardless of dosage of $CaCO_3$ was stated for strain AE05 and AE06. Strain AE02 was characterised by slower mycelium growth, however, the slowest mycelium growth was stated for strain AE11. Significant effect was found for interaction within the amount of $CaCO_3$ and mycelial growth of examined strains of black poplar mushroom. The fastest growth was found for strains AE05 and AE11 on substrate supplemented with the highest amount of $CaCO_3$ – 8 g/100g substrate DM. Strains AE02 and AE06 were growing fastest on the substrate without the addition of $CaCO_3$ (***Table 1, Figure 2***).

The amount of $CaCO_3$ addition to substrate had a significant effect on the black poplar mushroom yield. The hihgest yield was obtained with the highest addition of $CaCO_3$ in amount of

8 g/100 g substrates DM. On all other dosages of $CaCO_3$ obtained yield was significanlty lower. Strain reaction on the amount of $CaCO_3$ addition to substrates was different. The highest yield was obtained for strain AE05, lower yield was obtained for strain AE02 and AE11. Where the lowest yield was harvested from strain AE06. Moreover, significant influence of interaction between amount of $CaCO_3$ addition to the substrate and the yield of four investigated strains of black poplar mushroom. The highest yield was obtained, compared to other combinations, for addition of $CaCO_3$ in amount of 8 g/100g substrates DM for strain AE02. Strain AE05 and AE11 were yielding well both with addition of $CaCO_3$ in amount of 2.0 and 8 g/100g of substrates DM. However strain AE06 yielded well only with addition of 8 g/100g (***Table 1***).

Figure 2. Comparison of the growth of mycelium of *A. cylindracea* on substrates with addition of $CaCO_3$ at three doses (Ca1 – 2 g; Ca2 – 4 g; Ca3 – 8 g/100 g of DM substrate)

Conducted experiment reveled out that amount $CaCO_3$ added to the growing substrate did not affect dry matter content of carpophores of *A. cylindracea*. However, there were significant differences in dry matter content between strains. The highest amount of carpophores DM was stated in strain AE06. Strains AE11 and AE02 were characterized by a lower amount of carpophores dry matter. The lowest amount of dry matter content was obtained for fruiting bodies of strain AE05 (***Table 1***).

Amount of $CaCO_3$ addition to substrate siginficantly affected the morphological features of fruiting bodies of *A. cylindracea* i.e: stipe diameter and lenght, cap diameter, average cap weight, and whole carpophore. The stipe length and diameter was directly proportional to growing amounts of addition of $CaCO_3$ to the growing substrate. Fruiting bodies with longest and thickest stipes were harvested from strain AE02 and AE05. Fruiting bodies of strains AE06 and AE11 were characterized by thinner and shorter stipes. There was no significant interaction between the amount of additive, strain, and stipe thickness, however, the interaction was stated for the length of the stipe. The longest stipes were obtained for strains AE02 and AE05 with the addition of 8 g/100g of $CaCO_3$ to a growing substrate (***Table 2, Figure 3***).

The biggest cap diameter was obtained on substrates supplemented with $CaCO_3$ in amount of 8 g/100g substrates DM. Decreasing dosage of $CaCO_3$ – 4, 2 and 0 g/100g of substrate resulted in the development of caps with a smaller diameter. The biggest caps were obtained from strains AE02, followed by AE05 and AE11. The smallest caps developed strain AE06. There were no significant interactions between amoung of additive and cap diameter of tested strains (***Table 2, Figure 3***).

Average weight of the whole carpophore as well as average weight of cap depends on amount of $CaCO_3$ to growing substrate, and was invreasing with increasing amounts of the additive. No significant interaction was stated for those features. The biggest average weight of whole carpophore as well as cap was obtained for addition of 8 g of $CaCO_3$ per 100g of substrate. The feature was decreasing its value with decreased amounts of additive.The largest share of cap in whole carpophore was found for addition of $CaCO_3$ in amount of 8, 2 and 0 g per 100g. All the three examined strains; AE02, AE06 and AE11 were reacting similar and the share of cap in weight of whole fruiting body was large, however, in strain AE05 it was the smallest (***Table 3***).

Moreover, there were significant impact of interaction between the amount of additive and percent of share of carpophore weight and weight of whole carpophore of black poplar mushroom. The share was the largest with addition of highest dosage of $CaCO_3$ i.e. 8 g/100g for strains. Strain AE05 showed the highest share of cap in fruiting boddy weight on substrate supplemented with only 2 g of $CaCO_3$, whereas strain AE11 observed with similar cap share in weight of whole capropohore was found for all examined amounts of additive (***Table 3***).

Three dosage of $CaCO_3$ added to growing substrate were evaluated for the impact on storability of carpohores of black polpar mushroom after 3 and 7 days of storage in cold room at the temperature of 2 to 4 °C. Statistically, there were no signifficance differences between examined storage time. However there were signifficant and visible differences in the mass loss with different amounts of additive.

Lowest weight loss was observed with the addition of $CaCO_3$ at 4 g/100 g of growing substrate regardless time of storage (3 days – 35.7%; 7 days – 54.7%). The more weight loss was observed with caprophores harvested from substrate with addition of $CaCO_3$ in amount of 2 g/100 g (***Table 4, Figure 4-5***).

Based on this experiment it was found the addition of $CaCO_3$ to growing substrate affected the quality of carpophores of *A. cylindracea* after storage for 3 and 7 days in temperature of 2 to 4°C. The best quality of carpophores after 3 days of storage was maintained by fruiting bodies of strain AE06 harvested from substrate supplemented with 4 g and fruiting bodies of AE05 and AE11 with $CaCO_3$ addition 8 g/100 g. The worst quality was observed for strains AE02 and AE11 with addition of 4 g/100 g. of substrate. After 7 days of storage, the best quality was maintained by the carpophores of strain AE11 harvested from substrate supplemented with 8 g $CaCO_3$. The worst quality was observed in carpophores of strain AE02 with the addition of 4 g $CaCO_3$ (***Table 5, Figure 4-5***).

The supplementation of substrate with calcium carbonate influenced the composition of mineral elements such as nitrogen, phosphorus, magnesium and calcium in fruiting bodies of *A. cylindracea.* Increasing dosage of $CaCO_3$, increased the amount of nitrogen in carpophores of strains AE02, AE06 and AE11, whereas in strain AE05 it remained the same as in the control treatment. Content of phosphorus was increased with increasing amounts of $CaCO_3$ in all the examined strains. Potassium content increased in two strains (AE05 and AE06) but decreased in other two strains (AE02 and AE11) with increased dosage of $CaCO_3$ to growing substrate. With increasing amount of $CaCO_3$ in growing substrate elevated the amount of calcium and decreased amount of magnesium in caprophores of black poplar mushroom (***Table 6***).

Table 1. Influence of addition of $CaCO_3$ on mycelium growth (after 18 days of incubation) [cm], yield [g/100g] and dry matter of fruiting bodies [%]

Addition $CaCO_3$ (g/100g DM substrate)	Mycelium growth after 18 days of incubation				Yiel[d]				Dry mater of fruiting bodies			
	Strains				Strains				Strains			
	AE02	AE05	AE06	AE11	AE02	AE05	AE06	AE11	AE02	AE05	AE06	AE11
0.0	6.7	6.3	6.7	5.7	64.7	54.9	52.1	71.1	8.7	7.9	8.6	7.0
2.0	5.9	6.2	6.2	5.9	46.9	90.8	53.4	63.8	8.6	7.3	10.1	9.8
4.0	6.4	6.5	6.1	5.9	68.8	62.1	54.8	59.1	7.7	7.3	9.5	8.5
8.0	5.8	6.9	6.4	6.3	100.7	90.6	76.4	71.2	7.6	7.6	7.9	9.0
Average	6.2	6.5	6.4	5.9	70.2	74.6	59.2	66.3	8.2	7.5	9.0	8.6

[a] $LSD_{0.05}$ for strain = 0.3; $LSD_{0.05}$ for the amount of interaction $CaCO_3$ x strain = 0.6
[b] $LSD_{0.05}$ for amounts $CaCO_3$ = 9.7; $LSD_{0.05}$ for strain = 9.7; $LSD_{0.05}$ for amount of interaction $CaCO_3$ x strain = 19.5
[c] $LSD_{0.05}$ for strain = 0.9; $LSD_{0.05}$ for amount of interaction $CaCO_3$ x strain = 1.79

Table 2. Influence of addition of $CaCO_3$ on morphological features – stipe length and diameter, cap diameter [mm]

Addition $CaCO_3$ (g/100g DM substrate)	Stipe diameter					Stipe length					Cap diameter				
	Strains				Average	Strains				Average	Strains				Average
	AE02	AE05	AE06	AE11		AE02	AE05	AE06	AE11		AE02	AE05	AE06	AE11	
0.0	7.3	7.2	6.6	6.2	6.8	73.0	63.0	59.9	63.6	64.8	51.5	43.4	40.7	43.3	44.7
2.0	7.8	8.5	6.2	7.0	7.4	65.6	74.7	56.9	65.4	65.6	49.6	52.3	42.4	46.3	47.6
4.0	8.2	8.3	7.2	7.0	7.8	68.7	74.8	60.5	62.9	66.7	44.5	47.9	45.8	53.3	47.9
8.0	9.5	8.5	7.5	7.4	8.1	78.5	76.4	62.3	66.9	71.0	59.0	53.2	47.0	48.8	52.0
Average	8.2	8.3	6.8	6.9		71.1	72.2	59.9	64.7		51.2	49.2	44.0	47.9	

[a] $LSD_{0.05}$ for amounts $CaCO_3$ = 0.8; $LSD_{0.05}$ for strain = 0.8; $LSD_{0.05}$ for amount of interaction $CaCO_3$ x strain = 1.6
[b] $LSD_{0.05}$ for amounts $CaCO_3$ = 3.8; $LSD_{0.05}$ for strain = 3.8; $LSD_{0.05}$ for amount of interaction $CaCO_3$ x strain = 7.6
[c] $LSD_{0.05}$ for amounts $CaCO_3$ = 4.9; $LSD_{0.05}$ for strain = 4.9; $LSD_{0.05}$ for amount of interaction $CaCO_3$ x strain = 9.9

Figure 3. Fruiting bodies of black poplar mushroom obtained from substrate with the addition of $CaCO_3$ in an amount of 8 g/100g DM substrate

Table 3. Influence of addition of $CaCO_3$ on carpophores and cap weight and percent of share of carpophore weight and weight of whole fruiting bodies [g]

Addition $CaCO_3$ (g/100 g DM substrate)	Fruiting body [g]					Cap weight [g]					[%][c]				
	Strains				Average	Strains				Average	Strains				Average
	AE02	AE05	AE06	AE11		AE02	AE05	AE06	AE11		AE02	AE05	AE06	AE11	
0.0	12.4	6.3	5.5	7.9	8.0	8.6	4.0	3.7	5.5	5.5	65.5	63.1	65.1	66.8	65.1
2.0	9.8	2.1	6.0	7.5	8.8	6.7	8.2	4.0	5.0	6.0	68.5	67.6	64.5	65.5	66.5
4.0	8.7	9.6	7.2	10.7	9.1	5.4	6.2	4.9	7.6	6.0	60.4	58.9	67.1	66.7	63.3
8.0	17.3	13.9	11.2	8.8	12.8	12.4	9.0	8.0	5.9	8.8	70.6	61.0	67.9	66.4	66.5
Average	12.0	10.5	7.5	8.7		8.3	6.8	5.1	6.0		66.3	62.6	66.2	66.3	

[a] $LSD_{0.05}$ for amounts $CaCO_3$ = 3.5; $LSD_{0.05}$ for strain = 3.5; $LSD_{0.05}$ for amount of interaction $CaCO_3$ x strain = 6.9
[b] $LSD_{0.05}$ for amounts $CaCO_3$ = 2.6; $LSD_{0.05}$ for strain = 2.6; $LSD_{0.05}$ for amount of interaction $CaCO_3$ x strain = 5.2
[c] $LSD_{0.05}$ for amounts $CaCO_3$ = 1.7; $LSD_{0.05}$ for strain = 1.7; $LSD_{0.05}$ for amount of interaction $CaCO_3$ x strain = 3.5

Table 4. Influence of addition of $CaCO_3$ on storability of fruiting bodies [%]. Fruiting bodies weight loss after 3 and 7 days of storage in cold room at 2 to 4°.

Addition $CaCO_3$ (g/100g DM substrate)	3 days[a]					7 days[b]				
	Strains				Average	Strains				Average
	AE02	AE05	AE06	AE11		AE02	AE05	AE06	AE11	
0.0	42.7	59.7	49.4	31.5	45.8	56.9	74.8	62.2	52.9	61.7
2.0	49.8	45.5	56.3	47.3	49.7	63.2	63.0	64.9	57.3	62.1
4.0	36.6	22.8	35.5	48.0	35.7	52.6	41.0	61.5	63.8	54.7
8.0	50.2	39.1	53.5	41.3	46.0	65.4	54.5	62.9	55.6	59.6
Average	44.8	41.7	48.7	42.0		59.5	58.3	62.8	57.4	

[a] $LSD_{0.05}$ for amounts $CaCO_3$ = 13.33; $LSD_{0.05}$ for strain = 13.33; $LSD_{0.05}$ for amount of interaction $CaCO_3$ x strain = 26.66
[b] $LSD_{0.05}$ for amounts $CaCO_3$ = 11.2; $LSD_{0.05}$ for strain = 11.2; $LSD_{0.05}$ for amount of interaction $CaCO_3$ x strain = 22.2

Table 5. Influence of addition of $CaCO_3$ on quality of fruiting bodies after 3 and 7 days storage in cold room at 2 to 4° C [%]

Addition $CaCO_3$ (g/100g DM substrate)	3 days				7 days			
	AE02	AE05	AE06	AE11	AE02	AE05	AE06	AE11
0.0	4.0	4.0	4.3	3.7	2.8	2.8	3.3	2.8
2.0	3.8	4.0	3.8	4.0	3.0	3.0	2.9	3.2
4.0	3.3	4.0	4.3	3.5	1.8	3.0	3.3	2.8
8.0	4.0	4.2	3.8	4.2	3.0	2.8	3.3	3.5

Table 6. Influence of addition of CaCO3 on content of micro and macronutrients in the caps of the fruiting bodies [%]

Addition $CaCO_3$ (g/100g DM substrate)	N				P				K				Ca				Mg			
	Strains				Strains				Strains				Strains				Strains			
	AE02	AE05	AE06	AE11	AE02	AE05	AE06	AE11	AE02	AE05	AE06	AE11	AE02	AE05	AE06	AE11	AE02	AE05	AE06	AE11
0	5.78	5.9	5.33	5.2	0.9	0.86	0.8	0.86	3.41	3.27	3.25	3.39	0.032	0.031	0.032	0.03	0.17	0.15	0.16	0.17
2	5.88	5.9	6.0	5.76	0.9	0.89	1.04	1.0	3.41	3.29	3.3	3.19	0.034	0.032	0.034	0.032	0.19	0.18	0.19	0.2
4	5.95	6.0	6.11	6.02	0.97	0.98	1.04	1.0	3.32	3.4	3.9	3.22	0.038	0.04	0.039	0.042	0.17	0.17	0.18	0.17
8	6.07	5.99	6.1	5.98	1.2	1.07	0.99	1.1	3.29	3.87	3.9	3.0	0.042	0.044	0.041	0.045	0.16	0.15	0.17	0.15

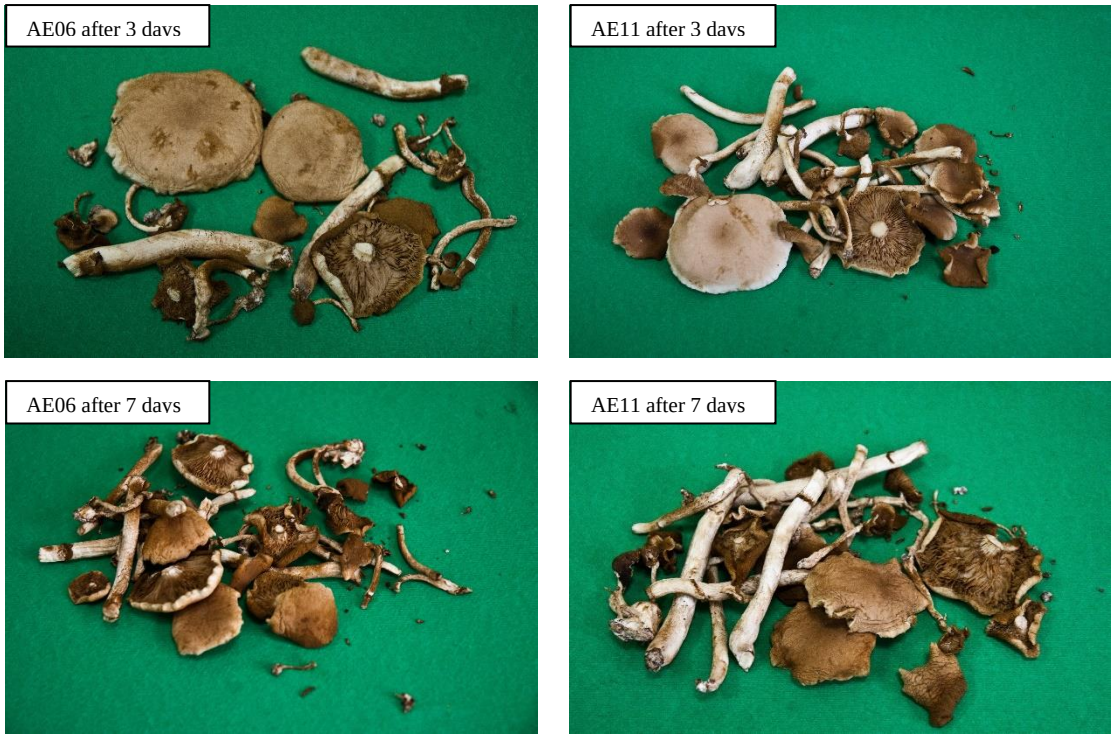

Figure 4. Fruiting bodies of strains AE06 and AE11 obtained from substrate with addition of 2 g of $CaCO_3$ per 100 g DM substrate after 3 and 7 days of storage

Figure 5. Fruiting bodies of strain AE06 obtained from substrate with addition of 4 g of $CaCO_3$ per 100 g DM substrate after 3 and 7 days of storage and fruiting bodies of strain AE11 obtained from substrate with addition of 8 g of $CaCO_3$ per 100g DM substrate after 3 and 7 days of storage

Discussion

The results from the experiments showed the influence of substrate supplementation with $CaCO_3$ on mycelial growth, yield and morphological and qualitative features as well as storability of carpophores of black poplar mushroom.

The growth rate of mycelia is characteristic of the species, the variety, the strain but also depends largely on the composition of growing substrate.

Besides such important elements such as nitrogen, carbon, potassium and magnesium, fungi require to grow also a certain amount of so-called trace elements. One of the main trace elements, from the perspective of the *Basidiomycetes* is calcium (Jennings, 1995). For the growth and development of most of the other fungi it is not a very important component but it plays a very important role in the formation of pinheads of carpophores, and the formation and development of mature fruiting bodies and affects storability. It is also a binder for organic acids arising in the metabolic processes and acts as a

buffer, maintaining the proper pH of substrates for metabolic processes (Treschow, 1944; Gadd, 1995). In our experiment, we compared mycelial growth and yield of the four doses of calcium carbonate: 0, 2, 4 and 8 g/100 g.

The investigated strains of *A. cylindracea* were characterized with the good growth of mycelium on sawdust with addition of calcium carbonate (***Table 1***), the same effect was noted by Tabata & Ogura (2003a,b) in *Hypsizygus marmoreus* (Peck) Bigelow and *Auricularia polytricha* (Mont.) Sacc. However supplementation does not significantly affect the rate of growth of mycelium what was also true for *Pleurotus eryngii* (Lee et al., 2006). Whereas, the efficiency was confirmed to increase with the addition of calcium carbonate. The inducing effect of $CaCO_3$ for mushroom yield may be related to the various gradients and stimuli created at the hyphal tips that ultimately influence growth and development of the mushroom what was described by (Gadd, 1995). Yield of fruiting bodies was higher compared with the control for all examined strains. The highest yields were obtained at the highest addition of calcium carbonate to the ground – 8 g/100 g DM (70 to 100 g depending on the strain). Lower doses of calcium carbonate into the substrate resulted in lower yield. Same effect of increased productivity of Shiitake mushroom was noticed by Royse & Sanchez-Vazquez (2003). However, our finding goes in contradiction with the research of Lee (2006) on King oyster mushroom, where yield decreased with the addition of calcium carbonate.

In our experiments, it was found that regardless of all kinds of factors, which are variable experiments, one of the main factors increasing yield was strain. Philippoussis et al. (2001) and Uhart & Albertó (2007) showed that strains differ in the size of the crop, which is confirmed by the author's own research. Two of tested strains of AE06 and AE11 yielded better than the other varieties of AE02 and AE05 (***Table 1***). Isikhuemhen et al. (2009) confirms the differences in height between crop varieties. The author also revealed differences in dry matter content fruiting varieties studied black poplar mushroom.

Fruiting bodies of black poplar mushroom are primarily earmarked for consumption in fresh state. The main factor influencing shelf life of mushrooms, is the temperature, because it determines intensity of physiological processes in fruiting bodies. However it is not the only factor affecting storability. The experiment demonstrated that addition of calcium carbonate to growing substrate also affect weight loss of fruiting bodies of black poplar mushroom during storage. The greatest weight loss was observed on substrates with the lowest addition of calcium carbonate in of 2 g/100 g substrate DM, and the lowest with the addition of calcium carbonate in an amount of 4 g/100 g substrate DM.

Fruiting bodies of black poplar mushroom are characterized by high morphological variability, which is manifested by the presence of individuals of a different color and a different structure of surface of the cap (Watling, 1992). Mostly these features are related to the type of substrate and the conditions of growth of fungus. Growing black poplar on substrate with wheat straw caused cap surface smooth and silky, while substrate with willow sawdust, casing from garden soil, conditioned the creation of caps concentrically wrinkled (Uhart & Alberto, 2007). In studies Uhart et al. (2008) stated the size of fruiting bodies was correlated with the type of supplement, but not with strains, which was not confirmed in present study. In the study of Royse and Sanchez-Vazquez (2003) on Shiitake, mushroom size (weight) was larger with non-supplemented substrate compared to substrate supplemented with $CaCO_3$. Whereas our study presents increased size and weight with the calcium carbonate supplementation. The largest and heaviest fruiting bodies obtained with the highest dose of calcium carbonate (8 g/100 g), while addition of calcium carbonate to the media did not affect dry matter content of fruiting of black poplar mushroom (***Table 3***).

Many researchers emphases existence of significant differences between the chemical composition of the fruiting bodies of different species and varieties of cultivated mushrooms (Crisan & Sands, 1978; Chang et al., 1981; Shin et al., 2007). Likewise, the chemical composition of fruiting bodies is changing under the influence of growing medium used (Chang & Miles, 1989; Cheung, 2008; Siwulski et al., 2011). Studies on the chemical composition of black poplar mushroom were performed by, among others, Bauer- Petrovská & Kulevanova (2000), Konuk et al. (2006), Tsai et al. (2007, 2008). In our study, content of nitrogen, phosphorus, potassium, calcium and magnesium in fruiting bodies of black poplar mushroom was evaluated. The nitrogen content of the fruiting bodies ranged from 4.97 to 7.36%, which is consistent with reports Bauer Petrovská & Kulevanova (2000) – 6.5% and Yildiz et al. (2005) – from 6.56 to 7.48%. The investigation of Tabata & Ogura (2003a,b) showed elevated content of calcium with addition of calcium carbonate, what was also true in our case. Moreover the author has shown that the nitrogen content increased with the amount of magnesium and calcium is added to the substrate.

In this study the content of macronutrients and micronutrients in the fruiting bodies of the tested strains of black poplar mushroom and the effect of the addition of calcium carbonate to growing substrate on their content in tested varieties of fruiting bodies. In world literature there is no information on the contents of the elements in the different varieties of fruiting bodies of black poplar mushroom.

Conclusions

1. The kind of applied substrate affected the mycelial growth rate, yield, morphological and qualitative features of carpophores of black poplar mushroom.
2. Strain influence yield and morphological and qualitative features. The highest yields was obtained for strain AE06 and AE11.
3. Substrate supplementation with calcium carbonate did not affect mycelial growth, however significantly influenced yield of black poplar mushroom.
4. The biggest yield was obtained on substrate supplemented with addition of calcium carbonate in amount 8 g/100 g.
5. Carpophores of biggest cap diameter and the highest average weigh was obtained on substrate supplemented with calcium carbonate in amount of 8 g/100 g.
6. Addition of calcium carbonate to growing substrate affected storability. The smallest weight loss after 3 and 7 days of storage was found for substrate supplemented with calcium carbonate in amount 4 g/100 g
7. The addition of calcium carbonate affected chemical composition of black poplar mushroom carpophores.

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