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SCREENING OF CONTENT AND DYNAMIC OF ACCUMULATION OF POLYPHENOLS IN SOME BASIDIOMYCETES SPECIES

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The ***aim*** of the study was to investigate the total content of polyphenolic substances in Basidiomycetes carpophores from 50 species, of which 27 belong to the order *Polyporales* and 23 to the order *Agaricales*. Introduced 23 strains of 8 species of Basidiomycetes. ***Methods.*** Gathered wild carpophores dried and crushed to a particle size of 0,1 till 0,01 mm and searching strains were cultured in Erlenmeyyers flasks by surface method on standard glucose-peptone culture medium. Determination of total content of polyphenolic compounds was carried out in ethanol extracts of mycological material by a modified method of Folin-Chokalteu. Completely dry biomass of carpophores and mycelium was determined gravimetrically. ***Results.*** There was identified the species of polyporal fungi *Ganoderma applanatum*, *Ganoderma lucidum*, *Laetiporus sulphureus* and *Fomes fomentarius* and types of agarical mushrooms *Stropharia rugosoannulata*, *Agrocybe cylindracea*, *Tricholoma flavovirens*, *Flammulina velutipes*, *Pleurotus ostreatus* and *Fistulina hepatica* high in polyphenolic compounds. It was determined the content of polyphenols ranging from more than 60 mg / g completely dry biomass. For introduced strains established dynamics of growth and accumulation of polyphenolic compounds in the mycelium and culture filtrate during fermentation on glucose-peptone medium. All cultures reach a maximum accumulation of biomass on the 12th day of growth. *Shizophyllum commune* Sc-1101 and 10 and *F. velutipes* F-202 have been identified as the most productive strains. The lowest accumulation of absolutely dry biomass was recorded for strain *P. ostreatus* P-192 and strain *F. fomentarius* Ff-09. Cultures have investigated individual value growth such as biomass accumulation in the applied cultivation conditions, which probably reflects the suitability of the medium for their growth and genotypic characteristics. Strains are overwhelmingly able to accumulate polyphenolic compounds in both mycelium and culture fluid during the whole period of cultivation. Maximum content of polyphenols in the mycelium to 96%, and in the culture fluid - for 91% of strains coincided with the end of their term cultivation. Calculated correlation coefficient between the content of polyphenols in the mycelium and culture fluid showed that there is a very high positive correlation of 73.2%, a positive high at 17.4% and the average 4.5% of experiment data. ***Conclusion.*** The strains of species *Shizophyllum commune*, *Pleurotus ostreatus*, *Fistulina hepatica* and *Laetiporus sulphureus* were selected for further research in order to obtain polyphenols mycelial and extracellular origin.

Key words: polyphenols, Basidiomycetes, carpophores, mycelium, cultural filtrate

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СКРИНІНГ ВМІСТУ ТА ДИНАМІКА НАКОПИЧЕННЯ ПОЛІФЕНОЛЬНИХ РЕЧОВИН У ДЕЯКИХ ВИДІВ БАЗИДІОМІЦЕТІВ

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Метою роботи було вивчення загального вмісту поліфенольних речовин у карпофорах 50 видів базидіоміцетів з яких 27 належать до порядку *Polyporales* та 23 – порядку *Agaricales*. Інтродуковано 23 штами 8 видів базидіальних грибів. ***Методи.*** Зібрані дикорослі карпофори висушували та подрібнювали до розміру часток 0,1±0,01 мм, а дослідні штами культивували поверхнево в колбах Ерленмейєра на стандартному глюкозо-пептонному живильному середовищі. Визначення загального вмісту поліфенольних речовин проводили у спиртових витяжках мікологічного матеріалу за модифікованою методикою Фоліна-Чокальтеу. Абсолютно суху біомасу карпофорів та міцелію визначали ваговим методом. ***Результати***. Виявлені види трутових грибів – *Ganoderma applanatum*, *Ganoderma lucidum*, *Laetiporus sulphureus* та *Fomes fomentarius* і види агарикових грибів – *Stropharia rugosoannulata*, *Agrocybe cylindracea*, *Tricholoma flavovirens*, *Flammulina velutipes*, *Pleurotus ostreatus* та *Fistulina hepatica* з високим вмістом поліфенольних речовин – понад 60 мг/г абсолютно сухої біомаси. Для інтродукованих штамів, встановлені динаміка росту та накопичення поліфенольних речовин в міцелії та культуральному фільтраті при ферментації на глюкозо-пептонному середовищі. Всі культури досягають максимуму накопичення біомаси на 12-ту добу росту. Найпродуктивнішими тут є штами *Schizophyllum commune* Sc-1101 і Sc-10 та штами *F. velutipes* F-202. Найнижчі значення накопичення абсолютно сухої біомаси зафіксовано для штаму *P. ostreatus* P-192 та штаму *F. fomentarius* Ff-09. Встановлено, що досліджені культури мають індивідуальні значення росту – накопичення біомаси в застосованих умовах культивування, що, ймовірно, відображає придатність цих умов для їх росту та генотипічні особливості. Штами в переважній більшості здатні до накопичення поліфенольних речовин як в міцелії, так і в культуральній рідині протягом всього терміну культивування. Максимум вмісту поліфенолів у міцелії для 96 %, та у культуральній рідині – для 91% від загальної кількості штамів співпадав із закінченням дванадцятидобового терміну їх культивування. Обчислення коефіцієнту кореляції між вмістом поліфенолів у міцелії та культуральній рідині одновікових культур показало, що спостерігається дуже висока позитивна кореляція у 73,2%, висока позитивна – у 17,4% та середня – у 4,5% дослідів. ***Висновок.*** Відібрано штами видів *Schizophyllum commune*, *Pleurotus ostreatus*, *Fistulina hepatica* та *Laetiporus sulphureus* – перспективні для подальших досліджень з метою отримання поліфенолів міцеліального та позаклітинного походження.

Ключові слова: поліфеноли, базидіоміцети, карпофори, міцелій, культуральний фільтрат

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СКРИНИНГ СОДЕРЖАНИЯ И ДИНАМИКА НАКОПЛЕНИЯ ПОЛИФЕНОЛЬНЫХ ВЕЩЕСТВ НЕКОТОРЫХ ВИДОВ БАЗИДИОМИЦЕТОВ

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Целью работы было изучение общего содержания полифенольных веществ в плодовых телах 50 видов базидиомицетов из которых 27 принадлежат к порядку *Polyporales* и 23 - порядка *Agaricales*. Интродуцировано 23 штаммы 8 видов базидиальных грибов. **Методы.** Собранные дикорастущие карпофоры высушивали и измельчали до размера частиц 0,1±0,01 мм, а исследуемые штаммы культивировали поверхностно в колбах Ерленмейера на стандартной глюкозо-пептонной питательной среде. Определение общего содержания полифенольных веществ проводили в спиртовых вытяжках микологического материала по модифицированной методике Фолина-Чокальтеу. Абсолютно сухую биомассу плодовых тел и мицелия определяли весовым методом. **Результаты.** Обнаруженные виды трутовых грибов - *Ganoderma applanatum*, *Ganoderma lucidum*, *Laetiporus sulphureus* и *Fomes fomentarius* и виды агариковых грибов - *Stropharia rugosoannulata*, *Agrocybe cylindracea*, *Tricholoma flavovirens*, *Flammulina velutipes*, *Pleurotus ostreatus* и *Fistulina hepatica* с высоким содержанием полифенольных веществ – более 60 мг / г абсолютно сухой биомассы. Для интродуцированных штаммов установлена динамика роста и накопления полифенольных веществ в мицелии и культуральном фильтрате при ферментации на глюкозо-пептонной среде. Все культуры достигают максимума накопления биомассы на 12-е сутки роста. Здесь самые продуктивные – штаммы *Schizophyllum commune* Sc-1101 и Sc-10 и штаммы *F. velutipes* F-202. Самые низкие значения накопления абсолютно сухой биомассы зафиксированы для штамма *P. ostreatus* P-192 и штамма *F. fomentarius* Ff-09. Установлено, что исследованные культуры имеют индивидуальные значения роста – накопление биомассы в примененных условиях культивирования, что, вероятно, отражает пригодность этих условий для их роста и генотипические особенности. Штаммы в подавляющем большинстве способны к накоплению полифенольных веществ как в мицелии, так и в культуральной жидкости в течение всего срока культивирования. Максимум содержания полифенолов в мицелии для 96%, и в культуральной жидкости - для 91% от общего количества штаммов совпадал с окончанием дванадцятидобового срока их культивирования. Вычисления коэффициента корреляции между содержанием полифенолов в мицелии и культуральной жидкости одновозрастных культур показало, что наблюдается очень высокая положительная корреляция в 73,2%, высокая положительная – в 17,4% и средняя – в 4,5% опытов. **Вывод.** Отобраны штаммы видов *Schizophyllum commune*, *Pleurotus ostreatus*, *Fistulina hepatica* и *Laetiporus sulphureus* – перспективные для дальнейших исследований с целью получения полифенолов мицелиальными и внеклеточного происхождения.

Ключевые слова: полифенолы, базидиомицеты, карпофоры, мицелий, культуральный фильтрат

In the last decades an actual problem is searching for new biologically active substances (BAS's) and their producers for the purpose of development and manufacturing application of modern drug and therapeutic products (Zaprometov, 1993; Nikitina, 2007).

In particular, polyphenol compounds, which are natural antioxidants preventing development of different pathogenic effects in a cell and as a result of different diseases, are the desired substances in different branches of industry and medicine (Zaprometov, 1993; Fedotov et al., 2012 Asatiani at al., 2010). These include phenolic acids and aldehyde derivatives, substances of polyphenoloxycarbon complex, carotenoids, flavonoids, melanins, tannins, etc. (Asatiani et al., 2010; Wasser, 2010).

It is established that these substances are synthesised by almost all plant and fungi organisms (Nikitina, 2007; Li Fu et al., 2011). The traditional sources of polyphenols are plant raw materials - *Camellia sinensis* and *Humulus lupulus*, as well as the fruits of *Vitis vinifera* (Li Fu et al., 2011, Halvorsen et al., 2011).

A number of studies are devoted to investigation of polyphenols in fungi, especially to investigation of total polyphenols in the fruit bodies of 49 species of edible fungi which belong to genera *Boletus*, *Suillus*, *Volvariella, Pleurotus* et al. (Guthalu Puttaraju Nethravathi et al., 2006; Guo Ya–Jun et al., 2012). However, these studies give a vague idea about the qualitative and quantitative content of polyphenols in higher basidial fungi and mycological material when cultivating them that makes it necessary to carry out further screening operations in this field.

The interest for basidiomycetes, including wood-destroying ones, is firstly associated with their ability to synthesise numerous BAS's. Especially while destructing lignin-cellulose complex, they produce antioxidant substances - oxidoreductases, vitamins, polyphenols, free radical blockers et al., which provide adaptive mechanisms for antioxidant protection of xylotrophs (Peyrat–Maillard et al., 2000; Fedotov, 2007; Wasser, 2010).

Secondly, mycelium cultures of these organisms are not demanding for a nutrient media composition, most of them are edible and non-poisonous, and can be used in the microbiological production of BAS's.

The aim of this study was to determine total polyphenols in the carpophores and mycelium and in the culture filtrate of some basidiomycetes species.

MATERIALS AND METHODS

The carpophores, mycelium and culture filtrate of 50 macromycetes species, 27 of which belong to order *Polyporales* and 23 – to order *Agaricales*, division *Basidiomycetes* were used as the materials for this investigation. The general information about the species of basidial fungi investigated is set forth in the published work (Fedotov et al., 2012) and relevant sections of the article.

Also 23 strains from a pileated fungi culture collection of the Physiology of

Plants Department, Donetsk National University: *Fomes fomentarius* (L. ex Fr.) Gill. – T-10, Ff-09, Ff-1201; *Laetiporus sulphureus* (Bull.) Murrill. – Ls-08, Ls-09; *Fistulina hepatica* Schff. ex Fr. – Fh-08, Fh-18; *Flammulina velutipes* (Curt.: Fr.) Sing. – F-03, F-06, F-1, F-202; *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm. – Hk-35, P-004, P-01, P-039, P-107, P-192, P-208; *Schizophyllum commune* Fr.:Fr. – Sc-10, Sc-1101, Sc-1102; *Trametes hirsuta* (Wulf.:Fr.) Pil. – Th-11 and *Trichaptum biforme* (Fr.) Ryv. – Tb-11.

Most of the introduced strains are isolated into a pure culture from the wild fruit bodies (FBs) of basidiomycetes collected in different areas of Donetsk oblast, the taxonomic position of which was established according to the modern literature (Kirk et al., 2001).

To determine total polyphenols, the collected FBs were dried and ground to the particle size of 0.1±0.01 mm, and the strains under investigation were cultivated superficially in Erlenmeyer flasks of 250 ml in a glucose-peptone growth medium (GPM, pH_0 6.5±0.2) of 50 ml with the following composition (g/l): glucose – 10.0; peptone – 3.0; KH_2PO_4 – 0.6; K_2HPO_4 – 0.4; $MgSO_4 \cdot 7H_2O$ – 0.5; $CaCl_2$ – 0.05; $ZnSO_4 \cdot 7H_2O$ – 0.001. 10-day mycelia strain cultures on wort agar were used as inoculums. The incubation temperature was 27.5°C.

The cultivation period was 6, 9 and 12 days. Upon completion of the cultivation period, a mycelium was separated from the culture broth by filtration at 5±1°C. The mycelium obtained was additionally dried a little on filtration paper and cooled down to 1±0.5°C.

The prepared mycelium was homogenized by grinding in a chilled mortar. The ground carpophores (GCs), homogenized mycelium (HM) and culture filtrate (CF) were used for further tests. Absolutely dry biomass (ADB) of the GCs and mycelium was determined by gravimetric method (State…, 1987).

The determination of total (W) polyphenols (PPs) was carried out in the alcoholic extracts of the mycological material using modified Folin–Ciocalteu method (Мусиенко et al., 2001) and calculated by the standard formula. The tests were performed in three replications.

Statistical manipulation was done using the programs for statistical manipulation of biological test results. A correlation analysis was performed to determine correlation level between PP concentration in the mycelium and CF of one-year cultures. A difference was considered as significant at confidence level $P>0.95$ (Prysedskiy, 1999).

RESULTS AND DISCUSSION

Total polyphenols in 225 carpophores of 27 species of Polyporales fungi and in 220 ones of 23 species of Agaricales fungi were evaluated at the first step of investigation (Table 1).

Table 1. **Total content of polyphenols in fruit bodies of some species of Basidiomycetes**

Species	Number of the samples	Polyphenols, mg/g
Order *Polyporales*		
Auricularia auricula-judae *	12	32,53 ± 3,52
Laeticorticium roseum *	3	20,02 ± 0,58
Chaetoporus ambiquus *	6	20,66 ± 0,95
Sparassis crispa *	9	10,54 ± 0,19
Fibuloporia mollusca *	6	10,54 ± 0,35
Tyromyces lacteus *	9	16,07 ± 0,76
Tyromyces revolutus *	3	12,09 ± 0,16
Tyromyces undosus *	6	10,33 ± 0,13
Irpex lacteus *	9	26,75 ± 0,43
Amyloporia lenis *	3	15,05 ± 0,21
Hydnum ochraceum *	3	10,02 ± 0,24
Trametes squalens *	6	15,07 ± 0,28
Trametes campestris *	6	20,14 ± 0,41
Trametes versicolor *	15	14,13 ± 0,71
Trametes zonatus *	9	15,06 ± 0,52
Fomes fomentarius *	12	248,29± 5,84
Heterobasidion annosum *	12	13,33 ± 0,64
Fomitopsis pinicola *	6	39,19 ± 0,58
Daedalea quercina *	6	9,02 ± 0,13
Piptoporus betulinus *	12	15,10 ± 0,10
Polyporus squamosus *	9	23,20 ± 0,37
Laetiporus sulphureus *	9	117,04 ± 0,56
Ganoderma applanatum *	9	161,08 ± 0,19
Ganoderma lucidum *	15	89,06 ± 1,5
Inonotus obliquus *	12	20,55 ± 0,31
Phellinus igniarius *	9	34,53± 0,55
Phellinus pomaceus *	9	19,04 ± 0,59
Order *Agaricales*		
Agaricus arvensis *	5	24,57 ± 4,07
Agaricus bisporus **	9	35,44± 0,63
Agaricus campestris *	5	23,46 ± 0,10
Agrocybe cylindracea **	9	75,85 ± 1,22
Coprinus comatus *	15	25,04 ± 0,58
Coprinus micaceus *	15	25,03± 0,15
Fistulina hepatica *	9	172,25 ± 0,20

Species	Number of the samples	Polyphenols, mg/g
Flammulina velutipes *	27	81,25 ± 7,75
Flammulina velutipes **	3	65,06 ± 0,92
Lentinus edodes **	9	35,47 ± 0,42
Marasmius oreades *	3	37,08 ± 0,65
Pleurotus citrinopileatus **	3	37,55 ± 0,11
Pleurotus eryngii **	6	15,03 ± 0,42
Pleurotus ostreatus *	34	100,56 ± 3,15
Pleurotus ostreatus var. Florida **	3	53,07 ± 2,01
Kuehneromyces mutabilis *	9	32,28 ± 0,83
Pholiota aurivella *	3	18,02 ± 0,35
Pholiota squarrosa *	3	12,04 ± 0,65
Schizophyllum commune *	21	19,29 ± 0,27
Stropharia aeruginosa *	3	32,53 ± 0,54
Stropharia rugosoannulata **	6	59,56 ± 1,85
Lyophyllum loricatum *	5	21,37 ± 0,63
Lyophyllum connatum *	5	20,42 ± 0,12
Tricholoma flavovirens *	5	79,08 ± 0,20
Tricholoma sejunctum *	5	31,48 ± 0,52

"*" – wild in nature CF, "**" – commercial CF

The analysis of polyphenols in the carpophores of Polyporales fungi showed the following. Most of their FBs (85%) contain little polyphenols being within the range of 9 mg/g (*D. quercina*) to 39 mg/g (*F. pinicola*). The other group comprises 3 species of Polyporales fungi (*G. lucidum, L. sulphureus* and *G. applanatum*) containing polyphenols in their FBs in the amount of 89 mg/g to 161 mg/g (ADB). The fruit bodies of tinder fungus *F. fomentarius* have the highest concentration of poliphenols - more than 248 mg/g (ADB). To compare the results obtained, it should be noted that the method of reactant extraction from the carpophores of shelf fungus *Inonotus obliquus* (Ach. ex Pers.) Pil. with the maximum PP concentration of 140 mg/g (ADB) by water extraction has been patented (Sysoeva et al., 2005; Patent 2448721).

The analysis of polyphenols in the carpophores of Agaricales fungi showed that most of them (74%) contain little polyphenols in their FBs but it is less compared to Polyporales fungi. Here they are within the range of 12 (*P. squarrosa*) to 37 mg/g (*P. citrinopileatus*). 5 species of Agaricales fungi (*P. ostreatus, S. rugosoannulata, T. flavovirens, F. velutipes* and *A. cylindracea*) can be put into a group with the medium polyphenol concentration of 53 to 101 mg/g in the fruit bodies. The highest polyphenol concentration of about 172 mg/g was registered in the wild fruit bodies

of *F. hepatica*. However, this parameter is more than 1.5 times lower than the concentration of phenolic substances in the fruit bodies of tinder fungus *F. fomentarius*. In contrast, it should be noted that the mean concentration of polyphenols amounts to 450 mg/g in the plant raw material of *Camellia sinensis*, and 70 mg/g in the mycological material (fruit bodies of *P. ostreatus*) (Li Fu et al., 2011; Halvorsen et al., 2011).

The analysis of polyphenols in the alcohol extracts from the fruit bodies of 50 basidiomycetes species made it possible to isolate some species of tinder fungi: *G. lucidum*, *L. sulphureus*, *G. applanatum* and *F. fomentarius*, and some species of Agaricales fungi: *S. rugosoannulata*, *A. cylindracea*, *T. flavovirens*, *F. velutipes*, *P. ostreatus* and *F. hepatica* having the high concentration of these substances of more than 60 mg/g (ADB).

The next step of the investigation was to isolate pure cultures from the carpophores as well as to study the growth and synthesis rate of polyphenols of some of them when cultivating in the GPM.

The results of ADB accumulation by the strains while their growing (on the 6th, 9th and 12th day of cultivation) are shown on Figure 1. As can be seen, all cultures reach the maximum of this parameter on the 12th day of their growth. The most productive strains here are those of *S. commune* Sc-1101 and Sc-10 and that of *F. velutipes* F-202. The lowest values of ADB accumulation were registered for *P. ostreatus* strain P-192 and *F. fomentarius* strain Ff-09. Thus, the investigated cultures have their individual growth values – biomass accumulation in the cultivation conditions used that probably reflects suitability of these conditions for their growth.

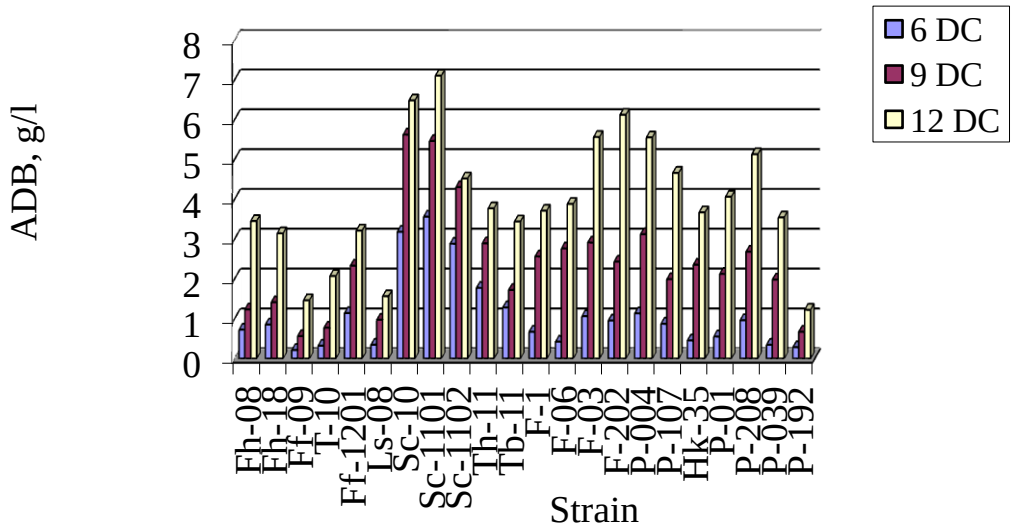

Fig. 1. Accumulation of absolute dry biomass of strains of Basidiomycetes on Day 6, 9, and 12 of cultivation (DC – day of cultivation)

The investigation results of total polyphenols in the mycelium and culture filtrate while growing some basidiomycetes strains are shown on Figure 2 and 3.

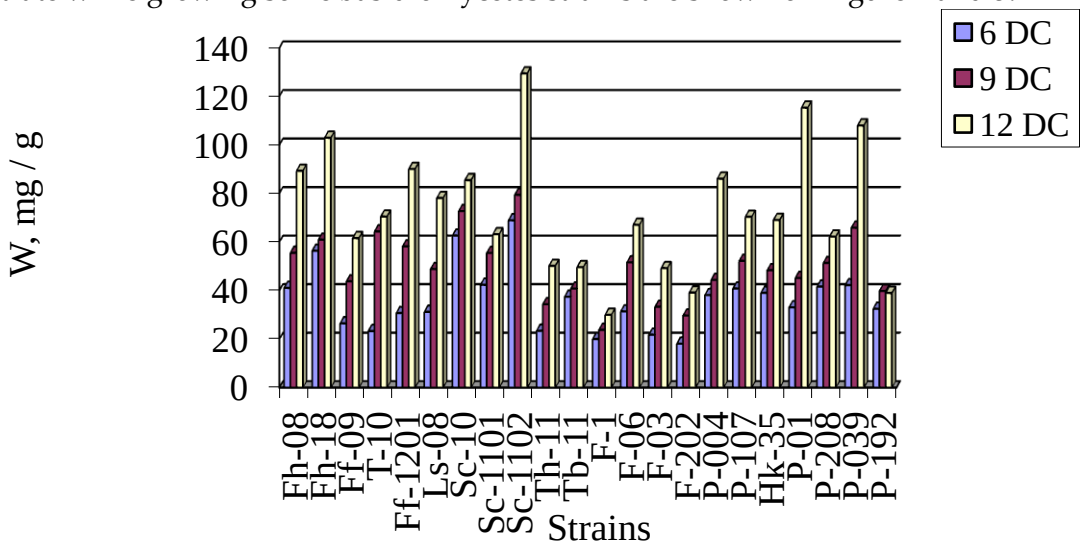

Fig. 2. The total content of polyphenols of mycelium of strains of Basidiomycetes on Day 6, 9, 12 of cultivation (DC – day of cultivation)

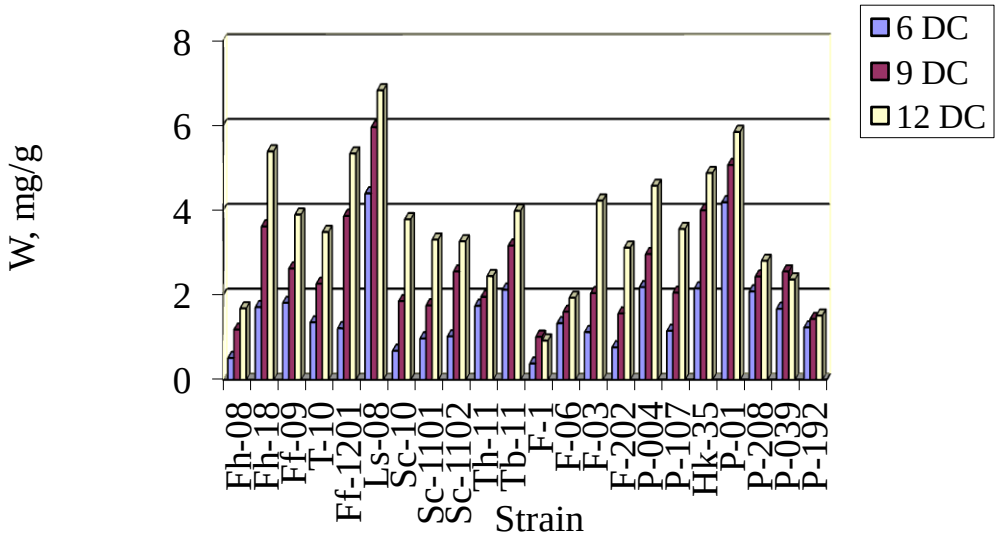

Fig. 3. The total content of polyphenols of culture filtrate of strains of Basidiomycetes on Day 6, 9, and 12 of cultivation (DC – day of cultivation)

It was established that most of strains were able to accumulate polyphenols both in the mycelium and CF during the whole cultivation period. The maximum of PP concentration in the mycelium for 96% and in the CF for 91% of the total strains coincided with the termination of their cultivation period.

The trend of polyphenols in the mycelium of the strains under investigation has the following characteristics. The highest concentration of these substances within the range of 107.9 to 129.4 mg/g was registered for *P. ostreatus* strains P-039 and P-208, and *S. commune* strain Sc-1102 on the 12th day of their growth. The lowest values of PP concentration of 29.9 to 50.1 mg/g were registered for *F. velutipes* strains F-1, F-202 and F-03, and *P. ostreatus* strain P-192 at the end of their cultivation period.

The trend analysis of polyphenols in the culture filtrate of the strains under investigation showed the following. The highest PP concentration was registered on the 12th day of their growth within the range of 5.4 to 6.8 mg/ml for the strains of *F hepatica* Fh-18, *L. sulphureus* Ls-08 and *P. ostreatus* P-01, and the lowest (0.9 to 1.7 mg/ml) - for the strains of *F. hepatica* Fh-08, *P. ostreatus* P-192 and *F. velutipes* F-1.

In all cases the concentration of polyphenols in the mycelium was much higher than the concentration of these substances in the culture filtrate and varied on the 12th day of cultivation from 11.4 times for *L. sulphureus* strain Ls-08 to 52.9 times for *F hepatica* strain Fh-08. A considerable difference between ability of strains to synthesise and accumulate PPs in the mycelium and CF may be most likely explained by their genotype realization under test conditions.

The calculation of a correlation coefficient between PP concentration in the mycelium and CF of one-year cultures showed the following. A very high positive correlation is observed in 73.2%, high positive one – in 17.4% and medium one – in 4.5% of tests.

Thus, the test results of total polyphenols in some basidiomycetes species enable us to make the following conclusions. The species of tinder fungi - *Ganoderma applanatum, Ganoderma lucidum*, *Laetiporus sulphureus* and *Fomes fomentarius*, and the species of Agaricales fungi - *Stropharia rugosoannulata*, *Agrocybe cylindracea*, *Tricholoma flavovirens*, *Flammulina velutipes, Pleurotus ostreatus* and *Fistulina hepatica* are characterized by the highest concentration of polyphenols in their carpophores. Most of introduced strains can accumulate polyphenols both in the mycelium and CF during the whole period of their cultivation.

The strains of *P. ostreatus* P-01, *F. hepatica* Fh-18 and *L. sulphureus* Ls-08 are perspective for further investigations aimed to obtain polyphenols of extracellular origin and the strains of *S. commune* Sc-1102, and *P. ostreatus* P-039 and P-208 – those of mycelial one.

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