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***Original Paper***

# Total flavonoid content, lipid peroxidation and total antioxidant activity of *Hericium coralloides*, *Fomes fomentarius* and *Schizophyllum commune* cultivated by the method of direct confrontation

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## Abstract

The patterns of culture growth, fruiting, total flavonoid content, lipid peroxidation and antioxidant properties were established for *Hericium coralloides*, *Fomes fomentarius* and *Schizophyllum commune*, and cultivated on Potato Glucose Agar by the method of direct confrontation. Co-cultivation is accompanied by the inhibition of the colonies' growth. The highest inhibitory effect was found to *H. coralloides*, and minimal inhibitory values were established to *S. commune*.

In the fruiting bodies of *H. coralloides*, *F. fomentarius* and *S. commune* that were formed on a different co-culture composition (except *H. coralloides* + *F. fomentarius* composition), an increase of total flavonoid content and total antioxidant activity were observed. Direct confrontation conditions are a stress for cultures because an increase of lipid peroxidation level in the fruiting bodies was observed.

Tendency of accumulation of total flavonoid content in the *H. coralloides*, *F. fomentarius* and *S. commune* fruiting bodies is an important antifungal aspect in co-cultivation of macromycetes, because the accumulation of flavonoids directly correlates to the magnitude of inhibition of the *H. coralloides* and *F. fomentarius* culture growth. This means that fungi have clear mechanisms of counteraction to competitors. However, practical application of this phenomenon is questionable.

**Keywords**
antagonism; culture; fruiting bodies; Malondyaldehyde; stress

## Introduction

*Hericium coralloides* (Scop.) Pers. is an edible fungus of great significance in medicine (Pallua et al. 2012). It produces whitish basidiomata growing mostly on dead wood of *Fagus sylvatica* L., causing white rot (Ko et al., 2005; Boddy et al., 2011; Pallua et al., 2012). *Hericium coralloides* is a rare fungus found in most countries of Europe and Asia http://iucn.ekoo.se/iucn/species_view/120231/, including Ukraine.

The main conservation measures for *H. coralloides* on the territory of Ukraine and other countries include the protection of its gene pool in culture collections (*ex-situ* method) (Saxena and Gupta, 2019) and in fungal populations of nature reserves and nature parks (*in situ* conservation method). *Re-situ* cultivation technology of rare mushrooms in nature has been developed and successfully tested in Hutsulshchyna National Nature Park (NNP), Kosiv, Ukraine (Petrichuk et al., 2017, Pasailiuk et al., 2018). *Re-situ* is a method that provides introduction and support of vital mushroom functions in nature by forming basidiomata (Pasailiuk et al., 2018). Some steps of growing *H. coralloides* by *re-situ*

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method include growing the mycelium in the laboratory and inoculating mycelium of *H. coralloides* in beech logs found nature. When *re-situ* technology of cultivation was initiated, it was established that beech logs were colonised by *Fomes fomentarius* (L.) Fr. and *Schizophyllum commune* Fr. and were not suitable for *H. coralloides*.

*Fomes fomentarius* has a wide distribution. It is a white-rot fungus that has been recorded on a wide range of hardwood trees (beech, birch, oak, poplar, maple, etc.) (https://mycocosm.jgi.doe.gov/Fomfom1/Fomfom1.home.html). It is found that *F. fomentarius* has significant properties, including its role in antioxidant enzyme activities, enzymatic activities, antimicrobial activities, antifungal activities (Bal and Akgül, 2018).

*Schizophyllum commune* is one of the most widely distributed and common mushrooms on Earth (Kuo, 2003). It grows anywhere that dead wood is found (http://www.michigannatureguy.com/blog/2013/11/28/split-gill-fungus/) causing white-rot (Ujor, 2010). *Schizophyllum commune* is an edible mushroom having medicinal value and it is also a very good source of protein, vitamins, lipids and mineral elements (Zahida et al., 2015).

We deduce that there is an antagonism between *H. coralloides* and other widely distributed xylotrophic fungi sharing the same ecological niche, on beech wood. This conclusion for *F. fomentarius* and *S. commune* was confirmed on malt extract agar and wort agar (Pasailiuk et al., 2019). The mechanisms providing the base of antagonistic effect and biochemical landscape of changes that occurred during direct confrontation remains unclear.

Competition between mushrooms is a phenomenon well-described between macromycetes and micromycetes within one Petri dish (Barinova et al., 2008). Recent works demonstrate the mechanisms of so-called "fungi wars" (Hiscox et al., 2018), the existence of which is possible in natural conditions due to the synthesis by certain fungal species of biologically active substances. In this case, obviously, mushrooms with better "vitality" will exhibit better adaptability and higher viability in the same conditions. The properties and ability to counteract other fungi are attributed to compounds of flavonoid nature. The process of such struggle, in our view, is stressful and must be accompanied by biochemical changes typical of stress.

Therefore, the aims of this study are: 1) to investigate the fungal mechanisms of counteraction to competitors; 2) to study the patterns of culture growth and opportunity of fruiting of *H. coralloides*, *F. fomentarius* and *S. commune* cultivated on Potato Glucose Agar (PGA) by the method of direct confrontation; 3) to determinate the total flavonoid content (as a possible antifungal factor), the MDA content (as the level of lipid peroxidation and the marker of stress), and the antioxidant properties of *H. coralloides*, *F. fomentarius* and *S. commune* fruiting bodies growing on PGA in the competition conditions.

## Materials and Methods

*Mycelial culture*

The pure cultures of *H. coralloides* (strain 2332), *S. commune* (strain 1763), *F. fomentarius* (strain (1528) were obtained from the Mushroom Culture Collection of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (IBK), #– 1152 in the international database of the World Federation for Culture Collections – WFCC (Bisko et al., 2016, http://www.wfcc.info/ccinfo/index.php/collection/by_id/1152/).

*Nutrient media and culturing fungi*

Single fungal isolates and their combinations were cultivated on PGA nutrient medium (Potato 200 g $l^{-1}$, Glucose 10 g $l^{-1}$, Agar 20 g $l^{-1}$, pH 5.8) at 22 ± 1 °C to complete overgrowth of the Petri dishes by the mycelium and at 14 ± 1 °C after complete overgrowth to fruiting.

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*Determination of antagonist activity*

The antagonist activity on PGA was studied according to Camporota (1985) and Bouziane et al. (2011). The method of direct confrontation was used. It means fungal cultures of *H. coralloides* (*Hc*), *F. fomentarius* (*Ff*) and/or *S. commune* (*Sc*) were placed in the same Petri dish (culture combinations: *Hc* + *Ff*; *Hc* + *Sc*, *Hc* + *Ff* + *Sc*). Each Petri dish contained 15 ml of PGA and two or three pieces of the inoculum (diameter of 5 mm), which were positioned in a diametrically opposite manner at a distance of 5 cm apart and 2 cm from the edge of the Petri dish. We assessed mycelial growth on the 66$^{th}$ days by measuring the diameter of the colony.

Inhibition of *H. coralloides* was quantified as the percentage of mycelial growth inhibition using the following formula:

$$I\% = (1 - Cn/Co) \times 100$$

where Cn is the average diameter of the *Hc* colonies in the presence of *Ff* and/or *Sc,* and Co is the average diameter of *Hc* colonies in the control (Camporota, 1985; Bouziane et al., 2011).

Inhibition of *Ff* (*Sc*) was quantified as the percentage of mycelial growth inhibition using the following formula:

$$I\% = (1 - Cn/Co) \times 100$$

where Cn is the average diameter of the *Ff* (*Sc*) colonies in the presence of *Hc* and/or *Sc* (*Ff*), and Co is the average diameter of *Ff* (*Sc*) colonies in the control.

*Sample Collection*

Fruiting bodies (teleomorphs) of *H. coralloides*, *F. fomentarius* and *S. commune* were collected in according fruiting time, i.e. on day 66 in co-cultures and on day 115 (*H. coralloides*), on day 68 (*F. fomentarius*) and on day 69 (*S. commune*) in single cultures.

*Preparation of Mushroom Extracts*

Organic extraction of the mushroom was carried out according to the method described in Jinting et al. (2017). Fruiting bodies (without medium) were minced and dried at room temperature for 2 hours. Accurately, 20 ml of methanol and chloroform were added at 1:1 volume ratio. The homogenate was agitated for 2 hours, filtered with normal filter paper, refluxed for 3 hours. The dried extract was re-dissolved in 100% dimethyl sulfoxide (DMSO) for highest concentration and finally stored at 4 ˚C.

*Determination of Total Flavonoid Content*

Total flavonoid content was determined following the method by Singh et al. (2012) and Jinting et al. (2017). Accurately, 1 ml of sample or standard was diluted with 4 ml distilled water and 0.3 ml of 5% sodium nitrate solution was added. After 6 minutes, 0.3 ml of a 10% aluminium chloride solution was added to the mixture. The mixture was incubated at room temperature for 5 minutes. Then, 2 ml of 1M sodium hydroxide was added to the mixture after 5 minutes of incubation. The mixture was vortexed thoroughly and the absorbance of the pink colour was measured at 510 nm against a blank by using UV-1100 Spectrophotometer. Quercetin was used for the calibration curve with a concentration range of 1 – 100 µg ml$^{-1}$. Results were expressed as micrograms quercetin equivalent (µg QE g$^{-1}$) of dried extract. All experiments were carried out in four replicates.

*Determination of total antioxidant activity by phosphomolybdenum assay*

Different concentration of Butylated Hydroxy Toluence (BHT) (5-125 µg ml$^{-1}$) in distilled water were prepared. 0.3 ml of mushroom extracts were taken in test tubes. The reagent solution was prepared by mixing 10 ml of 0.6 M sulphuric acid, 10 ml of 28 nM sodium phosphate and 10 ml of 4 mM ammonium molybdate into a beaker and 3 ml reagent solution was added to all the tubes. Three

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hundreds microliters of methanol served as blank. All the tubes were incubated at 95 ºC for 90 min. The tubes were cooled to room temperature and the optical density was measured at 695 nm using UV-1100 spectrophotometer. Results were expressed as a µg equivalent of Butylated Hydroxy Toluene × gram of fruiting body of *H. coralloides*, *F. fomentarius* and *S. commune* in phosphomolybdenum assay by the method of Prieto et al. (1999) as described by Imran et al. (2011).

*Estimation of lipid peroxidation*

The measurement of Malondialdehyde (MDA) has been used as an indicator of lipid peroxidation. MDA was measured according to the thiobarbituric acid reaction as described by Zhang and Qu (2004) and Chen et al. (2014). Two hundreds milligrams of fruiting bodies samples was homogenized with 5% trichloroacetic acid (TCA) and centrifuged at 4,000 g for 10 min. Two ml of extract was added to 2 ml 0.6% TBA placed in a boiling water bath for 10 min, and the absorbance at 532, 600, and 450 nm, namely, $A532$, $A600$, and $A450$, respectively, was determined spectrophotometrically. The MDA concentration was calculated according to the formula: $6.45 \times (A532 - A600) - 0.56 \times A450$ and expressed as nmol (nM) MDA × gram of fruiting body.

*Data analysis*

All experiments were carried out in four replicates. Data obtained were analyzed by using Statistica 8.0 (StatSoft Inc., USA) and Pearson's correlation coefficient was performed. The data are presented as mean ± standard deviation. Significant differences between means are indicated at $P \leq 0.05$ level.

## Results

*Effects of joint cultivation on PGA on mycelia growth*

To compare the mycelial growth of *H. coralloides* with and without competitive fungi, we inoculated mycelium of *F. fomentarius* and/or mycelium of *S. commune* on the same Petri dishes. After 66 days of joint cultivation, (the time where all competitive fungi had fruited on PGA) the colony diameters of *H. coralloides*, *F. fomentarius* and *S. commune* were measured. We found all culture growth was inhibited by joint cultivation. The most greater inhibition effects were observed for *H. coralloides* in using combination *Hc* + *Sc* – 90% and *Hc* + *Ff* – 84% (Table 1).

**Table 1 -** Growth of *Hericium coralloides* cultivated with confronting cultures on PGA, day 66

| Combination of cultures[1] | Colony diameter of *H. coralloides* (cm) | | Inhibition (%) |
|---|---|---|---|
| | Experiment | Control | |
| *Hc* + *Ff* + *Sc* | 3.93 ± 0.17* | 8.05 ± 0.21 | 51.24 ± 2.12 |
| *Hc* + *Ff* | 1.30 ± 0.14* | | 84.07 ± 1.81 |
| *Hc* + *Sc* | 0.80 ± 0.08* | | 90.06 ± 1.01 |

[1] *Hc* = *Hericium coralloides*, *Ff* = *Fomes fomentarius*, *Sc* = *Schizophyllum,* * Significant differences from the control, $P \leq 0.05$

Cultivation of *H. coralloides* in triple composition is accompanied by the less values of inhibition (51%), than in double (84% and 90%). Cultivation of *H. coralloides* and *F. fomentarius* together has significant inhibition effect on *F. fomentarius* growth (Table 2) not only on *H. coralloides.* To *S. commune* inhibition effect was the less (Table 3).

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**Table 2 -** Growth of *Fomes fomentarius* cultivated with confronting cultures on PGA, day 66

| Combination of cultures[1] | Colony diameter of *F. fomentarius* (cm) | | Inhibition (%) |
|---|---|---|---|
| | Experiment | Control | |
| *Hc +Ff +Sc* | 4.40 ± 0.08* | 9.00 ± 0.08 | 51.11 ± 0.91 |
| *Hc+Ff* | 3.00 ± 0.36* | | 66.67 ± 3.96 |
| *Ff +Sc* | 5.80 ± 0.08* | | 35.56 ± 0.91 |

[1] *Hc = Hericium coralloides*, *Ff = Fomes fomentarius*, *Sc = Schizophyllum*, * Significant differences from the control, $P \leq 0.05$

**Table 3** - Growth of *Schizophyllum commune* cultivated with confronting cultures on PGA, day 66

| Combination of cultures[1] | Colony diameter of *S. commune* (cm) | | Inhibition (%) |
|---|---|---|---|
| | Experiment | Control | |
| *Hc +Ff +Sc* | 6.95 ± 0.31* | 9.00 ± 0.08 | 22.78 ± 3.45 |
| *Hc+Ff* | 7.05 ± 0.13* | | 21.67 ± 1.43 |
| *Ff + Sc* | 6.90 ± 0.29* | | 23.33 ± 3.27 |

[1] *Hc = Hericium coralloides*, *Ff = Fomes fomentarius*, *Sc = Schizophyllum commune*
* Significant differences from the control, $P \leq 0.05$

*Effects of joint cultivation on PGA on culture fruiting*

Starting from day 61 to day 66 we observed fruiting of confronting cultures (Fig. 1). The combination of *Hc + Ff* was a single combination where fruiting bodies were not formed and the content of flavonoids, total antioxidant activity and MDA were not studied accordingly. We observed fruiting of single cultures, i.e., and the control, too (Fig. 2).

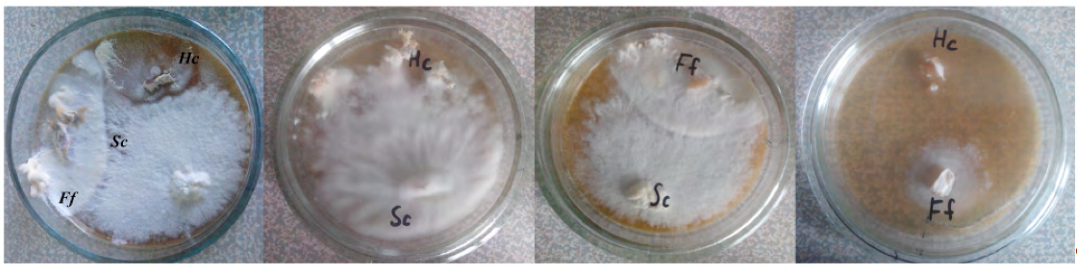


**Figure 1 -** Formation of teleomorphs of *Hericium coralloides* (*Hc*), *Fomes fomentarius* (*Ff*) and *Schizophyllum commune* (*Sc*) (except the combination *Hc+ Ff*) for direct confrontation between cultures, day 66

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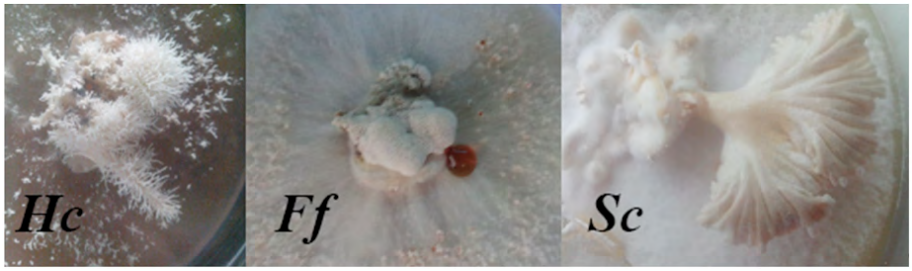


**Figure 2** - Formation of teleomorphs of *Hericium coralloides* (*Hc*, day115), *Fomes fomentarius* (*Ff,* day 68) and *Schizophyllum commune* (*Sc*, day 69) on PGA

We found a difference in the time frame of fruiting for monocultures compared to the cultures in direct confrontation conditions. For the monoculture of *H. coralloides* on PGA, almost total over growth of the Petri dishes happened on days 30 ± 1, with teleomorphs forming on days 110–115. For the monoculture of *F. fomentarius* on PGA, total overgrowth of the Petri dishes happened on days 15 ± 1, with teleomorphs forming on days 63–68. For the monoculture of *S. commune* on PGA, total overgrowth of the Petri dishes happened on days 18 ± 1, with teleomorphs forming on days 64–69. Therefore, fruiting bodies of *H. coralloides*, *F. fomentarius* and *S. commune* were harvested accordingly on days 115, 68 and 69 and total flavonoid content, total antioxidant activity and lipid peroxidation by content of MDA were studied.

Total overgrowth of the Petri dishes by confronting cultures happened on days 32 ± 2 (except the combination of *Hc* + *Ff*). Starting from day 61 to day 66, we observed fruiting of confronting cultures (except the combination of *Hc* +*Ff*). Therefore, fruiting bodies of *H. coralloides*, *F. fomentarius* and *S. commune* growing in competitive conditions were harvested on day 66 and total flavonoid content, total antioxidant activity and lipid peroxidation by content of MDA were studied.

*Comparison of total flavonoid content in fruiting bodies of confronting fungi*

Total flavonoid content (μg QE $g^{-1}$ dried extract) of fruiting bodies of control variants and their experimental combinations are presented in Figure 3.

In this study *H. coralloides* cultivated on PGA, exhibited the total flavonoid content in the teleomorphs with 1.14 ± 0.03 μg QE × gram dried extract, *F. fomentarius* cultivated on PGA exhibited the total flavonoid content in the teleomorphs with 0.44 ± 0.02 QE $g^{-1}$, *Schizophyllum commune* cultivated on PGA exhibited the total flavonoid content in the teleomorphs with 0.64 ± 0.16 QE $g^{-1}$ (Fig. 3).

The present study indicates that co-cultivation of xylotroph cultures is accompanied by a significant increase of total flavonoid content in the fruiting bodies compared to the monoculture conditions. Moreover, during the confrontation in all fruiting bodies, we observed the increase of total flavonoid content. Thus, the total flavonoid content for *H. coralloides* fruiting bodies in the conditions of the triple combination increased in 4.7 times, and for the combination of *Hc*+ *Sc,* it increased almost 10-fold (Fig. 3).

In the cases with high inhibition effect to *H. coralloides* culture, we detected higher total flavonoid content of *H. coralloides* fruiting bodies. Between these values, the coefficient of correlation was + 0.99. In a similar occurrence, the dependence of high total flavonoid content in the teleomorphs correlated to high levels of inhibition of culture growth for *F. fomentarius* (coefficient of correlation was + 0.84) but not for *S. commune* (coefficient of correlation was + 0.24).

We observed an increase of total flavonoid content in the teleomorphs of *F. fomentarius* in 14.8-fold (triple combination), and in 11.7-fold (combination of *Ff* + Sc). For *S. commune* the increase of

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total flavonoid content was maximal and ranged from 4.5 (in 7-fold) to 13.16 µg QE $g^{-1}$ dried extract (in 20.6-fold regarding control).

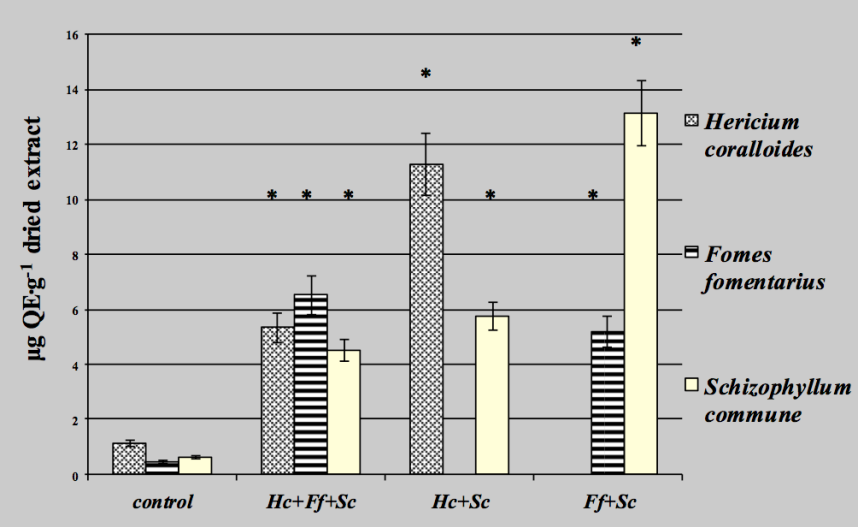


**Figure 3 -** Total flavonoid content in the fruiting bodies of fungi on PGAin single cultures (control) and in different co-culture combinations, day 66. * Significant differences from the control, $P \leq 0.05$

*Studying of total antioxidant activity and lipid peroxidation in the fungi competitive mechanism*

By studying of lipid peroxidation, we observed that the direct confrontation is accompanied by an increase MDA level in formed fruiting bodies, with the exception of *S. commune* fruiting bodies in the variant *Hc+ Sc.* MDA significantly increased, ranging from 15% for *S. commune* in the variant *Ff + Sc* to 35% for *H. coralloides* in the combination *Hc+ Sc* (Table 4).

**Table 4 -** Total antioxidant activity and lipid peroxidation in the teleomorphs of *Hericium coralloides*, *Fomes fomentarius* and *Schizophyllum commune*

| Combination of cultures | Sample | Total antioxidant activity (µgBHT/mg DW) | Lipid peroxidation (nM MDA/g fruiting body) |
|---|---|---|---|
| *Hc + Ff + Sc* | *Hc* | 16.33±0.32* | 365.00±15.34* |
| | *Ff* | 25.30±1.57* | 354.00±26.14* |
| | *Sc* | 15,56±1.02* | 531.25±17.97* |
| *Hc + Sc* | *Hc* | 32.35±5.57* | 428.00±24.10* |
| | *Sc* | 21,85±1.46* | 445.25±30.67 |
| *Ff + Sc* | *Ff* | 19,37±1.18* | 317.00±18.57* |
| | *Sc* | 56,65±1.54* | 487.25±21.84* |
| *Hc* (control) | *Hc* | 4.20±0.08 | 316.75±6.18 |
| *Ff* (control) | *Ff* | 2.06±0.06 | 263.25±12.31 |
| *Sc* (control) | *Sc* | 3.12±0.45 | 423.00±19.82 |

[1] *Hc = Hericium coralloides*, *Ff = Fomes fomentarius*, *Sc = Schizophyllum commune*
* Significant differences from the control, $P \leq 0.05$

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## Discussion

The co-cult vation of *H. coralloides, F. fomentarius* and *S. commune* cultures on PGA presented growth inhibition. Thus, testing cultures counteracting one another was important. Interestingly, each fungus is listed as a white rot fungi, therefore each of them has similar ecological-trophic requirements and this fact maybe is a reason of antagonism.

The inhibition of *H. coralloides* growth in triple composition was almost 2-fold lower than when *H. coralloides* was cultivated in dual combinations. This type of counteraction is similar to additive antagonism type (Folt et al., 1999). Not only was bilateral inhibition of culture growth established on the PGA medium for the *Hc* + *Ff* combination, but it was too fixed stopping of the colonies growth before their contact and fruiting bodies did'nt form. Therefore, no further studies (total antioxidant activity, total flavonoid content, MDA level) have been performed for this variant.

As it has been shown the chemical composition of the nutrient medium plays an important role in the manifestation of the antagonistic strengths of the cultures (Pasailiuk et al., 2019). Type of interaction ranged from inhibition *H. coralloides* growth on WA to stimulation *H. coralloides* growth on Czapek + cellulose medium. Thus, antagonistic interactions are determined by the type of combination, the chemical composition of the nutrient medium. It addition, these antagonistic interactions can be mediated at a distance and following contact. Similar conclusions are demonstrated by Boddy (2000), Boody et al. (2008) and Hiscox et al. (2015). Undoubtedly, the inhibitory effect is a consequence of the complex and diverse morphological, physiological and biochemical changes that are affected by the present species and environmental conditions.

The scholarly work of Griffith et al. (1994), Boddy (2000), Baldrian (2004), Hynes et al. (2007), Boddy et al. (2008), Evans et al. (2008) under score that the result of a culture's interaction could be determinated by pigment production, release of volatile organic compounds (which also act as info-chemicals), diffusible enzymes, toxins and antifungal metabolites. Additionally, it has been demonstrated that the interaction of fungi with plant hosts or microorganisms may be accompanied by oxidative stress (Breitenbach et al., 2015).

Our results demonstrate that co-cultivation of cultures is accompanied by antagonism, the inhibition of growth and oxidative stress too, because we register an increase of MDA (Table 4). Thus, cross-contamination of cultures is a stress factor.

Oxidative stress happens due to an increase of reactive oxygen byproducts. Free radicals induce lipid peroxidation, playing an important role in pathological processes. The stress mediated by free radicals can be measured by conjugated dienes, malondialdehyde, 4-hydroxynonenal, and others. However, MDA has been pointed out as the main product to evaluate lipid peroxidation (Grotto et al. 2009). In the last 20 years, MDA has been recognized as an important lipid peroxidation indicator, and could be used as a biomarker to detect damage in the body (Simioni et al., 2018).

An increase of MDA levels in *H. coralloides* fruiting bodies is directly related to the inhibitory effect on the *H. coralloides* mycelium growth which occurred in the presence of other cultures. When we observed a high percentage of growth inhibition, we also observed a hight MDA concentration, with the correlation coefficient of + 0.88. Consequently, co-cultivation can be considered a stress factor for *H. coralloides* culture. At the same time, the accumulation of MDA in the *F. fomentarius* fruiting bodies and its inhibition in the different combinations of *F. fomentarius* culture growth are values that correlate less closely, with the correlation indicator of + 0.64. In the *S. commune* fruiting bodies, the significant increase of MDA levels and the inhibition indicators of the growth of this culture, are not completely correlated. Therefore, co-cultivation is a reason the increase of MDA can be considered a stress factor for *F. fomentarius* and *S. commune* cultures too.

It is known that there are two principal mechanisms of protection against oxidative stress – regulation of membrane permeability and antioxidant potential (Lushchak, 2011). When cultures are

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grown together on the same Petri dish, a large number of stress factors can arise from competitive conditions. There is a limited surface of free space for growth, exhausting nutrient resources of the medium.

As a rule, many species of fungi form teleomorphs in the Petri dishes during the last stages of mycelium growth, in fact before death. Teleomorphs are important for macromycetes resettlement in nature, and formation of them is genetically programmed (Bukhalo, 1988). Thus, despite the limited resources of the nutrient medium and competition from each to other, mushrooms not only survive, but also perform a standard conservation program and form the fruiting bodies (Fig. 1). What are the mechanisms of the cultures counteraction (in fact antifungal) in this struggle?

The antifungal properties are attributed to compounds of flavonoids nature. *In vitro* experimental systems show that flavonoids possess anti-inflammatory, anti-allergic, antiviral, and anti-carcinogenic properties (Kavuru, 2008). Flavonoids promote physiological survival of plant by protecting it from pathogenic microorganisms and UV radiations (Hayat et al., 2017). Flavonoids are secondary metabolites. Phenolic and flavonoid molecules are important antioxidant components which are responsible for deactivating free radicals based on their ability to donate hydrogen (Aryal et al., 2019). Total flavonoid content is one of the most important quality indexes of living organisms and it is concerned with total antioxidant activity through the scavenging of oxygen – derived free radicals. Various health benefits arise due to the high proportion of antioxidants (Shi et al., 2019).

After studying total flavonoid content in the fruiting bodies of the monocultures of *H. coralloides*, *F. fomentarius* and *S.commune,* we found a relatively low level of flavonoid content. The established indicators are much less than the ones described in the literature for the content of flavonoids in the fruiting bodies of wild-growing exemplars of these species (Heleno et al., 2015; Vamanu and Voica, 2017). Co-cultivation was accompanied a significant increasing of total flavonoid content in the fruiting bodies of all cultures against to control (Fig. 3). Besides, the accumulation of total flavonoid content in the fruiting bodies of *H. coralloides* and *F. fomentarius* is a direct consequence of the culture growth inhibition.

An increase in total flavonoid content in our experiment is not the only example where the content of these compounds increases as a response to stress factors. Stress triggered by salinity and drought are also accompanied by an increase in total flavonoid content. For example, under salinity stress, total phenolics and flavonoids, contents of vanillin and protocatechuic acid in tolerant varieties of rice cultivar (*Oryza sativa* L.) increased (Minh et al., 2016). The result, influenced by drought on oxidative stress and flavonoid production in the cell suspension culture of *Glycyrrhiza inflata* Bat. indicated that water deficit enhanced biomass accumulation and flavonoid production, which was about 2-fold and 1.5-fold of the control, respectively (Yang et al., 2007). Literature has shown that drought stress elevates bioactive compounds, vitamins, phenolics, flavonoids and antioxidant activity in many leafy vegetables (Sarker and Oba, 2018). Abiotic stresses induce an increase of total phenolic, total flavonoid and antioxidant properties in endemic, Malaysian microalgae and cyanobacterium (Azim et al., 2018).

In addition, oxidative damage to cellular components is often associated with aging in a wide range of organisms, including fungi (Aung-Htut et al., 2012). Storage of postharvested mushrooms is accompanied by an increase of MDA and total flavonoid content in fruiting bodies (Meng et al., 2008). Therefore, we selected well-formed, mature (but not old) fruiting bodies of *H. coralloides*, *F. fomentarius* and *S. commune* from co-cultivation conditions and from monocultures in our experiment. Experiments were conducted after drying of fruiting bodies on the same day of collection of exemplars. Therefore, the changes we observed for total flavonoid content and MDA regarding the control are a reaction to competitive conditions, not age-related symptoms.

So, our study showed that co-cultivation positively affected flavonoid accumulation in mushroom, which may further promote the improvement of antioxidant status in the fruiting bodies.

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We assume that the increase of total flavonoid content is clearly evidence of the antifungal properties of flavonoids and their potential in scavenging oxygen-derived free radicals. This assumption seems plausible due to the fact that the composition of *Hc* + *Sc*, in the *S. commune* fruiting bodies, showed that the MDA level did not exceed the control values. Therefore, the higher level of total flavonoid content in the *S. commune* fruiting bodies exhibits a lower level of oxidative stress and ultimately a higher competitive ability relative to other cultures.

Total antioxidant capacity is an analysis frequently used to assess the antioxidant status of biological sample. It can evaluate the antioxidant response against the free radicals produced in a given situation. In physiological conditions, oxy-radicals are part of the normal regulatory course of the organism and the cellular redox state is closely controlled by antioxidants. When the levels of free radicals increases and both the enzymatic systems and low molecular antioxidants are not sufficient to protect the organism, these radicals attack membranes and cells (Rubio et al., 2016).

The results of studying total antioxidant activity supports the hypothesis that flavonoid accumulation in mushrooms positively affects total antioxidant activity and it was demonstrated that co-cultivation was accompanied by a significant increase of total antioxidant activity in the fruiting bodies of each studied culture (Table 4).

Therefore, a high level of oxidative stress in the joint cultivate subjects leads to an imbalance in the fruiting body antioxidant capacity. The increased antioxidant capacity may signify an increase of antioxidant protection in co-cultures and demonstrate high amounts of total flavonoid content.

## Conclusions

Co-cultivation of fungal cultures with the same ecological and trophic requirements is accompanied by the inhibition of their colonies' growth. On PGA, *F. fomentarius* and *S. commune* are confirmed to be antagonists to *H. coralloides*. Co-cultivation is a stress for cultures, and is accompanied by an increase of MDA and biologically active substances in the fruiting bodies. Our study suggest that accumulation of total flavonoid content in the *H. coralloides, F. fomentarius* and *S. commune* is an important antifungal aspect in co-cultivation of these fungi. This means that fungi have clear mechanisms of counteraction to competitors. However, practical application of this phenomenon is questionable. A full spectrum of metabolites of secondary synthesis and their ratios needs to be studied.

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## References

Aryal S, Baniya MK., Danekhu K, Kunwar P, Gurung R, Koirala N (2019) Total Phenolic Content, Flavonoid Content an Antioxidant Potential of Wild Vegetables from Western Nepal. Plants 8(4):E96. https://doi.org/10.3390/plants8040096

Aung-Htut MT, Ayer A, Breitenbach M, Dawes IW (2012) Oxidative stresses and ageing. Sub-cellulllar biochemistry 57:13–54. https://doi.org/10.1007/978-94-007-2561-4_2

Azim NH, Subki A, Yusof ZNB (2018) Abiotic stresses induce total phenolic, total flavonoid and antioxidant properties in Malaysian indigenous microalgae and cyanobacterium. Malaysian Journal of Microbiology 14(1):25–33.

DOI: 10.6092/issn.2531-7342/10668

Bal C, Akgül H (2018) Pharmacological properties of *Fomes fomentarius*. In: International Eurasian Conference on Science, Engineering and Technology (Eurasian SciEn Tech), 22-23 November 2018, Ankara, Turkey pp 1195 – 1198.

Baldrian P (2004) Increase of laccase activity during interspecific interactions of white-rot fungi. FEMS Microbiology Ecology 50(3):245–253. https://doi.org/10.1016/j.femsec.2004.07.005

Barinova KV, Vlasov DYu, Psurtseva NV (2008) Antagonism between Agaricales macromycetes and micromycetes – biodestructors in culture. In: Djakov Y.T., Sergeev Y.V., eds., Modern mycology in Russia: Proceeding of the 2nd congress of Russian mycologists, 27 March 2008, Moscow, pp. 217–218. [in Russia].

Bisko NA, Lomberg ML, Mytropolska NY, Mykchaylova OB (2016) The IVC Mushroom Culture Collection. Alterpress, Kyiv [in Ukrainian].

Boddy L, Crockatt ME, Ainsworth AM (2011) Ecology of *Hericium cirrhatum*, *H. coralloides* and *H. erinaceus* in the UK. Fungal Ecology 4: 163–173. https://doi:10.1016/j.funeco. 2010.10.001

Boddy L (2000) Interspecific combative interactions between wood-decaying basidiomycetes. FEMS Micriobiology Ecology 31(3):185–194. https://doi.org/10.1111/j.1574-6941.2000.tb00683.x

Boddy L, Frankland JC, van West P. von (2008) Ecology of Saprotrophic Basidiomycetes. Elsevier, Oxford.

Bouziane Z, Dehimat L, Abdel Aziz W, Benabdelkader M, Kacemchaouche N (2011) The antagonism between *Trichoderma viride* and other pathogenic fungal strains in *Zea mays*. Agriculture and Biology Journal of North America 2: 584–590. https://doi.org/10.5251/abjna.2011.2.4.584.590

Breitenbach M, Weber M, Rinnerthaler M, Karl T, Breitenbach-Koller L, Bähler J (2015) Oxidative Stress in Fungi: Its Function in Signal Transduction, Interaction with Plant Hosts, and Lignocellulose Degradation. Biomolecules 5(2):318–342. https://doi.org/10.3390/biom5020318

Bukhalo AS (1988) Higher edible Basidiomycetes in pure culture. Naukova dumka, Kiev, [in Ukrainian].

Camporota P (1985) Antagonisme in vitro de *Trichoderma* spp. vis-ŕ-vis d*e Rhizoctonia solani* Kühn. Agronomies 5:613–620. https://doi.org/10.1051/agro:19850707

Chen X, Song F, Liu F, Tian C, Liu S, Xu H, Zhu X (2014) Effect of different arbuscular mycorrhizal fungi on growth and physiology of maize at ambient and low temperature regimes. Scientific World Journal 1–7. https://doi.org/10.1155/2014/956141

Evans JA, Eyre CA, Rogers HJ, Boddy L, Muller CT (2008) Changes in volatile production during interspecific interactions between four wood rotting fungi growing in artificial media. Fungal Ecology 1(2-3):57–68. https://doi.org/10.1016/j.funeco.2008.06.001

Folt CL, Chen CY, Moore MV, Burnaford J (1999) Synergism and antagonism among multiple stressors. Limnology and Oceanography 44(3/2): 864–877. http://doi.org/10.4319/lo.1999.44.3_part_2.0864

Griffith G, Rayner A, Wildman H (1994) Interspecific interactions, mycelial morphogenesis and extracellular metabolite production in *Phlebia radiata* (Aphyllophorales). Nova Hedwigia, 59:311–334.

Grotto D, Maria LS, Valentini J, Paniz C, Schmitt G, Garcia SC, Pomblum VJ, Rocha JBT, Farina M (2009) Importance of the lipid peroxidation biomarkers and methodological aspects for malondialdehyde quantification. Química Nova 32(1). https://dx.doi.org/10.1590/S0100-40422009000100032

Hayat M, Abbas M, Munir F, Qasim HM**,** Keyani R**,** Rabia A (2017) Potential of plant flavonoids in pharmaceutics and nutraceutics. Journal of Biomolecules and Biochemistry 1(1):12–17.

Heleno SA, Barrosa L, Martinsa A, Queiroz MJRP, Moralesc P, Fernández-Ruizc V,. Ferreira ICFR (2015) Chemical composition, antioxidant activity and bioaccessibility studies in phenolic extracts of two *Hericium* wild edible species. LWT - Food Science and Technology 63:475–481.

DOI: 10.6092/issn.2531-7342/10668

Hiscox J, O'leary J, Boddy L (2018) Fungus wars: basidiomycete battles in wood decay. Studies in Mycology 89: 117–124. https://doi.org/10.1016/j.simyco.2018.02.003

Hiscox J, Savoury M, Vaughan IP, Müller CT, Boddy L (2015) Antagonistic fungal interactions influence carbondioxide evolution from decomposing wood. Fungal Ecology 14:24–32. https://doi.org/10.1016/j.funeco.2014.11.001

Hynes J, Muller CT, Jones TH, Boddy L (2007) Changes in volatile production during the course of fungal mycelial interactions between *Hypholoma fasciculare* and *Resinicium bicolor*. Journal of Chemical Ecology 33(1):43–57. https://doi.org/10.1007/s10886-006-9209-6

Imran MM, Mahroop RM, Abdul BJ, Asarudeen A (2011) Determination of total phenol, flavonoid and antioxidant activity of edible mushrooms *Pleurotus florida* and *Pleurotus eous*. International Food Research Journal 18:579–582.

Jinting A, Mohd NZ, Abdullah N, Mat NR (2017) Total Phenolic and Flavonoid Content and Antioxidant Activities of Ten Malaysian Wild Mushrooms. Open Access Library Journal 4:2333–9705. https://doi.org: 10.4236/oalib.1103987

Kavuru P (2008) Crystal Engineering of Flavonoids Crystal Engineering of Flavonoids. Master of Science thesis, University of South Florida.

Ko HG, Park HG, Park SH, Choi CW, Kim SH, Park WM (2005) Comparative study of mycelial growth and basidiomata formation in seven different species of the edible mushroom genus *Hericium*. Bioresourse Technology 96: 1439–1444. https://doi:10.1016/j.biortech.2004.12.009

Kuo M (2003) *Schizophyllum commune.* Retrieved from the *Mushroom Expert. Com* Web site: http://www.mushroomexpert.com/schizophyllum_commune.html

Lushchak VI (2011) Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. Comparative biochemistry and physiology, Toxicology & pharmacology: CBP 153(2):175–190. https://doi.org/10.1016/j.cbpc.2010.10.004

Meng DM, Zhang YX, Yang R, Wang J, Zhang XH, Sheng JP, Wang JP, Fan ZC (2017) Arginase participates in the methyl jasmonate-regulated quality maintenance of postharvest *Agaricus bisporus* fruiting bodies. Postharvest Biology and Technology 132:7-14. https://doi.org/10.1016/j.postharvbio.2017.05.018

Minh LT, Khang DT, Ha PTT, Tuyen PT, Minh TN, Quan NV, Xuan TD (2016) Effects of Salinity Stress on Growth and Phenolics of Rice (*Oryza sativa* L.). International Letters of Natural Sciences 57:1-10. https://doi.org/10.18052/www.scipress.com/ILNS.57.1

Pallua JD, Recheis W, Poder R, Pfaller K, Pezzei C, Hahn H, Huck-Pezzei V, Bittner LK, Schaefer G, Steiner E, Andre G, Hutwimmer S, Felber S, Pallua AK, Pallua AF, Bonn GK, Huck CW (2012) Morphological and tissue characterization of the medicinal fungus *Hericium coralloides* by a structural and molecular imaging platform. Analyst 137:1584–1595. https://doi.org/10.1039/c1an15615b

Pasailiuk MV, Petrichuk YV, Tsvyd NV, Sukhomlyn MM (2018) The aspects of reproduction of *Clathrus archeri* (Berk.) Dring by the re-situ method in the National Nature Park Hutsulshchyna. Leśne Prace Badawcze 79(3):287–293. https://doi.org/10.2478/frp-2018-0028

Pasailiuk MV, Sukhomlyn MM, Gryganskyi AP (2019) Patterns of *Hericium coralloides* growth with competitive fungi. Czech Mycology 71(1):49–63. https://doi.org/10.33585/cmy.71104

Petrichuk YV, Pasailiuk MV, Sukhomlyn MM (2017) The aspects of reproduction of *Hericium coralloides* (Scop.) Pers. by the re-situ method in the National Nature Park Hutsulshchyna. Biological Resources and Nature Management 9(1–2): 5–13. [in Ukraine]

Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry 269(2): 337–341. https:// doi.org/10.1006/abio.1999.4019

DOI: 10.6092/issn.2531-7342/10668

Rubio CP, Hernández-Ruiz J, Martinez-Subiela S, Tvarijonaviciute A, Ceron JJ (2016) Spectrophotometric assays for total antioxidant capacity (TAC) in dog serum: an update. BMC Veterinary reseach 12(1);166. https://doi.org/10.1186/s12917-016-0792-7

Sarker U, Oba S (2018) Drought stress enhances nutritional and bioactive compounds, phenolic acids and antioxidant capacity of *Amaranthus leafy* vegetable. BMC Plant biology 18(1):258. https://doi.org/10.1186/s12870-018-1484-1

Saxena A, Gupta S (2019) Experimental comparison: Methods for the preservation of fungal cultures. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 9(1):208–212. https://doi.org/10.5943/cream/4/2/8

Simioni C, Zauli G, Martelli AM, Vitale M, Sacchetti G, Gonelli A, Neri LM (2018) Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. Oncotarget 9(24): 17181–17198. https://doi.org/10.18632/oncotarget.24729

Singh V, Guizani N, Essa MM, Hakkim FL, Rahman MS (2012) Comparative Analysis of Total Phenolics, Flavonoid Content and Antioxidant Profile of Different Date Varieties (*Phoenix dactylifera* L.) from Sultanate of Oman. International Food Research Journal 19: 1063–1070.

Shi P, Du W, Wang Y, Teng X, Chen X, Ye L (2019) Total phenolic, flavonoid content, and antioxidant activity of bulbs, leaves, and flowers made from *Eleutherine bulbosa* (Mill.) Urb. Food Science & Nutrition 7(1):148–154. https://doi.org/10.1002/fsn3.834

Ujor VC (2010) The physiological response of the white-rot fungus, Schizophyllum commune to Trichoderma Viride, during interspecific mycelial combat. PhD thesis, University of Westminster School of Life Sciences

Vamanu E, Voica A (2017) Total Phenolic Analysis and Antioxidant Activity of Mushroom Tinctures from Medicinal and Edible Species, by in *Vitro* and in *Vivo* Tests. Scientific Bulletin. Series F. Biotechnologies XXI:318–324.

Yang Y, He F, Yu L, Chen X, Lei J, Ji J (2007) Influence of drought on oxidative stress and flavonoid production in cell suspension culture of *Glycyrrhiza inflate* Batal.Zeitschrift fur Naturforsch. C, Journal of bioscience 62(5-6):410–416. https://doi.org/10.1515/znc-2007-5-615

Zahida N, Khan SJ, Yasmeen A, Shafique M, Usman S, Ali S (2015) Optimization of Sub-Merged Culture Conditions for Biomass Production in *Schizophyllum commune*, a Medicinal Mushroom International Journal Current Microbiology and Applied Sciences 4(2): 258-266

Zhang ZL, Qu W (2004) Experimental Guidance of Plant Physiology, High Education Press, Beijing, China.