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Can lichen secondary compounds impact upon the pathogenic soil fungi *Fusarium oxysporum* and *F. avenaceum*?

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Abstract: The antifungal activity of secondary lichen metabolites extracted by means of acetone and ethanol from *Cetraria islandica*, *Cladonia mitis*, *C. rangiferina*, *Pseudevernia furfuracea* and *Usnea dasopoga* on the pathogenic fungi *Fusarium oxysporum* and *F. avenacuem* was examined. The activity of extracted compounds was tested after their mixture with PDA medium before solidification. The most active extracts inhibiting the growth of fungal mycelia contained fumarprotocetraric, salazinic and usnic acids, and atranorin. Unparalleled activity was demonstrated by an ethanol extract from mixed *C. islandica* and *P. furfuracea* thalli, which accelerated the growth of *F. avenaceum* compared with the control test with ethanol. The growth rate of the two *Fusarium* representatives was strongly inhibited by both alcoholic extracts from *C. mitis* and from *C. rangiferina*, more strongly than by the extracts from the mixed thalli of the two terricolous taxa. A dose of 1 ml of acetone added to the medium had no significant effect on the growth of the tested fungi; it indicates usefulness of acetone as a solvent for some lichen compounds in this type of experiment.

Keywords: lichen-fungi relationship, acetone and ethanol lichen extracts, lichen metabolites, fungal pathogen, inhibition, fungal cultures in vitro

INTRODUCTION

Lichens (lichenized fungi), a symbiosis of at least two basic components, a heterotrophic mycobiont and an autotrophic photobiont (Czarnota, 2009), biosynthesize secondary metabolites, many of which are unique to lichens (Boustie & Grube, 2005). According to various references, these substances vary in number from more than 850 (Elix, 2014) to more than 1000 (Molnar & Farkas, 2010), and have already been discovered in several thousand species of lichens (Ranković & Kosanić, 2015). The production of these substances occurs regardless of whether the fungal component of the lichen is a member of the *Ascomycota* or *Basidiomycota*, or whether the photobiont is composed of cyanobacteria or algae. It is now recognized that lichen symbiosis may also be formed by yeasts present in the thallus cortex layer (Spribille et al., 2016), endolichenized fungi (Chagnon et al., 2016) or bacteria (Grube et al., 2015); their single or cumulative effect on the biosynthesis of secondary components of this association is unknown.

The secondary metabolites in lichens are produced through three major pathways: (1) acetyl-polymalonyl, (2) mevalonic acid, and (3) shikimic acid (Boustie & Grube, 2005). They constitute a diversified chemical group, which can be divided into colourless aliphatic and acyclic compounds, as well as aromatic compounds (Elix, 2014). Their content in dry thalli usually ranges from 1 to 6% (Karunaratne et al., 2005), and occasionally can approach 30% (Ranković & Kosanić, 2015).

These compounds perform specific tasks that support adaptation and increase the lichen's chance of survival under changing environmental conditions by providing protection against biotic and abiotic factors (Huneck & Yoshimura, 1996). Therefore, it is not surprising that research into the ecological impact of lichen secondary metabolites in the natural environment includes, among many others, phenomena of allelopathy (Bialczyk et al., 2011), their antifungal properties (Halama & van Haluwin, 2004), as well as antibacterial (Ranković et al., 2012), anticancer (Liu et al., 2010) and anti-inflammatory effects on human disease (Vanga et al., 2017).

Despite the serious economic consequences caused by phytopathogenic fungi of the genus *Fusarium* (Ngaje et al., 2004) and the incontestable antifungal properties of many lichen substances, research on the impact of these substances on limiting the development of pathogenic fungi in forests and agricultural cultivations is still lacking, and there are few examples from the literature certifying the inhibitory effect of substances extracted from lichens against *Fusarium* fungi. The same substances, however, which may inhibit the growth rate of pathogenic fungi in the environment may also affect beneficial fungi, e.g., the genus *Trichoderma*, paradoxically changing resistance to the natural environment in an unexpected way. Research data on the effect of lichen substances on representatives of such soil fungi are limited. Considering the huge number of known lichen metabolites (e.g. Elix, 2014) and their possible chemical combinations in mixed extracts from the thalli of many thousands of species occurring worldwide, the challenges faced by scientists are enormous. The diversity of secondary lichen compounds present in the soil complex of a given ecosystem is conditioned by the presence of lichen species containing specific secondary metabolites. Allelopathic interactions of these compounds with fungal phytopathogens in nature constitute complex relationships between the lichen substances themselves. It can be assumed that the presence of a mixture of these compounds in the soil changes the biological activity of the soil substrate, and thus indirectly affects the quality of tree stands by regulating the dynamics of the growth rate of pathogenic species in the soil. The discovery of the biological activity of individual lichen compounds, such as usnic acid (Halama & van Haluwin, 2004; Goel & Singh, 2015) and physodic acid (Türk et al., 2006; Ranković et al., 2008, 2014), provides only an introductory image of the possibilities that may occur in the natural soil environment or after their artificial introduction as natural fungicides. More probably the microbiological activity of lichens depends on the extracted complexes of lichen substances contained in one or more species of lichen communities. Malicki (1965) and Dawson et al. (1984) pointed out the possibility of lichen compounds leaching out into the upper soil layer by rainwater, and García-Junceda & Filho (1986) as well as Zagoskina et al. (2013) found many water-extracted phenolic lichen compounds, but other researchers questioned such a soil-forming phenomenon (Stark et al., 2007). Therefore, most studies on the influence of lichen metabolites against living soil microorganisms have been focused upon the use of organic lichen extracts.

To date, antifungal properties against *Fusarium oxysporum* Schltdl. have been observed by Grujičić et al. (2014), who used compounds extracted by methanol from *Cetraria islandica* (L.) Ach. The weak activity of metabolites of *Cladonia rangiferina* (L.) Weber ex F.H. Wigg. extracted with water, ethanol and ethyl acetate was shown by Ranković & Mišić (2007) to inhibit mycelial growth of *Trichoderma harzianum* Rifai, and *Fusarium oxysporum*. *F. oxysporum* was also shown by Türk et al. (2006) to be inhibited by ethanol, chloroform and acetone extracted compounds from both chemical races of *Pseudevernia furfuracea* (L.) Zopf, namely *P. f.* var. *furfuracea* and *P. f.* var. *ceratea* (Ach.) D. Hawksw.

The aims of our work were to assess (1) the impact of dissolved secondary compounds and other extracted substances (as the sum total of interactions of substances in extracts) derived from the thalli of *Cetraria islandica*, *Cladonia mitis* Sandst., *C. rangiferina*, *Pseudevernia furfuracea* and *Usnea dasopoga* (Ach.) Nyl. as acetone and ethanol extracts on the soil pathogenic fungi *Fusarium avenaceum* (Fr.) Sacc. and *F. oxysporum*, (2) to identify possible interactions between secondary compounds coming from different lichen species on the examined fungi, and (3) to test if acetone or ethanol is a more useful solvent to extract the active substances. The aim of the experiment was also to investigate the possibility of using the obtained mixture of lichen substances in the extract as potential antifungal agents in agricultural or forestry practice. Antifungal activity of most of the tested lichen species against both these *Fusarium* fungi has never been previously researched. In essence, a search for natural fungicidal agents that inhibit the development of fungal diseases of plants has been undertaken. Undoubtedly, the undertaken experiments made it possible to confirm the antifusarial potential of the extract complexes of lichen substances, which in the future could help prevent fusarium disease and improve the sanitary condition of forest nurseries. The stimulating effect of mycelium growth described below is also the first in the literature on the

subject for the extract of mixed lichen species, which has measurable ecological consequences in the form of interaction of substances isolated from large lichen mats.

MATERIALS AND METHODS

Lichens and their secondary metabolites included in extracts

Three epigeic lichens, *C. islandica*, *C. mitis* and *C. rangiferina*, collected from a dry pine forest in Lasy Janowskie Forest (E Poland), and two epiphytes, *P. furfuracea* and *U. dasopoga*, from the Western Beskidy Mts (Polish Carpathians), have been used in the experiments. The decisive criterion for the selection of species was the presence of various lichen metabolites in their thallus based on literature sources (Smith et al., 2009; Table 1). The mixing of the thalli of two species was supposed to give an initial answer to the question: Do extracts from the mixed thalli of different species show stronger antifungal properties than the extracts of each of these species separately, i.e. is such an interaction the sum of the interactions of individual substances, or can it be the result of interactions between particular substances? The tested epiphytic species of *P. furfuracea* and *U. dasopoga* were used mainly from the point of view of using extracted-lichen substances as potential plant protection agents.

Selection of fungi for experiment, preparation of pure colonies and culture medium

Soil fungi were collected in 2013 from infected roots and shoots of tree seedlings (*Pinus sylvestris*, *Alnus glutinosa*, *Larix decidua*) grown in a forest nursery in the Kolbuszowa Forest District (Kotlina Sandomierska basin) of SE Poland. The presence of pathogens was indicated by phytopathological changes in the form of wilted leaves and needles, discoloration, and deformation of shoots. Due to the higher probability of disease symptoms in conditions of higher soil moisture, material for fungal isolation was collected in autumn.

Isolates of soil fungi were obtained from collected specimens of plants after their preparation. After washing under running water, seedlings were immersed in ethanol for at least 30 seconds, then washed twice with deionized water and placed on filter paper to dry. Thin slices of roots

Table 1. Chemical composition of lichens mentioned in the literature data (based on Smith et al., 2009) and the lichen secondary metabolites detected in acetone and ethanol (70%) extracts by using UPLC-MS method. Abbreviations: † – not detected, *m/z* – mass-to-charge ratio in a negative ion mode [M-H]; Rt – retention time

Lichen species	Chemical composition based on Smith et al. (2009)	Chemical composition based on UPLC-MS method			
		Solvent	Rt	*m/z*	Secondary metabolities
Cetraria islandica	fumarprotocetraric, protocetraric, protolichesterinic acids	acetone	†	†	†
		ethanol	5.191	471	fumarprotocetraric acid
			6.768		
Cladonia mitis	usnic, ±fumarprotocetraric, ±rangiformic acids	acetone	7.611	343	usnic acid
		ethanol	7.629	343	usnic acid
			5.191	471	fumarprotocetraric acid
Cladonia rangiferina	fumarprotocetraric acid, atranorin	acetone	5.250	471	fumarprotocetraric acid
		ethanol	4.467	373	atranorin
			4.577		
			5.183	471	fumarprotocetraric acid
Pseudevernia furfuracea	atranorin, physodic acids	acetone	†	†	†
		ethanol	6.750	469	physodic acid
Usnea dasopoga	usnic, salazanic acids	acetone	8.038	343	usnic acid
		ethanol	4.066	387	salazinic acid
			7.620	343	usnic acid

were removed from each plant with a scalpel. A single cut slice was placed with the aid of forceps onto the centre of the PDA medium in a Petri dish; these were left in a vaccination chamber with the UV light turned on. The fungal colonies were transplanted onto other Petri dishes until a homogeneous colony was formed on the medium. Repeated cultures of isolates at 22°C resulted in pure cultures of fungi, from which the more commonly occurring species, two of the more-or-less virulent plant pathogens, *F. oxysporum* and *F. avenaceum* (Wolny-Koładka, 2014), were selected for further study based on morphology of mycelium and conidia comparison with pure culture stored in Department of Agroecology of Rzeszów University, Poland. The same proportions of the medium were poured onto Petri dishes by means of automated (mixing, sterilization, maintenance of constant temperature, etc.) devices (Awel MP9 and Awel MD 320). The culture medium (39 g PDA per litre of demineralized water) was prepared automatically by the Awel MP9 device.

Extraction of secondary metabolites

Two solvents, acetone and ethanol, used in the experiment, were selected after testing the quality of extraction by several other solvents, including urine. The same experimental conditions were maintained by a 24 h extraction of slightly crushed 0.3 g (± 0.01 g) dry thalli of tested lichen specimens or their mixtures in 100 ml of solvent in closed plastic containers.

Ultraperformance Liquid Chromatography (UPLC) of alcohol extracts

UPLC was carried out to identify secondary compounds contained in the obtained alcohol extracts. Alcohol extracts (acetone and 70% ethanol) of all five lichen species were obtained by a 10-min. extraction of slightly crushed 0.3 g thalli in 10 ml of solvent. The extract was filtered with a MCE 0.45 µm filter. Then, 40 ml of distilled water was added to the filtered extract to retain the extracted compounds in the filter. The extract (10 ml extract + 40 ml of distilled water) was subjected to the solid phase extraction (SPE) method using Oasis Prime HLB Plus Light filters. Lichen compounds were washed out from the filter with 5 ml methanol, from this, 0.2 ml of each extract was diluted by adding 0.8 ml of distilled water and the UPLC method was conducted. A 0.1% aqueous solution of formic acid (eluent A) and acetonitrile (eluent B) was used as eluent. The following gradient was used: 20% B and 80% A to 100% B and 0% A in 8 min. The separations were performed at a mobile phase speed of 0.35 ml/min. and a column temperature of 50°C. The analysis time was 9.5 minutes. The molecular weight was taken from Huneck & Yoshimura (1996) to determine secondary metabolites on the basis of mass-to-charge ratio (*m/z*) obtained by mass spectrometry (UPLC-PDA-ESI-MS) in negative ions mode ionization $[M-H]^-$ (see: Tab. 1).

Supplementation of extracts, culture cultivation and measurement

The study consisted of four independent experiments, in which each of the two soil fungi tested was treated by acetone and ethanol extracted compounds of several species of lichens. Extracts were mixed to spread evenly within the medium via the Awel MP9 device; the volume of extracts was added into the agar medium before autoclaving (at 121°C for 20 min.) and was calculated so that their doses were 1 ml/Petri dish. Control test for supplementation of the 1 ml pure solvent were prepared with syringes; the solvent was added inside the medium before it was solidified.

The inoculation process began 24 h after supplementation, assuming that it was a sufficient period for the evaporation of the solvent. A 5 mm diameter inoculum was inoculated in the center of the medium with sterile forceps within the vaccination chamber. The Petri dishes were secured with parafilm to reduce the likelihood of contamination. The samples were cultured in an incubator at c. 22°C. The rate of growth of the fungus was determined every 12 h by measuring the mycelium diameter along the E-W and N-S axes using a caliper with an accuracy of 0.1 cm. Both diameters were averaged and the mean growth of the mycelium for all one-species cultures determined. All runs of mycelium measurements were performed at the same time, always in the same order of Petri dishes, in order to limit the possible influence of biological rhythms on the dynamics of mycelium growth. For the analysis, measurements were used from the beginning of the interval time in which the growth of mycelium in the control sample for the first time was detected up to the end of last

interval time preceding the moment in which the Petri dish was completely overgrown. Since growth measurements were determined every 12 h, it was impossible to determine precisely when the Petri dish was overgrown and thereby to accurately interpret the results graphically. The experiments lasted until the whole surface of medium was overgrown with pure cultures of the tested fungi in the control test without solvent. The sample size for each combination of fungal culture with lichen thallus extract was 30 replicates, while for control tests (control test with pure fungus culture and control test with solvent) was 20 replicates.

Statistical analyses

To determine the significance of the effect of lichen extracts on the growth of the soil fungi tested at specific time intervals, the statistical treatment of non-parametric test of Kruskal-Wallis analysis was applied with the STATISTICA software (version 13.1). The Average Absolute Deviation of the mean diameter of mycelium was calculated by Excel 2010 software (version 14.0.4760.1000).

RESULTS

Inhibition of *Fusarium oxysporum* culture by acetone extracted compounds

In the first 12 h of measurement, no mycelium growth was observed. Both control tests had overgrown the Petri dishes within 204 h. The combined extract of tested lichens *Cladonia mitis* + *C. rangiferina* had a smaller influence on *Fusarium oxysporum* than the extracted compounds of these species separately (Fig. 1).

The test sample of the *C. rangiferina* extract consisted of 30 replicates (from which one dish was removed because of contamination), of which fungicidal activity was observed on 17 Petri dishes (59%), and inhibitory activity on 12 (41%). Similar antifungal properties were also noted for the test sample of *C. mitis* extract; of 30 replicates, fungicidal activity occurred in 12 (40%) and inhibitory activity in 18 (60%). For 30 replicates of the test sample with mixed extracted compounds from *C. mitis* + *C. rangiferina*, only one mycelium was killed, and the mycelium growth in all other cultures was restricted.

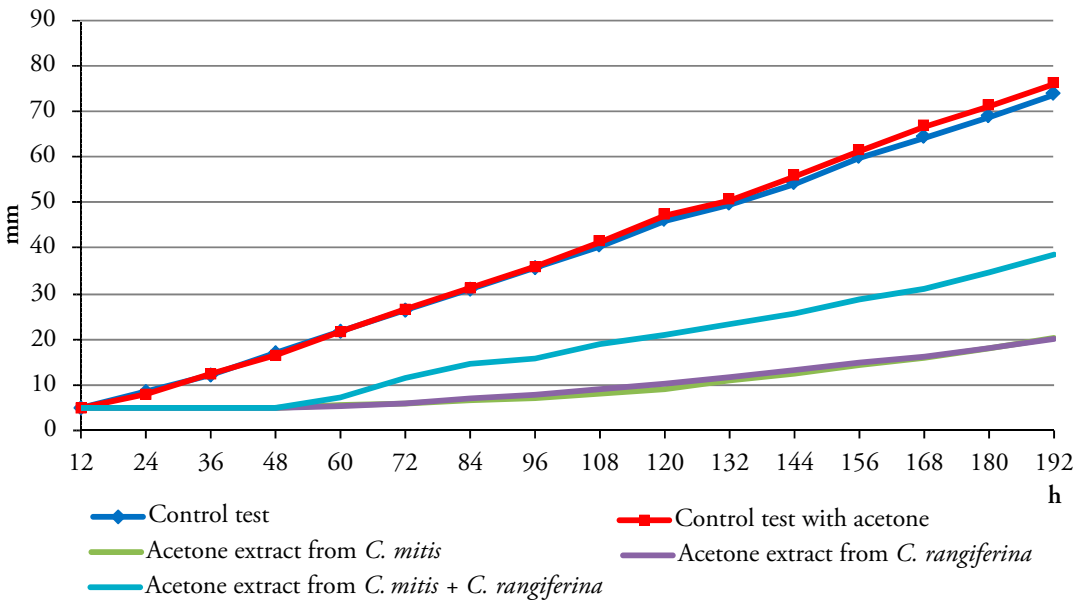

Fig. 1. Mean diameter [mm] of the *Fusarium oxysporum* mycelium over a time [h] of the *in vitro* experiment with acetone extracts of *Cladonia mitis* (N=30 replicates), *C. rangiferina* (N=29 replicates), and both species (N=30 replicates) supplemented into the culture medium (N=18 replicates for control test with acetone, and N=20 for control test without acetone).

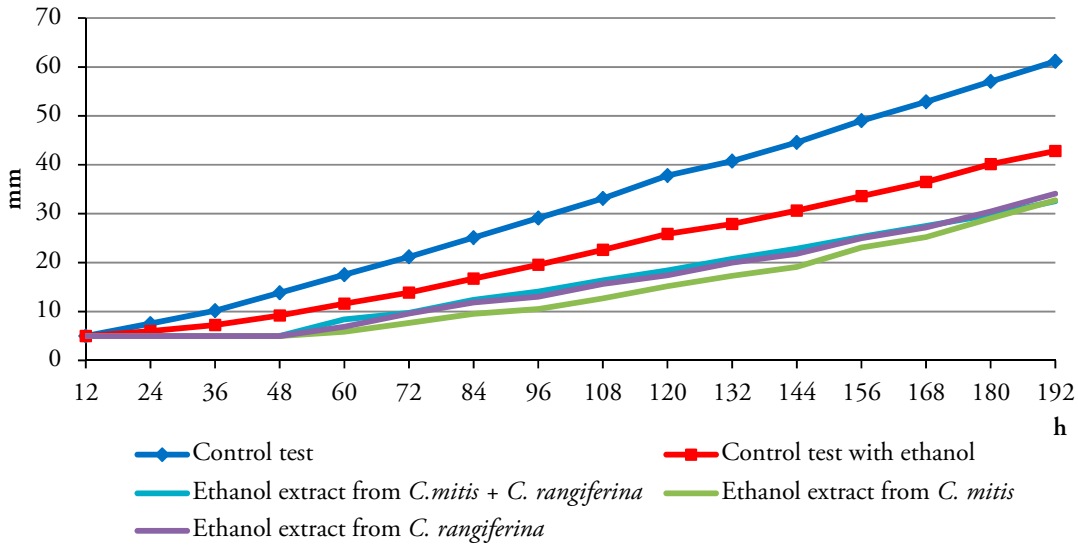

Fig. 2. Mean diameter [mm] of *Fusarium oxysporum* mycelium over a time [h] of the *in vitro* experiment with ethanol extracts of *Cladonia mitis*, *C. rangiferina*, and both *Cladonia* species supplemented into the culture medium (N=30 replicates for each tested ethanol extract; N=20 replicates for both control tests).

In general, both types of control tests did not show significant differences in the growth of the *F. oxysporum* mycelium, while the differences between them and samples with acetone extracts of substances derived from *C. mitis* and *C. rangiferina* separately and the extract from a mixture of both *Cladonia* species thalli were statistically significant (Appendix 1). A weaker degree of inhibition was recorded in the sample test with extract from the two mixed lichen species. As shown in the UPLC analyses (Table 1), usnic acid was probably mainly responsible for these results in the case of extract from *C. mitis* and fumarprotocetraric acid in the case of *C. rangiferina*.

Inhibition of *Fusarium oxysporum* culture by ethanol extracted compounds

The experiment with the ethanol extracted compounds was carried out with the same combination of test samples and control tests as for acetone extracts. The presence of ethanol in the control sample reduced the growth rate of the fungus (Fig. 2), but this relationship to the control test (pure culture) was not significant (Appendix 2). Statistically significant differences throughout the entire measurement period (from 24 h onwards) were recorded between both control tests and samples influenced by the two-species combined extract, as well as both single-species extracts (Appendix 2).

The control test with ethanol consisted of 20 replicates showing 100% fungal inhibition; no fungicidal activity was demonstrated. Over time, substances extracted from *C. mitis* inhibited mycelial growth of this pathogen most severely (i.e. fungicidal effect not observed) (Fig. 2); in contrast, 10% of replicates with extract from *C. rangiferina* showed a fungicidal effect. A combined extract from *C. mitis* + *C. rangiferina* killed fungal isolates in 5 replicates (17%) and inhibition of the mycelium was noted on 25 plates (83%).

Inhibition of *Fusarium avenaceum* culture by acetone extracted compounds

For the experiment involving acetone extracted compounds against *F. avenaceum*, *C. mitis* and *C. rangiferina* were used. Substances from both *Cladonia* species strongly inhibited the growth of *F. avenaceum* (Fig. 3). The difference in the mean mycelial diameter after 180 h between the control test and the sample supplemented with extract from *C. rangiferina* was c. 62 mm, compared with c. 68 mm for the sample with extract obtained from *C. mitis*. Statistically significant differences in the rate of growth between the

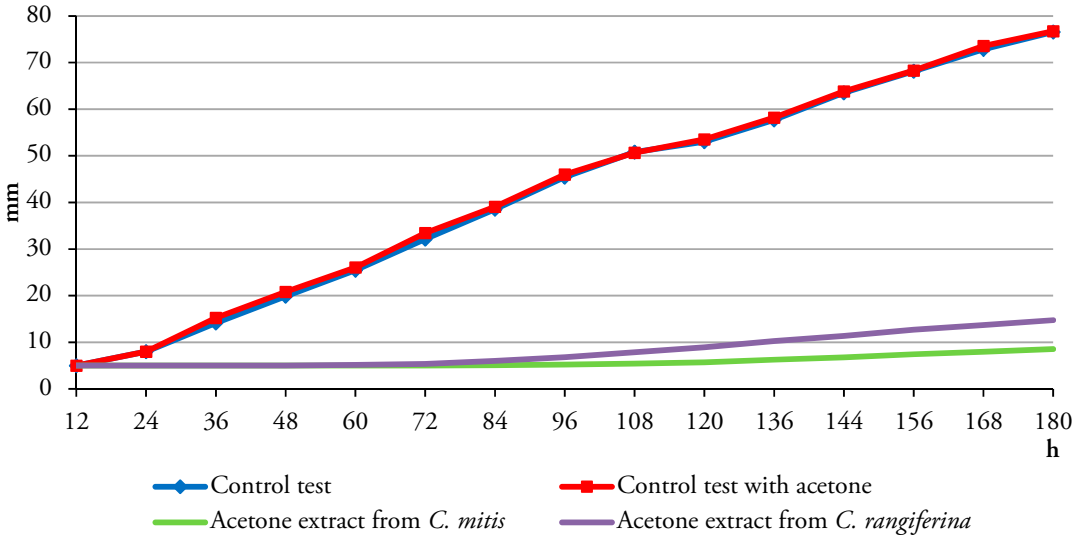

Fig. 3. Mean diameter [mm] of *Fusarium avenaceum* mycelium over a time [h] of the *in vitro* experiment with acetone extracts of *Cladonia mitis* and *C. rangiferina* supplemented into the culture medium (N=30 replicates for both tested acetone extracts; N=20 replicates for both control tests).

two control tests and both test samples involving *Cladonia* species were noted throughout the experiment (from 24 h onwards; Appendix 3). For the cultures influenced by extract from *C. rangiferina*, fungicidal activity of lichen compounds occurred in 14 (47%) and fungal inhibition in 16 (53%) cases, while for sample with the *C. mitis* extract, fungicidal activity was found in 21 (70%) and inhibition in 9 (30%) replicates; in both cases 30 biological replicates were considered.

Inhibition of *Fusarium avenaceum* culture by ethanol extracted compounds

Four different ethanol extracts derived from mixed thalli of (1) *U. dasopoga* + *P. furfuracea*, (2) *C. islandica* + *P. furfuracea*, (3) *C. mitis* + *C. rangiferina*, and (4) *C. islandica* + *P. furfuracea* + *U. dasopoga* were tested (Fig. 4).

The control test with ethanol showed an inhibition of the fungus in all 20 replicates. The same phenomenon for 30 replicates was noted for all test samples with extracted compounds from mixed thalli of the lichen species except in the case of an extract from the thalli of *C. mitis* + *C. rangiferina*, where fungicidal activity was obtained in 14 (47%), and inhibition in 16 (53%) replicates.

Statistically significant differences between the mean mycelial diameter of the control test and the samples subjected to ethanol extracts were found almost throughout all the measurements (Appendix 4). Only the effect of the extract derived from mixed *C. islandica* + *P. furfuracea* thalli, despite its visible inhibition, was not supported statistically for almost the entire experiment. The growth rate of the control tests with ethanol showed statistically significant differences over time from the tested combinations with the mixed extracts, except for the case of *U. dasopoga* + *P. furfuracea* and partly for *C. islandica* + *P. furfuracea* + *U. dasopoga*. The strongest growth restriction of this fungal species found for the *C. mitis* + *C. rangiferina* extract reflects its statistical significance compared to other tested cultures influenced by ethanol lichen extracts (Appendix 4). As demonstrated by UPLC analyses (Table 1), this result was affected by the presence of usnic acid and fumarprotocetraric acid (originated from both lichen species) and atranorin.

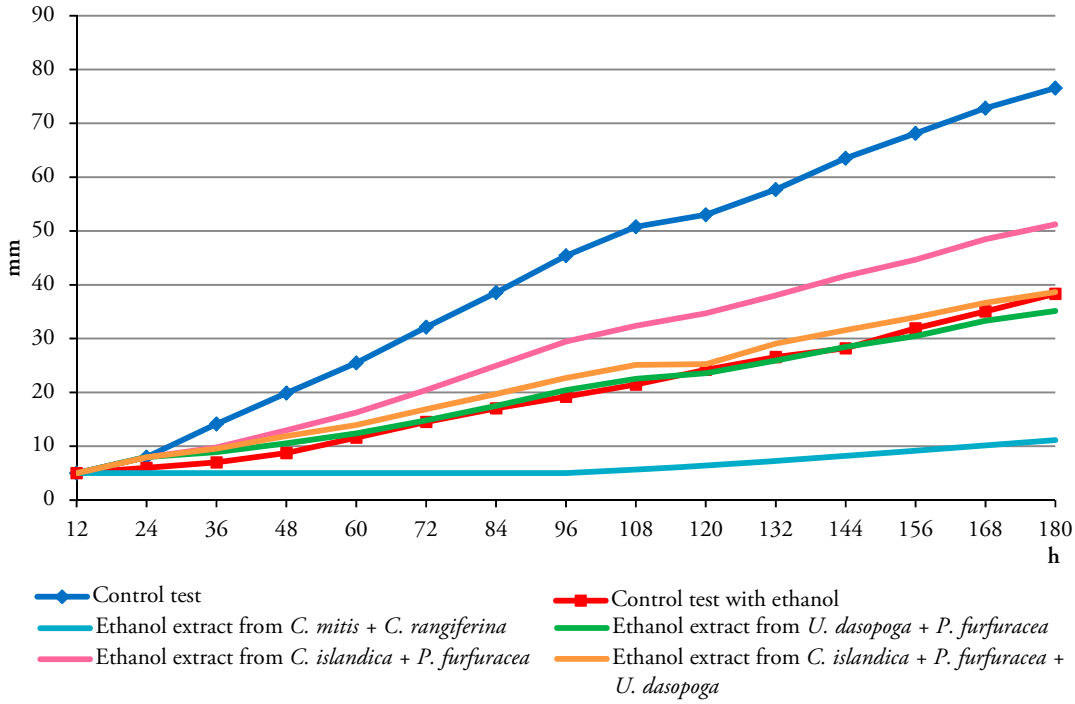

Fig. 4. Mean diameter [mm] of *Fusarium avenaceum* mycelium over a time [h] of the *in vitro* experiment with ethanol extracts of combined mixed thalli of *Cladonia mitis* + *C. rangiferina*; *Cetraria islandica* + *Pseudevernia furfuracea*; *Usnea dasopoga* + *P. furfuracea*; *C. islandica* + *P. furfuracea* + *U. dasopoga* supplemented into the culture medium (N=30 replicates for each tested ethanol extracts; N=20 replicates for both control tests).

DISCUSSION

General effects on tested *Fusarium* fungi

Analysis of the results show that lichen metabolites are biologically active substances which can inhibit the development of pathogenic fungi of the genus *Fusarium*. A high percentage of samples in these experiments showed fungicidal properties of tested lichen compounds (Figs 1–4). In the remaining cases, isolates of soil pathogens, neutralized the toxic effects of metabolites. Perhaps after conducting experiments over longer periods of time, it is possible that there will be no differences in the mycelial growth of control tests and samples with extracts. However, the resistance against lichen substances depends on the fungal species (Table 2).

Our experiments showed that one-species acetone extracts from lichens, *Cladonia mitis* and *C. rangiferina* strongly inhibited the mycelium growth of both *Fusarium* species (see Figs 1 and 2; Table 2). As showed UPLC analyses, both usnic and fumarprotocetraric acids, and atranorin would be mainly responsible for these effects (Table 1).

The difference in the diameter of the fungal colony between the two control tests indicate that ethanol inhibits the fungus, contrary to acetone, which showed a low or almost no inhibitory effect (compare Figs 1 and 2).

Fusarium oxysporum

Fusarium oxysporum showed a higher sensitivity in the case of ethanol (Fig. 2) *vs* acetone (Fig. 1) extract derived from a mixture of *C. mitis* + *C. rangiferina*, and for one-species extracts, the effect of acetone-extracted compounds was approximately twice stronger as for ethanol extracts. The comparison of the mycelium growth inhibition level with the chemical profile of the extracts (Table 1) indicate that acetone extracts are stronger despite of less number of active

Table 2. The effect of tested lichen extracts against two *Fusarium* species after 84 h and 180 h of experiment. The lichen inhibition potential is expressed as a percentage of the mean diameter of the mycelium in the control test after 84 h and 180 h.

Soil fungus	*Fusarium oxysporum*				*Fusarium avenaceum*			
Solvent	acetone		ethanol		acetone		ethanol	
Time [h]	84	180	84	180	84	180	84	180
Control test [%]	100	100	100	100	100	100	100	100
Control test with solvent [%]	101,9	93,1	53,3	58,2	101,4	100,2	52,5	59,5
C. mitis	8,6	23,3	26,3	42,1	1,6	6,6		
C. rangiferina	10,3	21,4	34,3	43,3	6,1	16,2		
C. mitis + *C. rangiferina*	45,5	50,3	37,3	41,8			0,0	13,7
U. dasopoga + *P. furfuracea*							54,0	54,7
C. islandica + *P. furfuracea*							77,5	79,5
C. islandica + *P. furfuracea* + *U. dasopoga*							60,8	59,9

secondary metabolites. Perhaps, this phenomenon may play some role in the high antifungal potential due to no chemical antagonistic interactions between extracted compounds that reduce the inhibitory potential. This is more visible by comparing effects of extracts derived from individual *Cladonia* species with extract from their mixed thalli. This issue requires further studies.

Fusarium avenaceum

This experiment repeated the high efficacy of acetone extracts from the mixture of *C. mitis* + *C. rangiferina* against *F. avenaceum* (Fig. 3). Mycelium was highly susceptible to the fungicidal potential of the tested extracts, which ultimately resulted in a high average level of its growth inhibition for test samples with *C. mitis* and *C. rangiferina*.

In the last of the conducted experiments, the ethanol solvent reduced the *F. avenaceum* growth by almost 50% (Fig. 4) but independently the extract from mixed *C. mitis* + *C. rangiferina* thalli strongly inhibited fungal growth, similarly to the case of *F. oxysporum* (compare Figs 2 and 4); the mean dimensional difference between this sample and the control test is c. 65 mm, and compared to the control test with ethanol it is c. 27 mm. The mixture of substances in the remaining ethanol extracts turned out to be practically ineffective in terms of inhibition, which seems to be related to various possible interactions between substances, which is also of ecological importance. The lack of analogous other alcohol and non-alcohol extracts from mixture of these species makes it impossible to compare their effectiveness against this fungal pathogen. Experiments in this area have yet to be undertaken.

Interactions of lichen compounds

The results obtained by using ethanol extracts from mixed thalli of lichen species do not explain in a direct way if synergism, antagonism or a cumulative effect occurs between extracted secondary compounds of lichens. The results obtained by the mixed ethanol extracted compounds from *C. islandica* + *P. furfuracea* against *F. avenaceum* were surprising; the growth rate of the fungus accelerated compared to the control test with ethanol (Fig. 4). It is possible that the reason for this is an antagonism of extracted secondary metabolites or a hormesis effect, previously found by Henningsson & Lundström (1970) and Brown & Mikola (1974) for certain species of macrofungi. The role of ethanol as a solvent for these substances should also be important. The ethanol extract from combined *C. islandica* + *P. furfuracea* + *U. dasopoga* thalli provided similar results to the control test with ethanol (Fig. 4), which may indicate neutralization of the previously observed phenomenon of stimulation under the influence of the greater antifungal potential of secondary metabolites derived from *U. dasopoga* (at least usnic acid). Furthermore, both visible effects were caused by a lesser number of metabolites included in

alcohol-extracts in our study compared to literature data (Table 1) concerning the presence of secondary metabolites in their thallus (Smith et al., 2009; except for *C. rangiferina* and *U. dasopoga* in ethanol extract).

Although the discussed issue cannot be unequivocally resolved, the comparison of the potential of extracts of mixed and single lichen species indicates indirect interactions. It seems that the more complex the extract is, the lower its inhibitory potential (see Figs 1 & 2), but the growth dynamics of *F. avenaceum* emphasizes that this aspect is more complicated (see Fig. 4).

It is not known how the high temperature of the sterilization process impacts on the chemical structure of lichen substances; possibly this factor may also affect the activity of secondary metabolites of lichens and thereby influence the growth of fungi. Regardless of this, the antifungal potential of the isolated lichen substances in the research methodology adopted turned out to be high, and even showed the opposite effect – stimulation of mycelium growth.

Composition of secondary metabolites

Most of the main compounds mentioned in the literature data for the examined lichen species have been detected in extracts used for the fungal inhibition experiments by using UPLC, but ethanol was a better solvent than acetone for the extraction of less concentrated accompanying substances (Table 1).

Despite additional substances being detected in thalli of both species by others (see Smith et al., 2009; Wirth et al., 2013), our UPLC analyses did not clearly show the presence of rangiformic and fumarprotocetraric acids in tested acetone extract of *C. mitis*, as well as atranorin in *C. rangiferina*. Perhaps, the concentrations of these substances were very low, so only those detected in a sufficiently high concentration can be considered effective inhibitors. Similarly, some acetone-soluble compounds which were referred in the literature were not found by Millot et al. (2017) in many lichen species, including those which were used in our studies, i.e. *C. rangiferina* and *C. islandica*.

The issue of identifying secondary metabolites based on the retention time value is problematic. In the case of ethanol-extracted metabolites from *C. islandica* and *C. rangiferina*, the identification of fumarprotocetraric acid for the former and atranorin for the latter is ambiguous. Due to the successful identification of fumarprotocetraric acid in the remaining tested ethanol extracts, it can be considered a compound with a retention time of c. Rt = 5.2. In the case of atranorin, this issue remains unresolved (see Table 1). The use of UPLC-MS for the separation and identification of lichen substances found in the available literature is a negligible part, as for the purposes of this study, so the presented results remain impossible to compare (e.g., HPLC, TLC; Millot et al., 2017; Voicu et al., 2019).

Ecological and practical issues

The observations related to antifungal effects of *Cladonia*-based extracts against both *Fusarium* species is clearly beneficial from the plant protection point of view. However, the obtained result does not show how the tested extracts would behave in the field and to what extent it would be possible to maintain the fungicidal effectiveness under the influence of biotic and abiotic factors. The obtained results confirm the premise of the antifungal potential of the substances present in the tested extracts, although the measured stimulating effect indicates that the potential of the lichen substances is more refined. Hence, there is a need to repeat the experiments using an extended methodology, e.g., with a greater range of the tested extract dose.

Due to the method of measuring mycelium growth (PFT) adopted in the experiments and the generally different research methodology used by other researchers, the available literature results for the tested fungal species (Türk et al., 2006; Ranković & Mišić, 2007; Grujičić et al., 2014) remain incomparable. For this reason, the results described herewith represent a further and extended step to determine the antifungal potential of the tested lichen extracts.

In the light of using measured mycelium growth (Fig. 4), an additional possibility is the use of lichen extracted substances as a source of carbon compounds for fungal isolates (Stark & Hyvärinen, 2003). On the other hand, degradation of these substances may occur under the influence of the microbial activity of certain soil organisms, including fungi, as demonstrated by Bandoni & Towers (1967) against usnic acid, although such an ability of species of the genus *Fusarium* has not been tested by them.

Moreover, Begora & Fahselt (2001) indicate that the high intensity of UV-A/B radiation may lead to the degradation of atranorin (and probably fumarprotocetraric acid) isolated from lichens, for instance from the species *C. rangiferina* tested herewith. Furthermore, extracts containing usnic acid and other secondary metabolites may underwent chemical degradation, and the usnic acid itself was degraded into compounds with a lower molecular weight. Interestingly, usnic acid seems to be an obligatory component of lichens (Burkin et al., 2013), and its minimum concentrations, even extracted by rainfall, may be important for the regulation of the dynamics of mycelium in soil species, especially in large-scale and multi-species lichen mats. In this context, it seems that the inhibitory potential of secondary lichen metabolites will depend on its relatively higher concentration in the soil, but even meeting this assumption may turn out to be insufficient to inhibit the growth of mycelium in soil. In addition, degradation of lichen substances by biotic and abiotic factors in nature may lead, contrary to intuition, to a concentration that induces a threshold effect on the mycelium of a given species, as noted by Rice (1987), paradoxically leading to stimulation effects rather than inhibition of growth dynamics of mycelium in the soil, which requires in-depth experiments.

CONCLUSIONS

None of the study extracts completely inhibits the growth of the tested fungi, but the short-term effectiveness of growth inhibition can be very high, limiting their natural growth potential to 50%; in a significant number of replicates, fungicidal activity of some extracts was also recorded.

The hypothesis regarding the possibility of the mixture-extracted compounds including secondary lichen metabolites affecting soil fungi in the natural environment would appear to be true, although the experiments do not answer the question of how the mixture of substances are extracted/eluted in nature.

The inhibition of *Fusarium* species cultures by extracts from various lichen species depends among others on the solvent used. Not only because of the different effects of acetone and ethanol on fungal *in vitro* cultures, but also perhaps due to obtaining different concentrations of the extracted complex of substances, its particular chemical profile and possible interactions between lichen compounds.

Acetone in a dose of 1 ml per Petri dish did not limit, or only slightly limited, the growth of the mycelium of the *Fusarium* species tested, which indicates its usefulness as a solvent for antifungal lichen compounds in this type of experiment.

The results of fungal growth inhibition by lichen metabolites extracted with ethanol can be masked by the inhibitory effect of the solvent itself. However, comparison of mycelium diameter in a test sample with a control test with solvent should alleviate this.

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Appendix 1

The comparison of a mean diameter of *Fusarium oxysporum* mycelium in cultures supplemented with tested acetone lichen extracts in the following measuring hours. There are no significant statistical differences (α = 0.05) between the mycelium growth of the control test and control test with acetone (blue colour) and between the samples influenced by extracts from *C. mitis* and *C. rangiferina*. The significant differences (red colour) are for all measure times from 24 h onwards between mycelium growth of both control tests (control test and control test with acetone) and mycelium growth of samples influenced by all tested acetone extracts and from 72 h onwards between the growth rate of samples influenced by extracts from *C. mitis* and mixed *Cladonia* species (combined extract) and between the samples of extracts from *C. rangiferina* and mixed *Cladonia* species, except 180 h and 192 h.

	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h	120 h	132 h	144 h	156 h	168 h	180 h	192 h
Control test	5	8.6	12.3	17.2	21.9	26.6	31.2	35.9	40.6	46.2	49.8	54.3	60.1	64.5	69.1	74
	±0	±0.43	±0.95	±1.22	±1.6	±2.15	±1.84	±1.64	±2.05	±2.27	±2.45	±2.98	±2.87	±2.75	±3.11	±1.88
	↑0	↑3.6	↑4	↑4.9	↑4.7	↑4.7	↑4.6	↑4.7	↑4.7	↑5.6	↑3.6	↑4.5	↑5.8	↑4.4	↑4.6	↑4.9
Control test with acetone	5	8	12.5	16.6	21.8	26.8	31.5	36.1	41.5	47.4	50.7	56	61.6	67	71.4	76.4
	±0	±0.48	±0.92	±1.04	±1.61	±1.97	±2.2	±2.07	±2	±2.32	±2.84	±2.7	±3.44	±2.81	±3.18	±2.79
	↑0	↑3	↑4.5	↑4.1	↑5.2	↑5	↑4.7	↑4.6	↑5.4	↑5.9	↑3.3	↑5.3	↑5.6	↑5.4	↑4.4	↑5
Acetone extract of *C. mitis*	5	5	5	5	5.6	6[a]	6.7[a]	7.2[a]	8.1[a]	9.1[a]	11[a]	12.6[a]	14.5[a]	16.1[a]	18.1[a]	20.4[a]
	±0	±0	±0	±0	±0.99	±1.55	±2.69	±3.44	±4.21	±4.47	±5.97	±6.92	±8.28	±9.36	±10.88	±12.54
	↑0	↑0	↑0	↑0	↑0.6	↑0.4	↑0.7	↑0.5	↑0.9	↑1	↑1.9	↑1.6	↑1.9	↑1.6	↑2	↑2.3
Acetone extract of *C. rangiferina*	5	5	5	5	5.4	6[b]	7.1[b]	7.9[b]	9.1[b]	10.3[b]	11.8[b]	13.3[b]	15[b]	16.3[b]	18.2	20.2
	±0	±0	±0	±0	±1	±1.59	±3.27	±4.26	±5.92	±7.21	±8.66	±10.08	±11.79	±13.26	±15.46	±17.85
	↑0	↑0	↑0	↑0	↑0.4	↑0.6	↑1.1	↑0.8	↑1.2	↑2.2	↑1.5	↑1.5	↑1.7	↑1.3	↑1.9	↑2
Acetone extract of mixed *C. mitis* + *C. rangiferina*	5	5	5	5	7.3	11.6[a,b]	14.7[a,b]	15.9[a,b]	19.1[a,b]	21.1[a,b]	23.5[a,b]	25.8[a,b]	28.9[a,b]	31.3[a,b]	34.9[a]	38.8[a]
	±0	±0	±0	±0	±1.07	±3.21	±3.61	±3.9	±4.15	±4.43	±4.45	±4.65	±4.73	±4.98	±5.15	±5.45
	↑0	↑0	↑0	↑0	↑2.3	↑4.3	↑3.1	↑1.2	↑3.2	↑2	↑2.4	↑2.3	↑3.1	↑2.4	↑3.6	↑3.9

[a] and [b] show significant differences between mycelium growth in cultures influenced by particular acetone extracts

±Average Absolute Deviation [mm]

↑ mean increment of mycelium diameter between two consecutive measurement hours [mm]

Appendix 2

The comparison of a mean diameter of *Fusarium oxysporum* mycelium in cultures supplemented with tested ethanol lichen extracts in the following measuring hours. There are no significant statistical differences (α = 0.05) between the mycelium growth of the control test and control test with ethanol (blue colour), between the sample influenced by extract from *C. rangiferina* and sample with extract from mixed both *Cladonia* species, between the samples with extracts from *C. mitis* and *C. rangiferina* and between the sample with extract from *C. mitis* and sample influenced by two-species extract, except results for 60 h. The significant differences (red colour) are for all measure times from 24 h onwards between mycelium growth of both control tests (control test and control test with ethanol) and samples influenced by extracts from *C. mitis*, from *C. rangiferina* and from mixed both *Cladonia* species.

	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h	120 h	132 h	144 h	156 h	168 h	180 h	192 h
Control test	5	7.5	10.2	13.8	17.5	21.2	25.1	29.1	33.1	37.8	40.8	44.6	49	52.9	57	61.2
	±0	±1.27	±2.39	±3.82	±4.96	±6.1	±6.98	±7.9	±8.62	±9.81	±10.67	±11.47	±12.87	±13.65	±13.93	±15.04
	↑0	↑2.5	↑2.7	↑3.6	↑3.7	↑3.7	↑3.9	↑4	↑4	↑4.7	↑3	↑3.8	↑4.4	↑3.9	↑4.1	↑4.2
Control test with ethanol	5	6	7.3	9.2	11.6	13.9	16.7	19.6	22.6	25.9	27.9	30.6	33.6	36.5	40.2	42.8
	±0	±0	±0.33	±0.41	±0.66	±0.75	±0.8	±0.93	±1.13	±1.15	±1.25	±1.38	±1.36	±1.45	±1.93	±1.93
	↑0	↑1	↑1.3	↑1.9	↑2.4	↑2.3	↑2.8	↑2.9	↑3	↑3.3	↑2	↑2.7	↑3	↑2.9	↑3.7	↑2.6
Ethanol extract of *C. mitis*	5	5	5	5	5.9[a]	7.7	9.6	10.5	12.7	15.2	17.3	19.1	23.1	25.2	29.1	32.8
	±0	±0	±0	±0	±0.75	±2.11	±2.91	±3.08	±3.65	±4.03	±3.99	±4.07	±3.82	±3.78	±3.71	±3.72
	↑0	↑0	↑0	↑0	↑0.9	↑1.8	↑1.9	↑0.9	↑2.2	↑2.5	↑2.1	↑1.8	↑2	↑2.1	↑3.9	↑3.7
Ethanol extract of *C. rangiferina*	5	5	5	5	6.9	9.6	11.9	13	15.6	17.4	20	21.8	25	27.2	30.5	34.1
	±0	±0	±0	±0	±1.32	±2.89	±3.79	±4.06	±4.72	±5.11	±5.56	±5.67	±6.28	±6.3	±6.88	±7.54
	↑0	↑0	↑0	↑0	↑1.9	↑3	↑2.3	↑2.1	↑2.6	↑1.8	↑2.6	↑1.8	↑3.2	↑2.2	↑3.3	↑3.6
Ethanol extract of mixed *C. mitis* + *C. rangiferina*	5	5	5	5	8.4[a]	9.7	12.4	14.1	16.4	18.4	20.8	22.9	25.3	27.5	29.8	32.6
	±0	±0	±0	±0	±2.31	±2.87	±3.96	±4.49	±5.14	±5.68	±6.27	±6.79	±7.55	±8.14	±8.78	±9.59
	↑0	↑0	↑0	↑0	↑3.4	↑1.3	↑2.7	↑1.7	↑2.3	↑2	↑2.4	↑2.1	↑2.4	↑2.2	↑2.4	↑2.8

[a] shows significant differences between mycelium growth in cultures influenced by particular ethanol extracts

±Average Absolute Deviation [mm]

↑ mean increment of mycelium diameter between two consecutive measurement hours [mm]

Appendix 3

The comparison of a mean diameter of *Fusarium avenaceum* mycelium in cultures supplemented with tested acetone lichen extracts in the following measuring hours. There are no significant statistical differences (α = 0.05) between the control test and control test with acetone (blue colour) and between the samples influenced by extracts from *C. mitis* and *C. rangiferina*. The significant differences (red colour) are for all measure times from 24 h onwards between mycelium growth of both control tests (control test and control test with acetone) and samples influenced by acetone extract from *C. mitis* as well as extract from *C. rangiferina* thalli.

	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h	120 h	132 h	144 h	156 h	168 h	180 h
Control test	5	8	14.2	19.9	25.5	32.1	38.6	45.4	50.8	53	57.7	63.5	68.2	72.8	76.6
	±0	±0	±1.49	±1.88	±1.84	±2.08	±1.94	±1.9	±1.52	±1.72	±2.34	±1.85	±2.14	±2.13	±1.8
	↑0	↑3	↑6.2	↑6.7	↑5.6	↑6.6	↑6.5	↑6.8	↑5.4	↑2.2	↑4.7	↑5.8	↑4.7	↑4.6	↑3.8
Control test with acetone	5	8	15.3	20.8	26.1	33.4	39.1	46	50.7	53.5	58.2	63.8	68.3	73.6	76.7
	±0	±0	±1.28	±0.93	±0.66	±0.73	±1.07	±0.7	±1.02	±1.22	±1.42	±1.1	±1.47	±1.74	±1.3
	↑0	↑3	↑7.3	↑5.5	↑5.3	↑7.3	↑5.7	↑6.9	↑4.7	↑2.8	↑4.7	↑5.6	↑4.5	↑5.3	↑4.1
Acetone extract of *C. mitis*	5	5	5	5	5	5	5.1	5.3	5.4	5.7	6.3	6.8	7.4	8	8.6
	±0	±0	±0	±0	±0	±0	±0.18	±0.45	±0.78	±1.19	±1.89	±2.59	±3.44	±4.15	±4.97
	↑0	↑0	↑0	↑0	↑0	↑0	↑0.1	↑0.2	↑0.1	↑0.3	↑0.6	↑0.5	↑0.6	↑0.6	↑0.6
Acetone extract of *C. rangiferina*	5	5	5	5	5.2	5.4	6	6.8	7.9	9	10.3	11.4	12.7	13.7	14.8
	±0	±0	±0	±0	±0.3	±0.64	±1.49	±2.4	±3.35	±4.31	±5.64	±6.81	±8.08	±8.96	±10
	↑0	↑0	↑0	↑0	↑0.2	↑0.2	↑0.6	↑0.8	↑1.1	↑1.1	↑1.3	↑1.1	↑1.3	↑1	↑1.1

±Average Absolute Deviation [mm]

↑ mean increment of mycelium diameter between two consecutive measurement hours [mm]

Appendix 4

The comparison of a mean diameter of *Fusarium avenaceum* mycelium in cultures supplemented with tested ethanol lichen extracts in the following measuring hours. There are no significant statistical differences (α = 0.05) between the control test and sample influenced by extract from mixed *C. islandica* + *P. furfuracea* (except 36 h and 48 h), and between the control test with ethanol and sample influenced by extract from mixed *U. dasopoga* + *P. furfuracea* (except 24 h and 36 h). The significant differences (red colour; α = 0.05) occurred for the mycelium growth in (1) the control test and: (i) control test with ethanol, and samples (ii) with extract from mixed *C. mitis* + *C. rangiferina* (from 24 h onwards); (iii) with extract from mixed *U. dasopoga* + *P. furfuracea*, and (iv) with extract from mixed *C. islandica* + *P. furfuracea* + *U. dasopoga* (from 36 h onwards); (2) control test with ethanol and samples: (i) with extract from mixed *C. mitis* + *C. rangiferina* (for 72, 120–180 h), (ii) with extract from mixed *C. islandica* + *P. furfuracea* (from 24 h onwards); (iii) with extract from mixed *C. islandica* + *P. furfuracea* + *U. dasopoga* (for 24-108 h); (3) sample with extract from mixed *C. mitis* + *C. rangiferina* and samples: (i) with extract from mixed *U. dasopoga* + *P. furfuracea* (for 24–168 h); (ii) with extract from mixed *C. islandica* + *P. furfuracea* (from 24 h onwards); (iii) with extract from mixed *C. islandica* + *P. furfuracea* + *U. dasopoga* (from 24 h onwards); (4) sample with extract from mixed *U. dasopoga* + *P. furfuracea* and samples: (i) with extract from mixed *C. islandica* + *P. furfuracea* (from 60 h onwards), and (ii) with extract from mixed *C. islandica* + *P. furfuracea* + *U. dasopoga* (for 72, 132, 156–168 h); (5) sample with extract from mixed *C. islandica* + *P. furfuracea* and sample with extract from mixed *C. islandica* + *P. furfuracea* + *U. dasopoga* (for 120, 156–180 h).

	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h	120 h	132 h	144 h	156 h	168 h	180 h
Control test	5	8	14.2	19.9	25.5	32.1	38.6	45.4	50.8	53	57.7	63.5	68.2	72.8	76.6
	±0	±0	±1.49	±1.88	±1.84	±2.08	±1.94	±1.9	±1.52	±1.78	±2.34	±1.85	±2.14	±2.13	±1.8
	↑0	↑3	↑6.2	↑5.7	↑5.6	↑6.6	↑6.5	↑6.8	↑5.4	↑2.7	↑4.7	↑5.7	↑4.7	↑4.4	↑3.8
Control test with ethanol	5	6	7	8.75	11.6	14.53	17.05	19.2	21.48	24.23	26.58	28.2	31.9	35.05	38.33
	±0	±0	±0	±0.33	±0.36	±0.93	±0.95	±0.75	±0.73	±0.83	±0.64	±0.87	±0.82	±0.64	±0.44
	↑0	↑1	↑1	↑1.75	↑2.85	↑2.93	↑2.52	↑2.15	↑2.28	↑2.75	↑2.35	↑1.62	↑3.7	↑3.15	↑3.28
Ethanol extract of mixed *C. mitis* + *C. rangiferina*	5	$5^{a,b,c}$	$5^{a,b,c}$	$5^{a,b,c}$	$5^{a,b,c}$	$5^{a,b,c}$	$5^{a,b,c}$	$5^{a,b,c}$	$5.7^{a,b,c}$	$6.4^{a,b,c}$	$7.3^{a,b,c}$	$8.2^{a,b,c}$	$9.2^{a,b,c}$	$10.2^{a,b,c}$	$11.2^{b,c}$
	±0	±0	±0	±0	±0	±0	±0	±0	±0.84	±1.71	±2.45	±3.22	±4	±4.82	±5.74
	↑0	↑0	↑0	↑0	↑0	↑0	↑0	↑0	↑0.7	↑0.7	↑0.9	↑0.9	↑1	↑1	↑1
Ethanol extract of mixed *U. dasopoga* + *P. furfuracea*	5	8^{a}	8.9^{a}	10.6^{a}	$12.4^{a,d}$	$14.8^{a,d,e}$	$17.5^{a,d}$	$20.4^{a,d}$	$22.6^{a,d}$	$23.6^{a,d}$	$25.9^{a,d,e}$	$28.5^{a,d}$	$30.5^{a,d,e}$	$33.3^{a,d,e}$	35.2^{d}
	±0	±0	±0.48	±0.5	±0.62	±0.7	±0.69	±0.91	±0.93	±1.07	±0.88	±1.17	±1.24	±1.29	±1.21
	↑0	↑3	↑0.9	↑1.7	↑1.8	↑2.4	↑2.7	↑2.9	↑2.2	↑1	↑2.3	↑2.6	↑2	↑2.8	↑1.9
Ethanol extract of mixed *C. islandica* + *P. furfuracea*	5	7.9^{b}	9.8^{b}	13^{b}	$16.3^{b,d}$	$20.5^{b,d}$	$25^{b,d}$	$29.5^{b,d}$	$32.4^{b,d}$	$34.7^{b,d,f}$	$38^{b,d}$	$41.7^{b,d}$	$44.7^{b,d,f}$	$48.5^{b,d,f}$	$51.2^{b,d,f}$
	±0	±0.13	±1.06	±1.91	±2.01	±2.07	±2.01	±2.3	±2.43	±2.35	±2.46	±2.54	±2.93	±3.21	±3.6
	↑0	↑2.9	↑1.9	↑3.2	↑3.3	↑3.8	↑4.5	↑4.5	↑2.9	↑2.3	↑3.3	↑3.7	↑3	↑3.8	↑2.7
Ethanol extract of mixed *C. islandica* + *P. furfuracea* + *U. dasopoga*	5	7.9^{c}	9.6^{c}	11.9^{c}	14^{c}	$16.9^{c,e}$	19.7^{c}	22.7^{c}	25.1^{c}	$25.3^{c,f}$	$29.1^{c,e}$	31.6^{c}	$34^{c,e,f}$	$36.7^{c,e,f}$	$38.6^{c,f}$
	±0	±0.13	±0.46	±0.77	±0.79	±0.85	±1.07	±1.1	±1.37	±1.49	±1.66	±1.57	±1.24	±1.71	±1.7
	↑0	↑2.9	↑1.7	↑2.3	↑2.1	↑2.9	↑2.8	↑3	↑2.4	↑0.2	↑3.8	↑3.5	↑2.4	↑2.7	↑1.9

[a], [b], [c], [d], [e] and [f] show significant differences between mycelium growth in cultures influenced by particular ethanol extracts

±Average Absolute Deviation [mm]

↑ mean increment of mycelium diameter between two consecutive measurement hours [mm]