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Effect of lichen substances complex on selected soil fungi

Summary

The main objective of the experiments was to determine the effect of a rainwater-extracted complex of lichen substances on the growth dynamics of mycelia of three selected representatives ofthe Basidiomycota with large fruit bodies, namely *Armillaria borealis*, *Heterobasidion parviporum* and *Hebeloma crustuliniforme*. In order to optimize the experimental conditions, preliminary experiments were conducted to determine: (1) medium type and temperature of fungal culture incubation, (2) usefulness of wood sawdust in the *A. borealis* cultures, (3) effective water extraction method, duration time of extraction and effect of solvent pH at three pH values – pH 3 (acidified), normal pH and pH 9 (alkalified), (4) lichen species as a source of various lichen substances in main experiments, and (5) determination of extract dose and time between supplementation and inoculation of fungal isolates on medium. Finally, six epigeic lichen species were selected for further experiments, namely *Cetraria islandica*, *Cladonia arbuscula*, *C. mitis*, *C. digitata*, *C. rangiferina* and *C. uncialis* and one epiphytic species, *Pseudevernia furfuracea*, from which single species extracts and mixed lichen species extracts were obtained in two sets: (1) *Cladonia mitis* + *C. digitata* + *C. rangiferina* and (2) *Cetraria islandica* + *Cladonia mitis* + *C. digitata* + *C. rangiferina* + *C. uncialis*. Finally, water extractions from lichens were obtained at pH 9 by two methods: (1) in a bulb-heated water bath, referred to as the "bulb method" and (2) in a bath with boiling water, referred to as the "tea method".

Prior to the establishment of the main *in vitro* fungal culture, all the water extracts from the lichen species were supplemented at 1 ml, 2 ml, 3 ml and 4 ml by two methods: 1) on the surface of solidified Hansen's medium and 2) by mixing extracts with the medium. The obtained results of mycelium growth dynamics were recorded using the Poisoned Food Technique (PFT). UPLC-PDA-ESI-MS chromatographic analysis of the extracts was performed to determine the chemical profile of lichen secondary metabolites affecting the growth of fungal cultures of the three species tested.

The results of *in vitro* experiments showed that the effect of "bulb extraction", which partly reflects the conditions possible to occur in nature, allows to reach such concentrations of complexes of lichen substances, including secondary metabolites, which more often stimulate than inhibit the growth of mycelia tested representatives of fungi. Cultures supplemented with extracts obtained by the "tea method" confirmed the stimulation effect of lichen substance complexes, especially in the case of *A. borealis* cultures.

The observed effects (stimulation-inhibition) were simultaneously determined by the level of dispersion of water-extracted substances in the medium. Higher growth-limiting potentials

of *H. parviporum* and *H. crustuliniforme* cultures were observed in extract mixed with medium, while *A. borealis* cultures grew comparatively in both supplementation methods. No fungicidal effects were observed in any of the combinations tested.

The reaction of the mycelium to lichen substances is a result of species specificity, but it also depends on the extraction method used or the method of distribution of lichen substances – on the surface of solidified medium or mixed within medium. In general, the highest potential to stimulate the growth dynamics of tested mycelia was measured in cultures with "bulb extract" from lichens of *Cetraria islandica*, *Cladonia arbuscula* and *C. uncialis*, while the highest reduction of mycelial growth dynamics was observed in samples with the extracts obtained by *Cladonia digitata* and *Pseudevernia furfuracea*. Similarly, cultures supplemented with "tea extract" grew to the greatest extent in samples with extracts from *Cetraria islandica*, *Cladonia rangiferina* and *C. digitata*; the weakest mycelial growth was measured in variants with extracts from *Cladonia arbuscula*, *Pseudevernia furfuracea*, and - with a slightly weaker level of mycelial growth inhibition - from *C. uncialis*. The extracts obtained from mixed-lichen epigeic species were similar to those based on single species in that they showed specific-methodological dependencies of impact upon mycelium growth dynamics.

The largest mycelial dimensions of cultured fungi in samples supplemented with water extracts reached almost 190% of the mean mycelial diameter in control samples, while the highest level of inhibition was c. 30% of the mean diameter of the cultures in control samples. Overall, the lowest amplitude of growth dynamics (highest resistance to the potential of the lichen substance complex) was observed for *H. crustuliniforme* isolates.

The results obtained from experiments based on "bulb extraction" indicate the necessity of changing the paradigm, to reflect the allelopathic role of lichen secondary metabolites in nature, as substances exclusively inhibiting the growth of microorganisms, including fungi. Water-extracted primary and secondary lichen metabolites (substance complex), in such concentrations as those obtained in the conducted experiments, seem to influence in the natural environment much more as stimulators of mycelium growth of soil macrofungi – at least in the light of the three tested species.

Extracts obtained by the "tea method" seem to be effective and safe fungistatic (in an inhibitory sense) agents against the pathogen *H. parviporum*. Simirly, against *A. borealis*, water extracts from the thalli of *P. furfuracea* seem to be promising. In terms of *H. crustuliniforme*, the use of low doses of lichen water-extracted substances as pro-mycorrhizal agents should be effective.

The results of the experiments described in this work establish a suitable basis for future research on the role and use of water-extracted lichen substances in a wide variety of fields, including those of an ecological, phytopharmacological, medical and economic nature.

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Data złożenia

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