

GROWTH SUPPRESSION OF PLANT PATHOGENIC FUNGI USING BRYOPHYTE EXTRACTS

SUPRESSÃO DE CRESCIMENTO DE FUNGOS PATOGENICOS DE PLANTAS USANDO EXTRATOS DE BRIÓFITAS

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ABSTRACT: Chemicals are often used in attempts to control diseases caused by plant pathogenic fungi during food production. However, chemicals can have adverse effects not just on food, but they also remain active for a long time within ecosystems, and thus are not environmentally friendly. Therefore, development of bio-treatment and avoiding use of chemicals are urgently needed. With the aim of studying and developing new environmentally-friendly treatments, we tested extracts from selected bryophyte species (*Porella platyphylla*, *Cinclidotus fontinaloides* and *Anomodon viticulosus*) on five plant pathogenic fungi under controlled conditions. The fungi (*Botryosphaeria dothidea*, *Phomopsis viticola*, *Calosphaeria* sp., *Colletotrichum acutatum* and *Monilinia laxa*) were selected based on common diseases they cause on fruits and grapevine. They were isolated in cultures and treated with bryophyte extracts. Bryophyte extracts were shown to be effective in suppression of certain plant pathogenic fungi growth and to have a huge potential in development of novel biotechnological treatments and biofungicides. The best results were achieved in inhibition of *B. dothidea*, *P. viticola* and *Calosphaeria* sp.

KEYWORDS: Plant fungal pathogens. Fruit and grapevine diseases. Moss. Liverworts. Natural chemistry.

INTRODUCTION

Bryophytes are a huge group of terrestrial plants (ca. 20 000 species) that have many interesting peculiarities - ecological, physiological, developmental or constitutional (SABOVLJEVIĆ et al., 2016). It is widely accepted that bryophyte species are seldom used as a food source but are also not harmed by other organisms. This can be due to their special chemical constituents (WOLTERS1964, SABOVLJEVIĆ et al., 2017). Indeed, bryophyte material in herbaria are rarely damaged by insects or overgrown with fungi. There are many reports of antiviral, antibacterial and antifungal activities of bryophytes (VAN HOOFF1981, SABOVLJEVIĆ et al., 2006, 2010, 2011). However, recently there have been reports of the growth of fungi on the bryophyte species named bryophilous fungi (DÖBBELER 1997). Thus, a taxonomically diverse suite of fungi interacts with bryophytes as pathogens, parasites, saprobes, and commensals (DAVEY; CURRAH 2006). However, there are so few data on the interaction between bryophytes and fungi that many new and interesting phenomena emerged (e.g. reports on ploidy level

changes in bryophyte caused by fungal extracts by Goga et al. (2018)).

Plant pathogenic fungal diseases can cause rather huge problems especially in growing crops or any kind of monocultures and each year many chemicals are used to combat fungal diseases (ELAD et al. 1980; COLLA et al. 2012). However, these are not always effective and need to be applied successively, depending on plant developmental stage as well as climate or weather conditions. Apart, this treatment is not environmental friendly, but it is widely applied in food production. The chemical accumulation in natural ecosystems causes many other problems and a search for bio-treatments of fungal plant diseases is urgently needed (GAMLIEL et al. 2000; GRUETSKY et al. 2002; EYLES et al. 2015).

In order to evaluate bryophyte-fungi relationships and to examine the potential of bryophytes in pathogenic fungi suppression, the effects of extracts from selected bryophyte species on five fungal pathogens that cause common fruit and grapevine diseases were studied. Isolates of the following pathogenic fungi were used: *Botryosphaeria dothidea* (Moug. ex Fr) Ces. & De Not, causing twig, branch and stem cankers, tip and

branch dieback, fruit rot of many woody plants; *Phomopsis viticola* (Sacc.) Sacc., a pathogen of grapevine causing *Phomopsis* cane and leaf spot disease; *Calosphaeria* sp. causing *Calosphaeria* canker in trunks of stone fruits; *Colletotrichum acutatum* Simmonds, causing anthracnose in a very wide host range, but economically most important regarding strawberries; *Monilinia laxa* (Aderhold & Ruhland) Honey, the causal agent of brown rot of stone and pome fruits, that is economically more important regarding stone fruits. So, we tested extracts from these selected bryophyte species on five plant pathogenic fungi under controlled conditions.

MATERIAL AND METHODS

Bryophyte sampling

Three bryophyte species were sampled randomly to test their chemical content activity. One leafy liverwort (*Porella platyphylla* (L.) Pfeiff.) was sampled near Morača monastery (Montenegro) on 12th February 2017. Additionally, two mosses, one aquatic (*Cinclidotus fontinaloides* (Hedw.) P. Beauv.) and one terrestrial (*Anomodon viticulosus* (Hedw.) Hook. & Tayl.) were collected in Rijeka Crnojevića (Montenegro) on 8th February 2017, by the authors. The vouchers were deposited in BEOU bryological collection (Belgrade University Herbarium, Serbia). This bryophyte material was used for preparation of extracts after short storage at room temperature.

Extract assay

The extracts were made by 5g of room-dried plant material ground into a powder. A total of 50mL of solvent (42mL of methanol and 8mL of water) was added to powder. The composite achieved was evaporated until it was dry and the powdery residue was dissolved into 5mL of methanol (HPLC grade). The bryophyte extract produced was used to test fungal growth inhibition.

Fungal isolates and culturing

All fungi used in the experiment were isolated from diseased plant material from different localities in Montenegro. *B. dothidea* was isolated from rotten olive fruits; isolate of *P. viticola* was taken from infected grapevine cane; *Calosphaeria* sp. was obtained from sweet cherry trees; *C. acutatum* isolate was obtained from diseased strawberry fruits and *M. laxa* was isolated from peach fruits with brown rot symptoms. All isolates

were maintained in the fungal collection in plant pathology laboratory of Biotechnical Faculty in Podgorica (Montenegro).

Experimental design

The influence of bryophyte extracts on mycelial growth of selected plant pathogenic fungi was studied according to a modified method described by Grgic et al. (2016). The experiment was set up in Petri dishes (90 mm) poured with 20 mL of potato dextrose agar (PDA). Sterile filter paper (diameter of 6 mm) was placed in centre of the Petri dishes while four fungal fragments (ø 6 mm) were placed crosswise at a distance of 5 mm from the edge of Petri dish. Each bryophyte extract was pipetted in amounts of 5, 10 and 15 µL and deposited on filter paper while distilled water was used as a control. The experiment was set up in two replicates. The cultures containing fungal fragments were incubated at 25°C in darkness. Inhibition of mycelial growth was evaluated when mycelium of each fungus was developed until it reached the filter paper in the centre of the Petri dish or very close to it. The distance from filter paper with bryophyte extract to developed fungal mycelium was measured and used to calculate the suppression rate caused by bryophyte extract on each fungus.

Statistics

Data analysis was carried out using the statistical program Origin 6.1. Results are presented as mean values, evaluated by technical repetition. The distances between filter paper with bryophyte extract and fungal mycelium as rating criteria of the growth inhibition effect were statistically analysed by analysis of variance, and mean values were compared using LSD test. If their difference was greater than the LSD test, they were considered statistically significant.

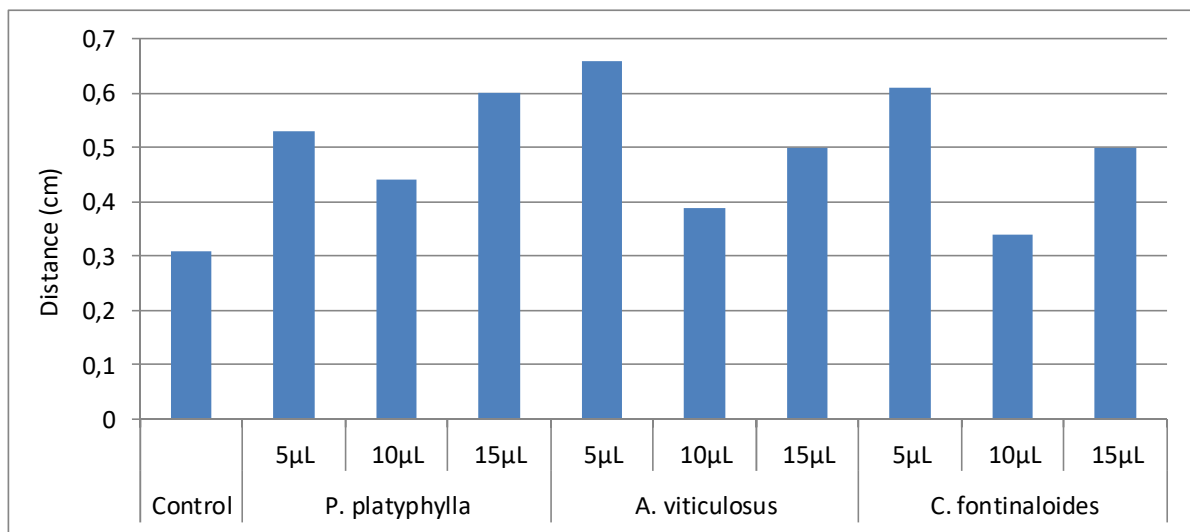
RESULTS AND DISCUSSION

The bryophyte extracts obtained showed different effects on selected fungi. The results obtained are presented in Table 1 while inhibitory effects of bryophyte extract to each fungus are given in the separate figure.

B. dothidea was affected by growth suppression by all three bryophyte extracts applied in certain dosage (Figure 1). The extract of *P. platyphylla* inhibited fungal growth when applied at 15µL, while extracts of *A. viticulosus* and *C. fontinaloides* achieved statistically significant inhibitory effects at a dose of 5µL.

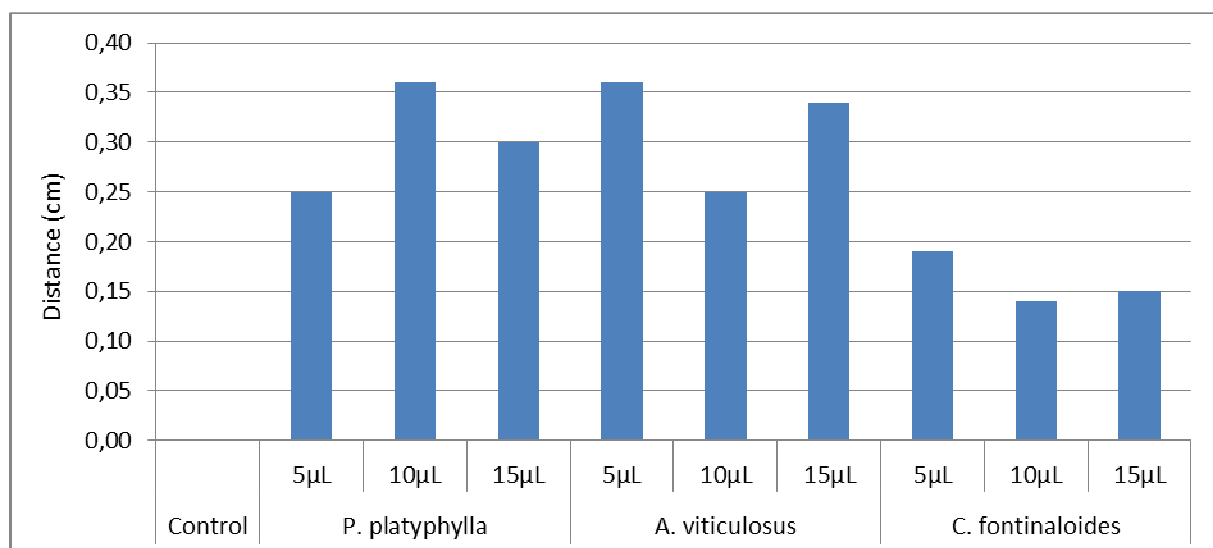
Table 1. Inhibition zone (cm) as distances between filter paper with bryophyte extract and fungal mycelium

Fungi	<i>Porella platyphylla</i>			<i>Anomodon viticulosus</i>			<i>Cinclidotus fontinaloides</i>			LSD	
	Amount of extract			Amount of extract			Amount of extract				
	Control	5 μ L	10 μ L	15 μ L	5 μ L	10 μ L	15 μ L	5 μ L	10 μ L		15 μ L
<i>B. dothidea</i>	0.31	0.53	0.44	0.60	0.66	0.39	0.50	0.61	0.34	0.50	0.243
<i>P. viticola</i>	0.00	0.25	0.36	0.30	0.36	0.25	0.34	0.19	0.14	0.15	0.244
<i>Calosphaeria</i> sp.	0.00	0.09	0.19	0.10	0.03	0.18	0.13	0.20	0.11	0.11	0.165
<i>C. acutatum</i>	0.64	0.61	0.83	1.15	0.65	0.79	0.50	0.90	0.43	0.61	0.309
<i>M. laxa</i>	0.30	0.26	0.31	0.14	0.51	0.06	0.08	0.31	0.10	0.30	0.338

**Figure 1.** Influence of different bryophyte extracts and dosage on growth inhibition of *B. dothidea* mycelium.

Statistically significant inhibition of *P. viticola* was recorded when treated with *P. platyphylla* and *A. viticulosus* extracts in all three doses tested. Among the dosages used there were

no statistically significant differences in achieved fungal inhibition. On the other hand, extract of *C. fontinaloides* exhibited no inhibitory activity no matter the dosage applied (Figure 2).

**Figure 2.** Influence of different bryophyte extracts and dosage on growth inhibition of *P. viticola* mycelium

Mycelial growth of *Calosphaeria* sp. was suppressed when all three bryophyte extracts were applied in certain amount (Figure 3). Inhibition of the fungus was recorded when *P. platyphylla* and *A.*

viticulosus extracts were applied at 10 µL while the extract of *C. fontinaloides* suppressed fungal development at 5 µL.

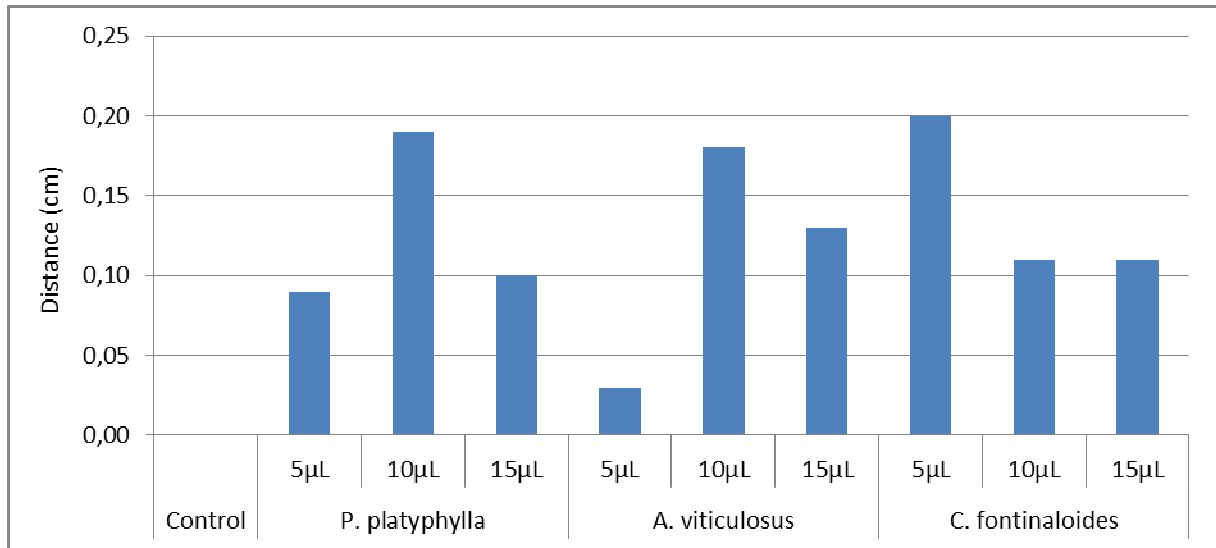


Figure 3. Influence of different bryophyte extracts and dosage on growth inhibition of *Calosphaeria* sp. mycelium

C. acutatum mycelium was suppressed when extract of *P. platyphylla* was applied at the highest dosage of 15 µL. In other dosages and other bryophyte extracts there were no statistically

significant differences compared to control (Figure 4).

The extracts of bryophytes applied to mycelium of *M. laxa* showed no inhibitory effects in any of the applied dosages (Figure 5).

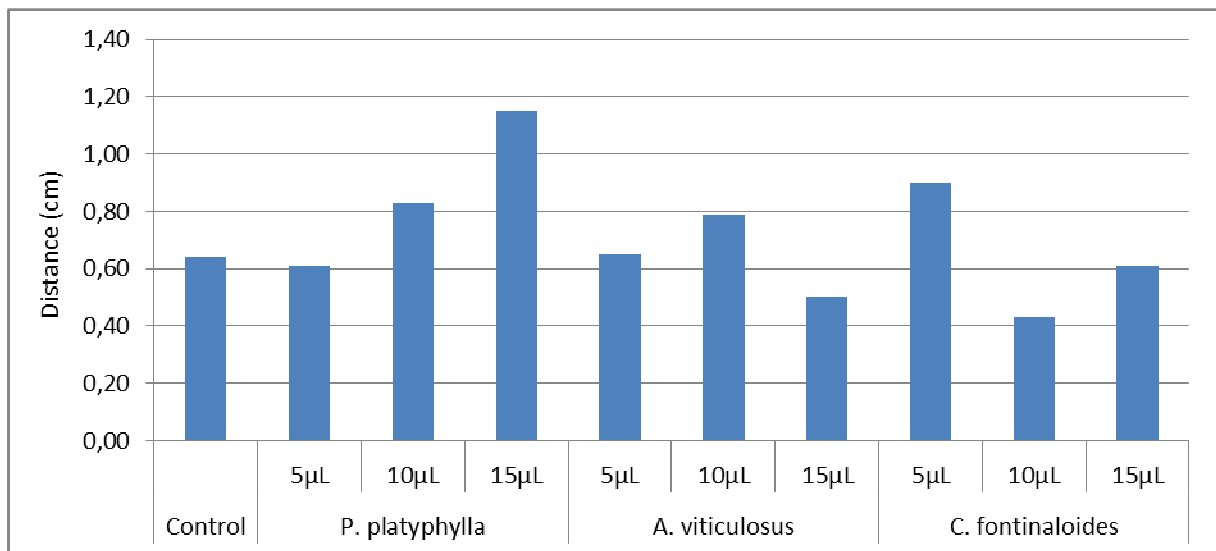


Figure 4. Influence of different bryophyte extracts and dosage on growth inhibition of *C. acutatum* mycelium

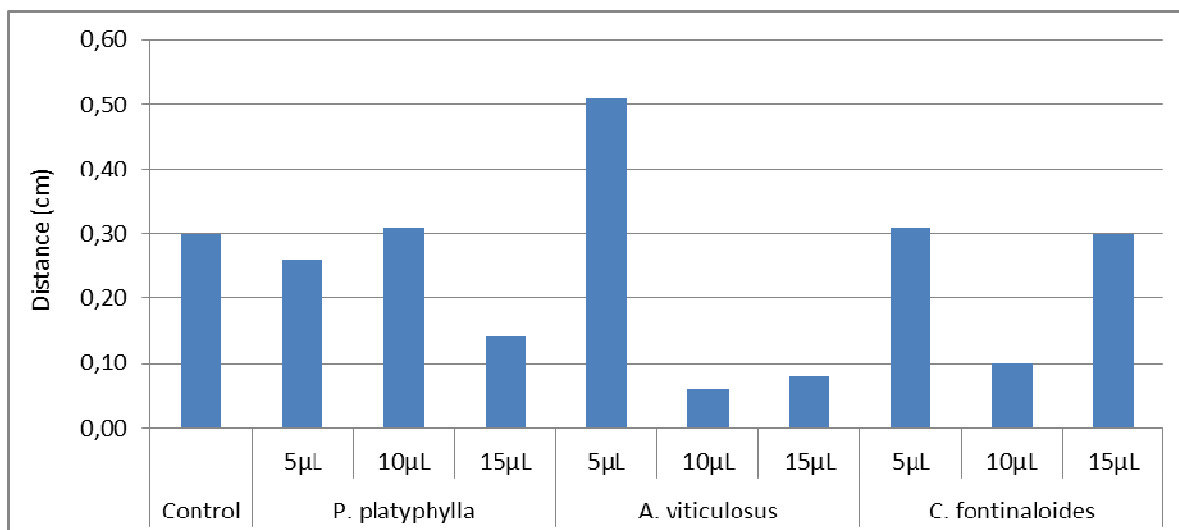


Figure 5. Influence of different bryophyte extracts and dosage on growth inhibition of *M. laxa* mycelium

It is generally accepted that bryophytes possess significant activity against plant pathogenic fungi, though data on these remained rather limited. Srivastava (2015) reported that moss *Funaria hygrometrica* extracts show activity against *Alternaria* species causing diseases of garden plants, field crops, vegetables, and ornamentals. Also, some bryophyte extracts inhibited development of *Botrytis cinerea* (grey mould), *Phytophthora infestans* (late blight of tomatoes) and *Blumeria graminis* (powdery mildew of wheat) in *in vitro* experiments carried out by Tadesse et al. (2003). A bryophyte aqueous extract was found to be effective against seven postharvest phytopathogens tested and reported by Alam et al. 2011. Shivom Singh et al. (2010) studied antimicrobial properties of a liverwort extracts which showed bioactivity against several important fungal plant pathogenic species, namely *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium oxysporum* and *Tilletia indica*.

There are only a few records about antimicrobial activity of the three bryophyte species in this study. The extracts of *P. platyphylla* showed antimicrobial effects on some gram-positive bacteria (BEIKE et al. 2010), while Glime (2017) reported its antifungal properties.

Ethanol extract of *C. fontinaloides* was observed to have significant antibacterial effect (YAYINTAS et al. 2017) but no antifungal activity was tested. In regard to *A. viticulosus*, to the best of our knowledge there are no data published about its antimicrobial activity as stated also by Vollar et al. (2018).

Our findings present valuable information within the area studied since there are no data on antifungal effects of the three moss species to the selected plant pathogenic fungi.

CONCLUSIONS

The results are the first ones in Montenegro on efficacy of selected bryophyte extracts in suppressing growth of plant pathogenic fungi (*B. dothidea*, *P. viticola*, *Calosphaeria* sp., *C. acutatum* and *M. laxa*). The inhibitory effects of tested bryophyte extracts indicate the potential as a source of natural active substances in management of certain fungal plant pathogens.

The best antifungal activity was achieved by *P. platyphylla* and *C. fontinaloides* extracts, which were the most effective on *P. viticola* mycelial growth. Significant results were also obtained by extracts of *P. platyphylla*, *C. fontinaloides* and *A. viticulosus* in some dosages applied to suppress mycelial growth of *B. dothidea* and *Calosphaeria* sp.

Achieved results could be important for development of environmentally safe alternatives in integrated plant disease management.

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RESUMO: Fungos fitopatogênicos são controlados com produtos químicos para combater doenças causadas por eles durante a produção de alimentos. Tais produtos são ruins não apenas para a alimentação, mas

também podem permanecer por muito tempo nos ecossistemas, portanto, não são ecologicamente corretos. Desta forma, os biotratamentos e a prevenção de usos químicos são urgentemente necessários no futuro próximo. Com o objetivo de estudar e desenvolver nova alternativa de controle de doenças de plantas, testamos os extratos de espécies de briófitas selecionadas (*Porella platyphylla*, *Cinclidotus fontinaloides* e *Anomodon viticulosus*) em cinco fungos fitopatogênicos (*Botryosphaeria dothidea*, *Phomopsis viticola*, *Calosphaeria* sp., *Colletotrichum acutatum* e *Monilinia laxa*) em condição controlada. Estes fungos, selecionados com base nas doenças que causam em frutos e videiras, foram isolados em culturas puras e tratados com os respectivos extratos de briófitas. Os extratos de briófitas demonstraram ser eficazes na supressão de certos fungos fitopatogênicos e têm um enorme potencial no desenvolvimento de novos biofungicidas e tratamentos biotecnológicos. Os mais interessantes resultados foram obtidos na inibição de *B. dothidea*, *P. viticola* e *Calosphaeria* sp.

PALAVRAS-CHAVE: fitopatógenos fúngicos, doenças de frutas e videiras, musgo, hepáticas, química natural

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