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Original Scientific Paper

Bryophyte extracts suppress growth of the plant pathogenic fungus *Botrytis cinerea*

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ABSTRACT: In this study, the extracts of three selected bryophyte species are shown to have inhibitory effects on grey mould disease (*Botrytis cinerea*). Methanol extracts of one leafy liverwort (*Porella platyphylla*) and two mosses, one aquatic (*Cinclidotus fontinaloides*) and one terrestrial (*Anomodon viticulosus*), were applied *in vitro* to *Botrytis cinerea*, after which tests showed suppression of fungal development.

Keywords: grey mould disease, biofungicide, mosses, liverwort

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INTRODUCTION

Fungal diseases of plants cause significant losses of both quantity and quality in agricultural production. In order to control the diseases, chemical treatments still have a major role. However, due to some important negative effects of applied synthetic fungicides, effects such as residues in food, environmental pollution, fungal resistance to fungicides and appearance of new resistant pathogenic races, there is a justifiable need to search for natural antifungal compounds.

Grey mould disease caused by the necrotrophic fungus *Botrytis cinerea* Pers. affects many plant species, including crops. It can be found on over 200 dicotyledonous and a few monocotyledonous plants (Elad *et al.* 2004). This fungus causes much damage in viticulture, horticulture and production of small fruit crops and vegetables. It can infect mature or senescent tissues, plants prior to harvest, or seedlings. The given species is known to produce an arsenal of degrading enzymes that enable it to feed on different plant tissues. Thus, this necrotrophic and polyphagous pathogenic ascomycete is capable of killing host cells by production of reactive oxygen species and toxins or by induction of a plant-produced oxidative burst (Choquer *et al.* 2007).

Although the grey mould agent (i.e., *Botrytis cinerea*) is one of the most studied of pathogenic fungi (van Kan 2006), there are not many reports on its interaction with bryophytes. However, there are some publications indicating infection of mosses by the fungus (Ponce de León *et al.* 2007, 2012). These facts suggest that early land-growing plants, i.e., bryophytes like *Physcomitrella patens*, developed cellular and molecular responses to interact and cope with microbial pathogens during their life cycle. There are also reports on both the general and specific response of mosses to fungal attack (Veljić *et al.* 2008, 2009; Ponce de León 2011; Bukvički *et al.* 2012a, b; Ponce de León & Montesano 2013, 2017; Ponce de León *et al.* 2015).

In addition to the specific response of bryophytes to fungal infection, study of features like specific chemical constituents and secondary metabolism in bryophytes (Sabovljevic *et al.* 2016) can lead to the discovery of biofungicides capable of improving food production and safety.

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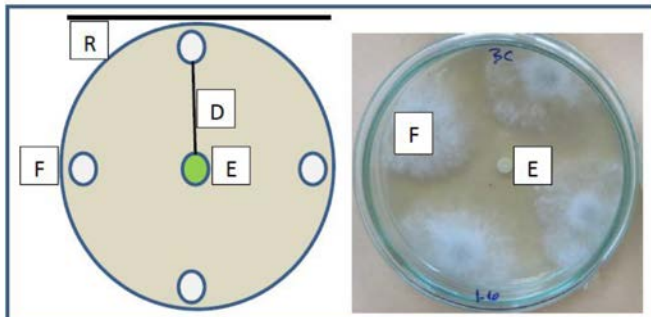

Fig. 1. Model of the experiment´s design: R – diameter of Petri dishes, D – diameter of fungal growth towards disc, E – disc with extract, F – fungal colony.

With the aim of studying suppression of the widespread occurrence of grey mould disease, randomly selected bryophyte extracts were applied to *B. cinerea* in the present investigation.

MATERIAL AND METHODS

Bryophyte sampling. Three bryophyte species were sampled randomly to test the activity of their chemical content. One leafy liverwort [*Porella platyphylla* (L.) Pfeiff.] was sampled near Morača monastery (Montenegro) on 12 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 8 February 2017 by the authors. The vouchers are deposited in the BEOU bryological collection (voucher numbers 05829, 05780 and 05779, respectively). This bryophyte material was used for preparation of extracts after brief storage at room temperature.

Extract assay. Extracts were made using 5 g of room-dried plant material ground into a powder and filtrated. A total of 50 ml of solvent (42 ml of methanol and 8 ml of water) was added to the powder. The composite achieved in that way was boiled down until dry and the remaining powder was dissolved in 5 ml of methanol (HPLC grade). The bryophyte extract obtained in this way was used to test inhibition of fungal growth. The amounts tested were 5, 10 and 15 µl. Distilled water served as a control (C).

Experimental design. *Botrytis cinerea* was isolated from infected strawberry fruits collected in a field near Podgorica (Montenegro). This is the most common disease of strawberry and grapevine in Montenegro (Latinović & Latinović 2011; Latinović *et al.* 2011). Four fragments (∅ 6 mm) of a developed fungal colony were placed near the edges of Petri dishes (∅ 9 cm) containing 20 ml of potato dextrose agar (PDA) as the nutrient medium, and discs (sterile filter paper, ∅ 6 mm) with different amounts of bryophyte extracts were settled in the middle. The growth of *B. cinerea* was measured from the edge of developed mycelium towards the disc, and the distance covered was taken as the inhibitory parameter (Fig. 1, left). The employed inhibition methodology follows Grgic *et al.* (2016) but was slightly modified here. Each concentration was tested eight times after 48 and 120 h from the beginning of fungal inoculation. The time of the second measurement (120 h) from fungal inoculation was chosen according to a preliminary time measurement in the same conditions, i.e., the time needed for *B. cinerea* to achieve the disc edge in the control Petri dishes. The Petri dishes were incubated at 25±2°C in a fungal growth chamber under conditions of constant darkness. The control discs contained no additional substance.

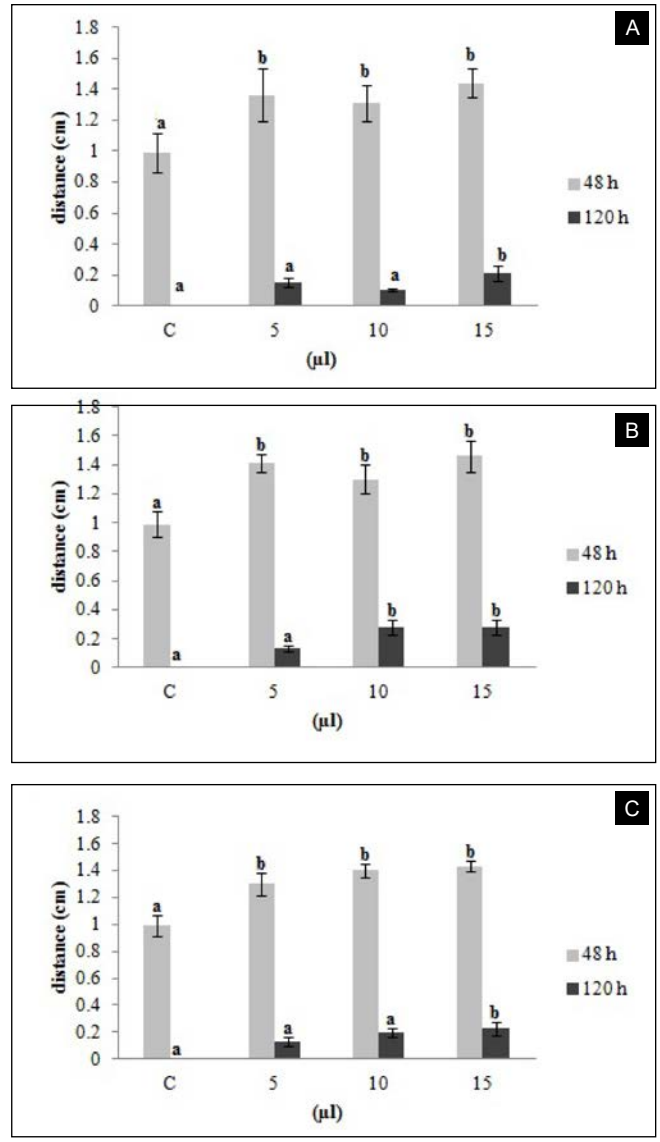

Fig. 2. A) Effects of *P. platyphylla* extracts on fungal growth; B) effects of *C. fontinaloides* extracts on fungal growth; C) effects of *A. viticulosus* extracts on fungal growth.

Statistics. Data analysis was performed in the Origin 6.1 statistical program. Results are presented as mean values, evaluated by technical repetition.

RESULTS AND DISCUSSION

The obtained results clearly showed that selected bryophyte extracts affect the growth of *B. cinerea*. All three of the tested species caused inhibition of fungal growth. This is not surprising since Wolters (1964) previously reported antifungal properties of bryophytes. Recent results confirm significant antifungal activity of methanol extracts of various mosses against different fungi (e.g., *Aspergillus* spp., *Penicillium* spp., *Trichoderma viride* and *Candida albicans*) (Veljić *et al*. 2008, 2009; Bukvički *et al*. 2012b) and food microorganisms (Bukvički *et al*. 2012b).

The amounts tested did not differ significantly in their inhibition of *B. cinerea* growth after two days. However, after five days from the test start, it could be seen that the greatest amount (15 µl) caused the strongest inhibition of fungal growth. This was indicated by failure of the mycelium to spread to the disc soaked in selected bryophyte extracts.

Some distance to the disc with bryophyte extracts is already visible two days after treatments (Fig. 1, right) and is definitely confirmed at the moment when the fungus in treatment without bryophyte extracts reached the disc but was suppressed in its counterparts treated with various amounts of bryophyte extracts (Fig. 2).

The potential of bryophyte extracts in bio-treatment of grey mould disease is high and further studies are needed. Such extracts offer a promising alternative to chemical compounds used in production and protection of food. It is clear that extracts of all three bryophyte species inhibited *B. cinerea* growth under *in vitro* conditions. Further investigation is also needed in order to develop biotechnological protocols for large-scale production of bryophyte biomass yielding a quantity of plant material that greatly surpasses the small biomass of bryophytes in nature and to avoid shrinkage of natural populations or threats to their existence.

CONCLUSIONS

The herein presented studies of the influence exerted by selected bryophyte extracts on *B. cinerea* mycelial growth are the first ones in Montenegro. Extracts of one leafy liverwort (*P. platyphylla*) and two mosses (*C. fontinaloides* and *A. viticulosus*) caused inhibition of fungal growth under *in vitro* conditions. The results are important since the tested bryophyte extracts have the potential to be a source of natural active substances in control of grey mould disease.

Development of biofungicides will be essential in future agricultural production in order to obtain healthy products with no environmental threats.

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Botanica SERBICA

REZIME

Primena ekstrakata briofita u suzbijanju fitopatogene gljive *Botrytis cinerea*

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Ekstrakti tri odabrane vrste briofita su ispoljile inhibitorno dejstvo prema sivoj truleži (*Botrytis cinerea*). Metanolni ekstrakti jedne vrste jetrenjače (*Porella platyphylla*) i dve mahovine, jedne vodene (*Cinclidotus fontinaloides*) i jedne terestrične (*Anomodon viticulosus*), primenjeni su *in vitro* na *B. cinerea*, prilikom čega je utvrđena inhibicija razvoja gljive.

Ključne reči: siva trulež, biofungicid, mahovine, jetrenjače