UDC 575
DOI: 10.2298/GENSR1601037J
Original scientific paper

FILAMENTOUS FUNGI ISOLATED FROM GRAPE MARC AS ANTAGONISTS OF *Botrytis cinerea*

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Jovicic-Petrovic P. J., I. M. Stankovic, A. R. Bulajic, B. B. Krstic, D. D. Kikovic, V. B. Raicevic (2016): *Filamentous fungi isolated from grape marc as antagonists of Botrytis cinerea.*-Genetika, vol 48 , no.1, 37-48.

In this paper we report on the isolation and identification of three filamentous fungi from grape marc, and antifungal effect of their cell-free culture filtrates on the growth of *Botrytis cinerea*, causal agent of gray mold. Grape marc is a waste material that has been used as soil amendment in sustainable agriculture. Isolates originating from grape marc were identified on the basis of morphological features and internal transcribed spacer rDNA or β-tubulin gene sequencing. The presence of three different species, *Penicillium paneum*, *Penicillium chrysogenum* and *Aspergillus fumigatus* has been detected expressing different effect on the growth of *B. cinerea*.

The effect of crude culture filtrates of selected fungi on *B. cinerea* growth was tested. Heat sensitivity of the established inhibition effect was examined by autoclaving the crude culture filtrate prior to testing. Additional aim was to determine whether antifungal effect was influenced by previous exposure to *B. cinerea* in dual liquid cultures. Crude culture filtrate of **A. fumigatus** K16/2 showed the lowest suppression of **B. cinerea** growth. A maximal percentage inhibition achieved within the study was 38.2%, 39.8% and 23.8 for crude filtrates of *P. paneum* K7/1, *P. chrysogenum* K11/1 and *A. fumigatus* K16/2, respectively. Presence of *B. cinerea* in dual liquid culture induced significant increase in antifungal capacity of the culture filtrates in comparison to pure culture filtrates of the chosen isolates. The antifungal activity of all of the isolates' culture filtrates retained after heat treatment suggesting the presence of some thermostable antifungal metabolites. The results indicate the complexity and specificity of the interaction between filamentous fungi and *B. cinerea*. Grape marc is a good source for

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isolation od *B. cinerea* fungal antagonists and their antifungal metabolites. Specificity of fungal-fungal interactions suggests that further research on the antagonistic mechanisms and factors affecting them should be studied separately for each pair of antagonists.

Key words: Aspergillus, *Botrytis cinerea*, culture filtrate, growth inhibition, *Penicillium*

INTRODUCTION

Botrytis cinerea, the causal agent of gray mold, is economically very important plant pathogen causing the significant yield and quality losses in vineyards. Although the control of *B. cinerea* has relied on chemical fungicides in recent decades, requirements relating to human and environment health include different sustainable approaches in agriculture practise (ELMER and REGLINSKI, 2006; JOVICIC-PETROVIC, 2014), including compost amendment to soil. Compost amendment is important in terms of waste management, since in this way wine industry residual by-products become an important resource in sustainable and organic agriculture (PARADELO *et al.*, 2011). Soil compost amendment has been proved beneficial as organic substrate addition and for suppression of plant diseases (ABBASI *et al.*, 2002; PÉREZ-PIQUERES *et al.*, 2006; SIDDIQUI *et al.*, 2009).

Grape marc has recently been introduced for agricultural use, usually following a period of composting. Although it is considered to be fertilizer and conditioner, its suppressive effects against plants pathogens have also been reported. The suppressive effect of compost is considered to be a result of complex interaction of both abiotic and biotic factors (NTOUGIAS *et al.*, 2010; CASTANO *et al.*, 2011). It has been reported that sterile composts exhibited lower suppressive effect and did not induce plant resistance, implying that compost microbial populations play a crucial role (ELMER and REGLINSKI, 2006).

Thus, composition of fungal population and diversity in agricultural waste, grape marc in particular, is important because different fungi exhibit various interactions with other soil microorganisms, including soil-borne phytopathogenic fungi (JOVIČIĆ-PETROVIĆ *et al.*, 2012). *Aspergillus* and *Penicillium* species have been isolated from grapes that have been colonized by *B. cinerea* (BENE and MAGYAR, 2009) and fungal diversity of grape marc was evaluated by NTOUGIAS *et al.* (2010).

Interactions between different microorganisms are influenced by both biotic and abiotic factors. Thus, induction of the activity of cellulase, chitinase and protease by addition of the corresponding substrate can increase the effect of *Trichoderma viride* on some post-harvest rot fungi (TERNA *et al.*, 2013). Considering this, hypothesis of the research was that fungi occurring in the grape marc can affect the growth of *B. cinerea*, and that the mechanisms of interaction can be induced by the preculturing fungi isolated form grape marc in the presence of *B. cinerea.* Therefore, the aim of this research was to isolate and identify fungal antagonists of *B. cinerea* from grape marc, to test the effect of their metabolites contained in cell-free culture filtrates on the growth of *B. cinerea*, and to examine if the previous exposure of the isolates to *B. cinerea* in dual liquid cultures increase the eventual inhibitory effect. In addition, the aim was to examine the thermotolerance of the inhibitory effect of culture filtrates.

MATERIALS AND METHODS

Fungus isolates collection

Isolation and enumeration of filamentous fungi from grape marc waste (experimental field “Radmilovac”) was performed using serial dilution method on Rose-Bengal selective

medium with streptomycin (PEPER *et al.*, 1995). After enumeration, colonies with different morphology properties were isolated, obtained monosporial cultures were maintained at 4ºC on Potato dextrose agar (PDA) (Merck, Germany). The isolate of *B. cinerea* originating from the culture collection of the Department of Phytopathology, University of Belgrade, Faculty of Agriculture, was used as representative plant pathogenic fungi in order to the evaluate antifungal effect of selected fungi from grape marc on its growth rate.

Confrontation test

Preliminary selection of the isolates with antagonistic potential towards *B. cinerea* was performed by confrontation test. 5 mm diameter disks of each isolate were placed at a distance of 3 cm apart from the *B. cinerea* in Petri dish containing PDA medium. Plates incubated with pure culture of *B. cinerea* were used as control. Experiment was performed in three replications. After 5 days of incubation at 25°C in dark, antagonistic isolates were selected according to the reduction of the mycelial diameter of *B. cinerea* . The percentage of mycelilal growth inhibition of *B. cinerea* was calculated as: (colony diameter of control – colony diameter of treatment)/colony diameter of control x 100. Isolates causing *B. cinerea* growth inhibition of 25% and more were selected for further study.

Identification of B. cinerea antagonists from grape marc

Fungi isolated from grape marc were preliminary identified according to their macroscopic and microscopic morphological features on PDA medium by observation of colony morphology and conidiophores and spores arrangement, respectively. Colony appearance and microscopic examination (Leica DMLS, Germany) was performed on 7-day-old culture grown at 25°C in dark. The growth on temperatures higher than 40°C was additionally followed for distinction of *Aspergillus* species (SAMSON *et al.*, 2004a)

After identification of selected isolates on the basis of their morphological features, molecular identification was applied.

Genomic DNA of all selected fungi was isolated directly from 100 mg of mycelia from 7-day-old cultures grown in Potato dextrose broth (PDB, Sigma Aldrich, USA), using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), following manufacturer's instructions. DNA was extracted in 100 µl of elution buffer and further used as a target in PCR reaction. Molecular identification of selected isolates was performed on the bases of ITS sequences, and for the isolate K11/1 of β-tubulin region sequences. PCR amplifications of ITS region was performed using ITS1/ITS4 primer pair in 25 µl reaction volume (12.5 µl of Master Mix Fermentas, Lithuania, 1 µl of each primer, 1 µl of sample, and 6.5 µl of RNase free water). These reactions were subjected to an initial denaturation of 2 min at 94°C, followed by 35 cycles of 2 min at 94°C, 30 s at 57°C, and 1 min at 72°C, with a final extension of 10 min at 72°C in Thermocycler T-1 (Biometra, UK).

Amplification of β-tubulin region was carried out with Bt2a/Bt2b primer pair (SAMSON *et al.*, 2004b) with the same reaction mixture as previously described under the following amplification protocol: initial denaturation of 1 min at 94°C; 33 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C; final extension of 10 min at 72°C. A negative control where template DNA was replaced by molecular water was included in each reaction. Amplified products were analyzed by 1% agarose gel electrophoresis, stained with ethidium bromide, and visualized under a UV transilluminator.

PCR products of a predicted size, obtained in PCR assay were sequenced directly after purification with a QIAquick PCR Purification Kit (Qiagen), and sequenced on ABI 3730XL Sequencer (Macrogen, Inc., Korea) in both directions. All generated sequences were deposited in GenBank database and compared with respective sequences (http://www.ncbi.nlm.nih.gov/BLAST/) using the ClustalW program (THOMPSON *et al.*, 1994) and MEGA5 software (TAMURA *et al.*, 2011).

Effect of crude culture filtrates on growth of Botrytis cinerea

Sterilized liquid culture filtrates were prepared as previously described (MA *et al.*, 2008). Six Erlenmeyer flasks containing 200 ml of sterile PDB were prepared. Three of those were used for preparation of pure liquid culture filtrates of each fungus isolate (named fK7/1, fK11/1 and fK16/2), and three for dual culture filtrates (isolate grown together with *B. cinerea*, named fK7/1+B, fK11/1+B and fK16/2+B).

Flasks intended for incubation of pure liquid cultures were inoculated with five 5-mm-diameter mycelial disks of selected isolates, while flasks with PDB intended for dual cultures were inoculated with five mycelial disks of the respective isolate, and five mycelial disks of *B. cinerea*. Disks were obtained from the edge of actively growing colonies of fungi, previously grown on PDA for 7 days. Flasks were than incubated in dark, at 25±1ºC on a rotary shaker at 160 rpm for 7 days. The liquid cultures were filtered through filter paper and the filtrates were centrifuged at 8000 rpm for 15 min (Centrifuge U-320, BOECO, Germany). The supernatant was sterilized by filtration through 0.22 µm Milipore membrane or by autoclaving at 121°C for 15 min was stored at 4°C and used for further studies.

The effect of cell-free culture filtrates of all three selected isolates/species separately as well as of dual culture filtrates were investigated by growing *B. cinerea* on PDA amended with respective filtrate. 5-mm-diameter disks cut from edge of actively growing colony of *B. cinerea* were plated in the center of plates containing 10 and 50% of sterilized culture filtrates that were added in melted PDA (45-55ºC). The effect of filtrate sterilized by high temperature was tested to examine the presence of thermostable metabolites that can affect *B. cinerea* growth in culture filtrates. As negative control treatment PDA without amendments was used for growing *B. cinerea* under the same conditions. All plates were incubated in dark at 25±1Cº for five days before colony diameters were measured. The experiment was performed in three replicates.

Growth inhibition percentage (GI, %) was calculated as equal to the: (colony diameter of control – colony diameter of treatment)/colony diameter of control x 100. Results were subjected to analysis of variance (ANOVA) by Statistica software (StatSoft, Tulsa, OK, USA). Comparison of mean values of data was done by Fisher's LSD test at significance level of p=0.05.

RESULTS AND DISCUSSION

Isolation, selection and identification of fungal species from grape marc

The quantity of fungal spores in grape marc was estimated to $7.8x10^5$/g dry weight. Monosporial cultures of 16 morphologicaly different fungal isolates were obtained. On the basis of confrontation tests three of the isolates were selected as antagonists of *B. cinerea*. Those isolates were named as K7/1, K11/1, and K16/2 and used for further study. The presence of the isolates K7/1 in paired culture plate induced significant reduction of *B. cinerea* colony diameter for average 77%3%. K11/1 isolate inhibited *B. cinerea* for average 43% in comparison to control.

Also, K11/1 overgrow *B. cinerea* colony after seven days of incubation. Isolate K16/2 reduced *B. cinerea* colony for 35% comared to the control plate.

The remaining 13 isolates grown with shown mutual inhibition, or inhibited *B. cinerea* for less than 25%. After this preliminary screening, the isolates that showed antifungal effect towards *B. cinerea* were further characterized in purpose of identification.

Isolate K7/1 is identified to belong to *Penicillium roqueforti* group on the bases of following morphological features: colonies blue green, later become darker, reverse greenish, conidiophore quaterverticilate, stipe typically ornamented, metulae rough walled bearing 5-7 flask shaped phialides, conidia in loose chains, globose, greenish, smooth walled with 4 µm average diameter. Isolate K11/1 is identified to belong to the *Penicillium chrysogenum* as colonies exhibited velvety appearance, at first yellow-green changing to darker green shades yellow exudate and reverse side, with terverticilate conidiophores, cylindrical metulae bearing 3 to 6 flask shaped phialides, with globose to ellipsoid, slightly green, smooth-walled conidia whose average diameter was 2.9 µm. Isolate K16/2 was identified as *Aspergillus fumigatus* based on its morphological features - colonies on PDA powdery in texture blue-green to gray, slight yellow reverse side, grows well at temperatures over 40°C, micromorphologicaly smooth-walled conidiophores, uncoloured, terminate in broadly clavate vesicle, greenish closely compacted philaides occurring on the upper portion of the vesicle, green subglobose conidia around 2.7 µm in diameter, produced in chains basipetally.

Identification on the basis of morphological features as well as major cultural characteristics was further confirmed with molecular identification. After amplification of selected genomic regions of all three isolates, the fragments of approximate size between 500-600 bp were obtained (Fig. 1.), and sequenced.

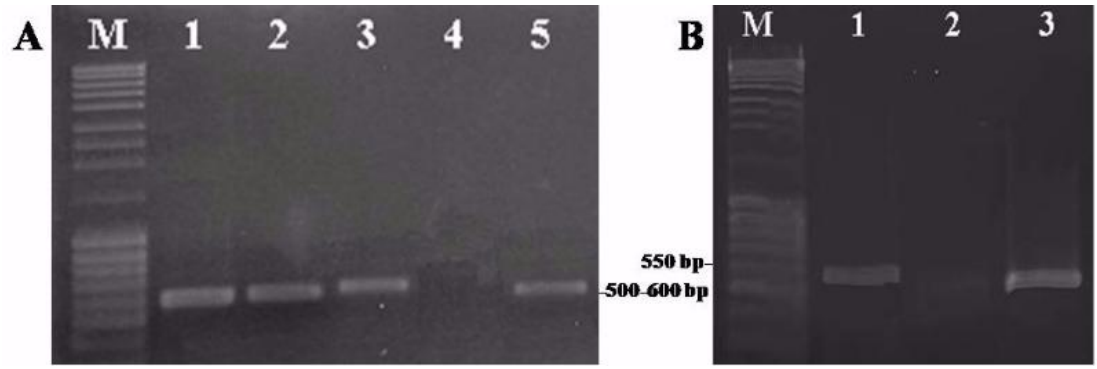

Figure 1**.** Molecular detection of selected isolates by polymerase chain reaction (PCR) **A,** ITS region using primers ITS1/ITS4 (Line: 1-isolate K7/1, 2-K11/1, 3-K16/2, 4-negative control, 5-positive control), and **B,** β-tubulin region using primers Bt2a/Bt2b (Line: 1-K16/2, 2-negative control, 3-positive control).

During this work following ITS sequences were generated: isolates K7/1 (KF267253), K11/1 (KF413577) and K16/2 (KF267254) as well as β-tubulin sequence of isolate K11/1 (KF516018). ITS sequence of isolate K7/1 exhibited 99-100% nucleotide identity with 29 isolates of *P. paneum*, while ITS sequence of isolate K16/2 exhibited 99-100% nucleotide identity with 37 isolates of *A. fumigatus* originating from different parts of the world, providing their respective identification. ITS sequence of K11/1 exhibited 99-100% nucleotide identity with 37 isolates of different species of *Penicillium* sp., while β-tubulin sequence exhibited 99-100% nucleotide identity with 19 isolates of *P. chrysogenum* originating all over the world, providing successful identification.

Effect of crude filtrate of fungi from grape marc on the Botrytis cinerea growth

Culture filtrates of the fungal isolates from grape marc showed different effect on the *B. cinerea* growth (Fig. 2., Fig. 3., Fig. 4.)

Obtained results showed that dual culture filtrates caused significantly higher growth inhibition compared to pure culture filtrates.

Inhibition of *B. cinerea* growth by *P. paneum*, K7/1 pure culture filtrates depended on the filtrate concentration. Inhibition percentage amounted to 38.3% in the case of 50% dual culture filtrate sterilized by filtration. All of the examined filtrates significantly reduced their activity after heat treatment, but they still induced *B. cinerea* growth inhibition. Higher filtrate concentration in media induced higher inhibition, but the opposite was noted with pure culture filtrates. (Fig. 2.)
Figure 2.

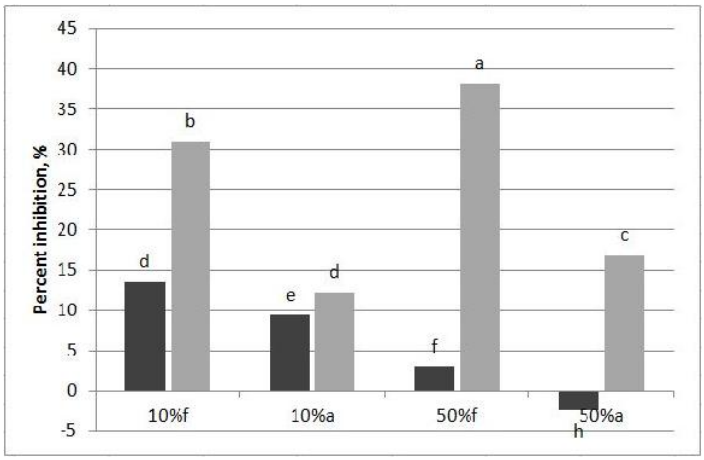

Fig. 2. Effect of 10% and 50% sterile culture filtrates of *Penicillium paneum*, K7/1 on *Botrytis cinerea* growth (f – filtrates sterilized by filtration; a – filtrates sterilized by autoclaving; ■ – pure culture filtrates, K7/1; ■ – dual culture filtrates, K7/1+B). Bars with the same letter represent means that are not significantly different according to the Fisher's LSD test (p=0.05).

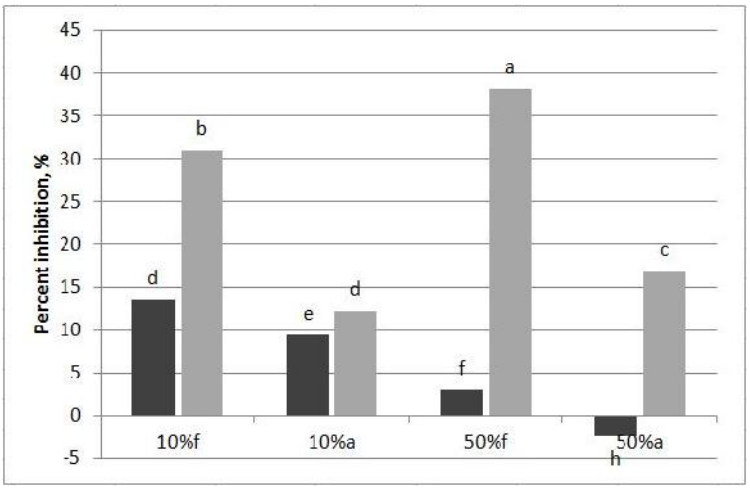

Fig. 3. Effect of 10% and 50% sterile culture filtrates of *Penicillium chrysogenum*, K11/1 on *Botrytis cinerea* growth (f – filtrates sterilized by filtration; a – filtrates sterilized by autoclaving; ■ – pure culture filtrates, K11/1; ■ – dual culture filtrates, K11/1+B). Bars with the same letter represent means that are not significantly different according to the Fisher's LSD test (p=0.05).

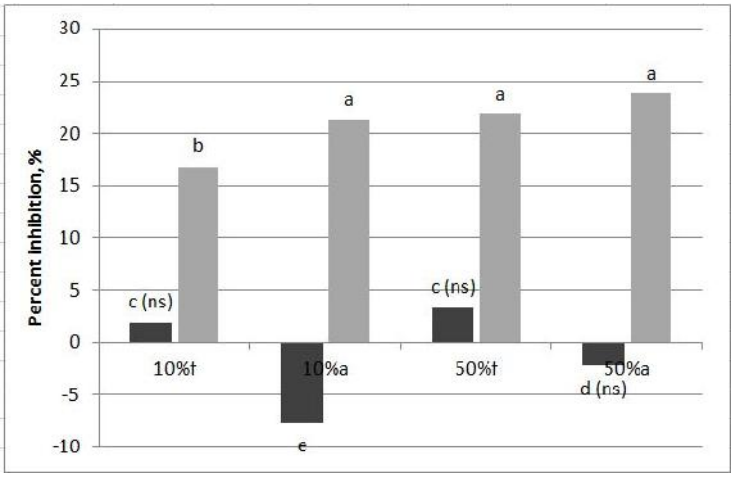

Fig. 4. Effect of 10% and 50% sterile culture filtrates of *Aspergillus fumigatus*, K16/2 on *Botrytis cinerea* growth (f – filtrates sterilized by filtration; a – filtrates sterilized by autoclaving; ■ – pure culture filtrates, K16/2; ■ – dual culture filtrates, K16/2+B). Bars with the same letter represent means that are not significantly different according to the Fisher's LSD test (p=0.05). (ns) – Effect is not statistically significant in comparison to control.

The presence of sterile pure culture filtrates of *P. chrysogenum*, K11/1 in media did not result in the pathogen growth inhibition (Fig. 3.). The only exception was autoclaved pure culture filtrate applied in the concentration of 50%. 10% of autoclaved pure culture filtrate even led to the stimulation of fungal growth. On the other hand, presence of *B. cinerea* in the dual culture induced production of extracellular antifungal compounds contained in liquid filtrate. Range of *B. cinerea* growth inhibition by dual culture filtrates (K11/1+B) was 32.3-79.3%. Inhibition percentage depended on concentration and sterilization technique (Fig. 3.). As a surprising result, higher inhibition was achieved with dual culture filtrates sterilized by autoclaving in comparison to the filtrates that are not heat treated. As in the case of the activity of autoclaved pure culture filtrate that appear to have some inhibitory effect, such result could not be explained for now. It can be assumed that some additional antifungal compounds were synthesized during the heat treatment.
Figure 3.

A. fumigatus, K16/2 is the fungal isolate that has shown the lowest effect on *B. cinerea* growth. Its dual culture filtrates caused *B. cinerea* inhibition. Plates amended with 50% of autoclaved dual culture filtrate had the highest effect, with 23.8% growth inhibition. (Fig. 4.). The presence of pure culture filtrates had no inhibitory effect on mycelyal diameter of *B. cinerea*, autoclaved pure culture filtrates even exhibited some stimulative effect. Dual culture filtrates inhibited *B. cinerea* growth and such effect retained after heat treatment, indicating that presence of the phytopathogen induced production of some thermostabile antifungal metabolites by *A. fumigatus*, K16/2.
Figure 4.

The inhibitory effect of sterile crude culture filtrates indicate that indirect modes of antagonistic mechanisms are involved in *B. cinerea* inhibition. Previous exposure of all of the three selected antagonists to the presence of *B. cinerea* induces changes in the profile and/or quantities of produced metabolites that inhibit growth of this plant pathogen, thus enhancing the inhibition

DISCUSSION

Sustainable agriculture faces the challenge to produce enough food while maintaining the environmental quality. Among other, it relies on soil biological processes and soil biodiversity, so the practices affecting the contribution of soil micro-flora are important part of this approach. Beneficial soil microorganisms interact with plants and phytopathogens and play an important role in the sense of biofertilisation and plant disease control (SINGH *et al.*, 2011). Compost and agro-industrial waste amendments to soil may contribute to soil biodiversity acting like microbial inoculums. In this study, three filamentous fungi from grape marc were isolated and the effect of their extracellular metabolites towards one of the most important pathogen of grapevine was tested.

Worldwide, annual yield loss in grape production caused only by *B. cinerea* has been estimated to be at least 2 billion $US (ELMER and MICHAILIDES, 2004). Fungicidal treatments against *B. cinerea* cost about 540 million euros in 2001, which represents 10% of the world fungicide market (Annual Report, UIPP 2002). Furthermore, the magnitude of the fungicidal treatments against this fungus has provoked the appearance of resistant strains, necessitating the development of new molecules (LEROUX *et al.*, 2002).

Market concerns about fungicide residues and the need to manage fungicide resistance (BRENT and HOLLOMAN, 2007) resulted in a global reorientation towards sustainable disease

management and alternative measures for disease suppression. Microbial antagonists of *B. cinerea* have been reviewed by ELMER and REGLINSKI (2006). Filamentous fungi are important group of microorganisms with different modes of action towards *B. cinerea*, including the indirect effect of their metabolites. Knowing that *Penicillium* and *Aspergillus* are both genera known producers of antibiotics (DIÁNEZ *et al.*, 2007), hydrolytic enzymes (KHOKHAR *et al.*, 2011) and antifungal peptides (JOVICIC-PETROVIC *et al.*, 2016), metabolites contained in their culture filtrate may have important role in *B. cinerea* suppression.

Considering the fact that during composting, microorganisms may survive in the outer most layers, at lower temperatures (ALVÉS *et al.*, 2011), versatility of microorganisms present in compost is closely related with the nature and microbial diversity of agro industrial waste that was subjected to composting. Composts are known as a rich source of plant disease suppressive microorganisms which includes *Pseudomonas* sp., *Klebsiella* sp., *Enterobacter* sp., *Bacillus* sp. and *Streptomyces* sp. among bacterial agents; and *Penicillium* sp., *Trichoderma* sp. and *Gliocladium* sp., among filamentous fungi biocontrol organisms (NELSON *et al.*, 2002).

Fungal diversity of grape marc has been evaluated by NTOUGIAS *et al.* (2010), while fungal diversity of grape marc compost and grape marc compost tea has been evaluated by SANTOS *et al.* (2008). Predominance of *Penicillium* and *Aspergillus* species in grape marc compost found in this studies is in accordance with already reported data and is considered to be a consequence of the chemical composition (high sugar, low cellulose content) (SANTOS *et al.*, 2008; DIÁNEZ *et al.*, 2007).

In this paperwork, the presence of three different species *P. paneum*, *P. chrysogenum* and *A. fumigatus* were isolated and identified from grape marc and their capacity to act as agents in biological control was documented.*Penicillium roqueforti* group has been reclassified recently on the basis of molecular features and *P. paneum* has been defined as new species (SAMSON *et al.*, 2004b). Its presence in grape marc is important in order to evaluate the safety issue of the grape marc utilization in agriculture, because of its secondary metabolites such as patulin might be hazardous to human and animal health (O'BRIEN *et al.*, 2006). Antibacterial properties of *P. chrysogenum* are well known, but recent papers have emphasized the attention to its antifungal activity. It has been reported that cysteine and lysine-rich protein secreted by *P. chysogenum* exhibits antifungal activity towards variety of filamentous fungi *in vitro*, including plant-pathogenic *B. cinerea* (MARX *et al.*, 2008; KAISERER *et al.*, 2003). Presence of *A. fumigatus* in grape marc as a result of the present paper is not unexpected, considering that due to the possibility of utilizing various carbon sources and ability to persist in composting conditions, the presence of *A. fumigatus* is noted in different environments (TEKAIA and LATGÉ, 2005). Based on complete ITS1-5.8S rRNA gene-ITS2 sequencing, NTOUGIAS *et al.* (2010) also reported the presence of *A. fumigatus* in grape marc.

Considering the result that 3 out of 16 isolates of fungi from grape marc have shown substantial antagonistic effect on *B. cinerea* in confrontation test, it can be concluded that grape marc have some biological potential in suppression fungal growth of and it should be tested on additional plant pathogens. The effect of the liquid culture filtrates' concentration as well as the effect of the previous exposure of the isolates to *B. cinerea* was generally different in three examined species suggesting that their different capacity in antifungal activity towards *B. cinerea* rely on different mechanisms. Presence of *B. cinerea* during the cultivation of selected antagonists induced changes in production of antifungal metabolites by the isolates, so that dual culture filtrates had significant effect in suppressing growth of *B. cinerea.* The extracellular metabolites

had no equally high inhibitory effects if the same isolates were grown in pure cultures. It turned out that all of the tree selected isolates may produce some thermostable metabolites, because their autoclaved culture filtrates caused significant inhibition of the growth of *B. cinerea*. However, *P. chrysogenum*, K11/1 and *A. fumigatus*, K16/2 produced thermostable antifungal metabolites only if they were grown in the presence of *B. cinerea. P. chrysogenum* K11/1 pure culture filtrate showed significant inhibitory effect if 50% of autoclaved filtrate was applied but such effect could be explained by the synthesis of some antifungal compounds during the heat treatment.

The effects of sterile filtrates of *P. paneum* and *P. chrysogenum* on *B. cinerea* growth indicate that those isolates generally demonstrate significant efficiency. MISHRA (2010) reported that the strain *Trichoderma viride* – 1443 is found effective to *Pythium aphanidermatum*. *T. viride* 20% culture filtrate inhibited *P. aphanidermatum* growth for 44%, which is the result comparable to the present study results.

The obtained results indicate the complexity and specificity of the interaction between filamentous fungi, their metabolites and *B. cinerea*. Considering that, fungal-fungal interactions and factors affecting them, should be addressed separately for each pair of antagonists. Further research will be directed toward isolation, identification and characterization of specific metabolites with prominent effect on the growth of *B. cinerea* and testing their effect on other important plant pathogens.

ACKNOWLEDGEMENTS

Presented results are part of the research that has been conducted under the project TR 31080 and III 43001 financed by the Ministry of Education, Science and Technological development, Republic of Serbia, and international FP7 Project AREA 316004.

Received March 23th, 2015
Accepted December 22th, 2015

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FILAMENTOZNE GLJIVE IZOLOVANE IZ KOMINE GROŽĐA KAO ANTAGONISTI *Botrytis cinerea*

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Izvod

Komina grožđa je otpadni material koji se može koristiti kao dodatak zemljištu u održivoj poljoprivredi. U ovom radu predstavljeni su rezultati izolacije i identifikacije gljiva iz komine grožđa koje pokazuju antagonizam prema *Botrytis cinerea*. Antagonisti *B. cinerea* odabrani na osnovu konfrontacijskog testa, identifikovani su na osnovu morfoloških karakteristika i sekvenci ITS rDNA ili β-tubulin regiona. Utvrđeno je prisustvo tri različite vrste - *Penicillium paneum*, *Penicillium chrysogenum* i *Aspergillus fumigatus* koji pokazuju antagonizam prema *B. cinerea*. Testiran je efekat filtrata tečnih kultura odabranih gljiva na rast *B. cinerea*. Ispitana je i stabilnost postignute inhibicije nakon termičkog tretmana filtrata. Dodatni cilj istraživanja bio je ispitati da li na antifungalni efekat filtrata utiče prethodna izloženost izolata patogenu u dvojnoj tečnoj kulturi. Filtrati tečnih kultura odabranih antagonista doveli su do različite inhibicije rasta *B. cinerea*, ali se u njihovom delovanju mogu uočiti i određene sličnosti. Do najnižeg procenta inhibicije rasta doveo je *A. fumigatus*, K16/2. U slučaju sva tri ispitivana izolata postignuti procenti inhibicije bili su značajno viši ukoliko je primenjen filtrate dvojne kulture, odnosno ukoliko je izolat odgajan u prisustvu *B. cinerea*. Maksimalni procenti inhibicije rasta *B. cinerea* koji su postignuti iznosili su: 38,2% u slučaju *P. paneum* K7/1, 39,8% u slučaju *P. chrysogenum* K11/1 i 23,8% u slučaju *A. fumigatus* K16/2. Efekat filtrata tečnih kultura kod sva tri izolata nije izostao nakon termičkog tretmana, što ukazuje na prisustvo termostabilnih metabolita. Komina grožđa u okrviru diverziteta gljiva poseduje potencijal za suzbijanje *B. cinerea* i predstavlja dobar izvor za izolaciju antagonista ovog patogena.

Primljeno 23. III. 2015.
Odobreno 22. XII 2015.