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Colletotrichum orbiculare ON WATERMELON: IDENTIFICATION AND *IN VITRO* INHIBITION BY ANTAGONISTIC FUNGI

ABSTRACT: Anthracnose caused by the fungus *Colletotrichum orbiculare* is one of the most significant diseases of *Cucurbitaceae.* In Serbia watermelon fruits with typical anthracnose lesions were collected during the year of 2015. Affected fruits showed sunken, dark brown to black lesions with orange conidial masses produced in black acervuli. In an attempt to identify the causal organism, small pieces of necrotic tissue were surface sterilized and placed on potato dextrose agar (PDA). Macroscopic and microscopic morphologicaly characteristics of three isolates were observed after growth on PDA for 7 days at 25 ºC under a 12 h light/dark cycle. Fungal colonies developed white, grey to black dense aerial mycelium. Conidia were hyaline, aseptate, straight and cylindrical to clavate, 9–12.5 µm × 4–5.5 µm. Fungal isolates were also characterized by sequencing of the internal transcribed spacer (ITS) rDNA region using ITS1F/ITS4 primers and β-tubuline 2 gene using T1/Bt2b primers. The nucleotide sequences were deposited in GenBank (ITS Acc. No. KT454386, KT454387 and KT454388; β-tubuline 2 gene Acc. No. KT581236, KT581237 and KT581238). BLAST analysis of ITS and β-tubuline 2 gene sequences showed that our isolates were 100% identical to other *C. orbiculare* in NCBI GenBank. Pathogenicity test was conducted on symptomless, detached watermelon fruits. All tested isolates caused anthracnose lesions on watermelon fruits after 10 days of incubation. *Trichoderma harzianum* (DSM 63059) and *Gliocladium roseum* (DSM 62726) were evaluated *in vitro* for their antagonistic potential against *C. orbiculare*. The results of this study identify *T. harzianum* and *G. roseum* as promising biological control agents (BCAs) for further testing against anthracnose disease on watermelon fruits.

KEYWORDS: *Colletotrichum orbiculare*, watermelon, identification, antagonistic fungi

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INTRODUCTION

Anthracnose caused by the fungi *Colletotrichum orbiculare* species complex is one of the most significant diseases of cucumber (*Cucumis sativus* L.), melons (*Cucumis melo* L.), pumpkin (*Cucurbita pepo* L.) and watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai). The disease is widespread under both greenhouse and field cultivation and can occur on seedlings, leaves, petioles, stems and fruits of *Cucurbitaceae* and other herbaceous host, belonging to the *Asteraceae, Fabaceae* and *Malvaceae* (Farr and Rossman, 2013). In Serbia, *C. lagenarium* (synonym of *C. orbiculare*, von Arx, 1957) has been reported as pathogen on several *Cucurbitaceae* (Spasić, 1963; Stojanović *et al.* 2002).

Differentiation between *Colletotrichum* species based on host range or host of origin may not be a reliable criterion for fungi of this genus (Freeman *et al.* 1998). The host plants of species of the *C. orbiculare* complex can be attacked by other *Colletotrichum* species: *C. melonis* (*C. acutatum* complex), *C. karstii* (*C. boninense* complex) and *C. coccodes* (Damm *et al.* 2012a; Liu *et al.* 2013). However, due to their morphological variability, the ample range of hosting crops and the wide variety of isolates are partially difficult to identify as *Colletotrichum* spp. by traditional taxonomic methods, which must be complemented with molecular techniques and multilocus phylogenetic studies (Whitelaw-Weckert *et al.* 2007; Cannon *et al.* 2012; Damm *et al.* 2012a; Weir *et al.* 2012). In a major taxonomic reorganization of 42 strains of *C. orbiculare* and related species, Damm *et al.* (2013) identified 9 distinct clades within the *C. orbiculare* species complex based on multilocus phylogenetic analysis (ITS, GAPDH, CHS-1, HIS3, ACT, TUB-2 and GS). The results of analysis confirmed the four species previously known as belonging to this species complex: *C. lindemuthianum*, *C. malvarum, C. orbiculare* and *C. trifolli*, and recognized four new species from weeds: *C. bidentis*, *C. sidae*, *C. spinosum* and *C. tebeestii*.

Watermelon is susceptible to numerous plant pathogenic fungi. The main concern is related to leaf blight (*Alternaria cucumerina*), gummy stem blight (*Didymella bryoniae*), anthracnose (*Colletotrichum lagenarium*) and fusarium wilt (*Fusarium oxysporum* f.sp. *niveum*) (Bulajić *et al.* 2008). The occurrence of anthracnose on watermelon fruits has been found in Serbia during several last years. Economic losses caused by the disease are mainly attributed to lower fruit quality and marketability.

Controls of anthracnose on watermelon are currently limited to the use of cultural and chemical control methods. In search of alternatives, biological control has emerged as a way of managing this disease. *T. harzianum and G. roseum* are the most common fungal biological control agents (BCAs) that have been comprehensively researched and deployed throughout the world (Janisiewicz and Korsten, 2002).

The objectives of the present study were: (a) identifying the species of *Colletotrichum* causing the anthracnose on watermelon fruit using both classical and molecular techniques, and (b) evaluate the antagonistic effect of *T. harzianum* and *G. roseum* against *Colletotrichum* spp. originated from watermelon fruits.

MATERIAL AND METHODS

Isolates

Watermelon fruits with typical anthracnose lesions were collected during 2015 in the area of Ašanja, Srem district. Symptoms on infected fruits appeared as sunken, dark brown to black lesions with orange conidial masses produced in black acervuli (Figure 1). Pieces of the diseased tissues were sterilized in 3% NaOCl for 3 min, followed by several rinses with sterile distilled water, and placed on PDA in Petri plates at 25 ºC for 5 days. Monoconidial cultures were produced for each isolate and mainteined on PDA slants at 4 °C.

Pathogenicity test

Pathogenicity tests with three representative isolates (LC1, LC2 and LC3) were conducted on mature and symptomless watermelon fruits. The fruits were cleaned and surface sterilized with ethanol (70%). Mycelial PDA discs of 5 mm were taken from a 14-day-old culture of each isolate and deposited on watermelon fruits superficially wounded with a sterile scalpel. In control fruits, only PDA disks without fungal mycelia were deposited onto wounds. The fruits were then incubated in a plastic container at 25 °C and >95% relative humidity, and examined for lesion development 10 days after inoculation. After 14 days, spores from diseased fruits were aseptically transferred onto PDA plates, which were incubated at 25 °C in darkness. The resultant cultures were checked for colony and spore morphology to confirm Koch's postulates.

Morphological identification

Macroscopic and microscopic morphology characteristics of three isolates were observed after growth on PDA for 7 days at 25 ºC under a 12 h light/dark cycle. Appressoria were produced using a slide culture technique (Johnston and Jones, 1997). Microscopic preparations were made in clear lactic acid. Length and width were measured for 100 conidia and shape of characteristic structures (conidiophores, conidia, setae, appressoria) was recorded using Olympus BX51 microscope.

Molecular identification

The selected isolates were transferred on PDA medium and allowed to grow for 7 day at 25 ºC. The isolation of DNA was performed with DNeasy Plant Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. All isolates were identified at the species level using a molecular strategy based

on sequences of the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) and partial sequences of the beta-tubulin 2 gene (TUB2).

The ITS and partial sequences of the TUB2 gene were amplified and sequenced using the primer pairs ITS1-F (Gardes and Bruns, 1993) and ITS4 (White *et al.* 1990); T1 (O'Donnell and Cigelnik, 1997) and Bt-2b (Glass and Donaldson, 1995). The PCRs were performed in a 2720 Thermal Cycler (Applied Biosystems, Foster City, California) in a total volume of 25 µl. The ITS and TUB2 PCR mixture contained 12.5 µl 2 X PCR Master mix (K071, Fermentas, Lithuania), 9 µl RNase-free water, 1.25 µl each of both forward and reverse primers (100 pmol/µl, Metabion International, Deutschland) and 1 µl template DNA. Amplification conditions of ITS gene constituted an initial denaturation of 3 min at 94 °C followed by 35 cycles of 1 min denaturation at 94 °C, 1 min annealing at 50 °C, 1 min elongation at 72 °C and a final extension of 10 min at 72 °C, while the TUB2 PCR was performed at an initial denaturation step of 5 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 52 °C and 30 s at 72 °C, and a final extension step of 7 min at 72 °C.

Amplified products were analyzed by 1% agarose gel electrophoresis, stained with Midori Green DNA Stain (Nippon Genetics) and visualized under a UV transilluminator. Sequencing in both directions was performed on an automated sequencer (ABI 3730XL Automatic Sequencer Macrogen, Korea). Sequence generated in this study was subjected to a Megablast search analysis at NCBI's GenBank nucleotide database for sequence similarity. Sequence of Serbian representative isolates (LC1, LC2, LC3) was aligned by using ClustalW algorithm implemented in MEGA6 (Tamura *et al.* 2013).

Alignment consisted of ITS and TUB2 sequences from all available isolates of species of the *Colletotrichum* group with outgroup species. Gene regions were aligned separately and concatenated into a single alignment. Sequences were initially aligned using Clustal W algorithm (Thompson *et al.* 1994) and manually adjusted in MEGA6 (Tamura *et al.* 2013). Phylogenetic analyses were constructed by the Neighbor-Joining (NJ) algorithm implemented in MEGA6 using 46 isolates of *C. orbiculare* and related *Colletotrichum* species and the outgroup *C. gloeosporioides* (Table 1). Sequences from isolates LC1, LC2 and LC3 were included in the analysis. The reliability of the obtained tree was evaluated using the bootstrap method based on 1,000 replicates and bootstrap values <50% were omitted.

Antagonistic activity *in vitro*

T. harzianum (DSM 63059) and *G. roseum* (DSM 62726), employed for *in vitro* antagonistic activity were obtained from German Collection of Microorganisms and Cell Cultures (DSMZ). The assay was performed on PDA by dual culture method. Plates inoculated only with three tested isolates served as controls. After 10 days at 25 ºC the percent growth inhibition (PGI) was calculated using the formula: PGI (%) =KR-R1/KR x 100, where KR is the colony

diameter in control plate without antagonist, and R1 is the colony diameter in treated plate (Skidmore and Dickinson, 1976). Hyphal interaction and morphology were observed with Olympus BX51 microscope.

Table 1. Stains of *Colletotrichum* spp. with collection details and GenBank accessions. Strains studied in this paper are in bold.

Taxon	Isolate name[a]	Host	Country	GenBank accessions	
				ITS	TUB2
C. bidentis	COAD 1020, CPC 21930	*Bidens subalternans*	Brazil	KF178481	KF178602
C. gloeosporioides	IMI 356878, CBS 112999, ICMP 17821, STE-U 4295	*Citrus sinensis*	Italy	JQ005152	JQ005587
C. lindemuthianum	CBS 523.97, LARS 798	*Phaseolus coccineus*	Costa Rica	JX546815	JX546861
C. lindemuthianum	CBS 524.97, LARS 800	*Phaseolus coccineus*	Costa Rica	JX546816	JX546862
C. lindemuthianum	CBS 571.97, LARS 83	*Phaseolus vulgaris*	Brazil	JX546818	JX546864
C. lindemuthianum	CBS 569.97, ATCC 56897, LARS 9	*Phaseolus vulgaris*	Europe	JX546817	JX546863
C. lindemuthianum	CBS 151.56, IMI 063364, ATCC 12611, UCLAF 230	*Phaseolus vulgaris*	France	JX546812	JX546858
C. lindemuthianum	CBS 143.31	*Phaseolus vulgaris*	Germany	JX546808	JX546854
C. lindemuthianum	CBS 144.31	*Phaseolus vulgaris*	Germany	JQ005779	JQ005863
C. lindemuthianum	CBS 146.31	*Phaseolus vulgaris*	Germany	JX546809	JX546855
C. lindemuthianum	CBS 147.31	*Phaseolus vulgaris*	Germany	JX546810	JX546856
C. lindemuthianum	CBS 150.28	*Phaseolus vulgaris*	Germany	JX546811	JX546857
C. lindemuthianum	CBS 151.28	*Phaseolus vulgaris*	Germany	GU227800	GU228094
C. lindemuthianum	CBS 152.28	*Phaseolus vulgaris*	Netherlands	JX546813	JX546859
C. lindemuthianum	CBS 153.28	*Phaseolus vulgaris*	Netherlands	JX546814	JX546860
C. lindemuthianum	CBS 130841, CIKY1	*Phaseolus vulgaris*	USA	JX546819	JX546865
C. lindemuthianum	CBS 131.57	*Phaseolus vulgaris*	USA	JX546805	JX546851
C. lindemuthianum	CBS 132.57	*Phaseolus vulgaris*	USA	JX546806	JX546852
C. lindemuthianum	CBS 133.57	*Phaseolus vulgaris*	USA	JX546807	JX546853
C. malvarum	CBS 521.97, LARS 720, Lav-4	*Lavatera trimestris*	UK	KF178480	KF178601
C. malvarum	CBS 123.24	*Malvaceae*	unknown	KF178479	KF178600
C. orbiculare	CBS 129432, USYD-2008-01	*Benincasa hispida*	Australia	KF178469	KF178590
C. orbiculare	CBS 133194, KTU-H1	*Cucumis melo*	Japan	KF178459	KF178580
C. orbiculare	CBS 133195, KTU-H2	*Cucumis melo*	Japan	KF178460	KF178581
C. orbiculare	CBS 133196, KTU-H5	*Cucumis melo*	Japan	KF178461	KF178582
C. orbiculare	CBS 133197, KTU-K5	*Cucumis melo*	Japan	KF178468	KF178589
C. orbiculare	CBS 133198, KTU-K6	*Cucumis melo*	Japan	KF178458	KF178579
C. orbiculare	CBS 570.97, LARS 73	*Cucumis sativus*	UK	KF178466	KF178587
C. orbiculare	CBS 514.97, 104-T, LARS 414	*Cucumis sativus*	Japan	JQ005778	JQ005862
C. orbiculare	CBS 274.54	*Cucumis sativus*	Netherlands	KF178462	KF178583
C. orbiculare	CBS 172.59	*Cucumis sativus*	Netherlands	KF178464	KF178585
C. orbiculare	CBS 173.59, IMI 213974	*Cucumis sativus*	Netherlands	KF178463	KF178584
C. orbiculare	CBS 122.24	*Cucumis sativus*	UK	KF178467	KF178588
C. orbiculare	CBS 107.17	unknown	unknown	KF178465	KF178586
C. orbiculare	**LC1**	***Citrullus lanatus***	**Serbia**	**KT454386**	**KT581236**
C. orbiculare	**LC2**	***Citrullus lanatus***	**Serbia**	**KT454387**	**KT581237**
C. orbiculare	**LC3**	***Citrullus lanatus***	**Serbia**	**KT454388**	**KT581238**
C. sidae	CBS 504.97, LARS 76, ATCC 58399, NRRL 8096	*Sida spinosa*	USA	KF178472	KF178593
C. sidae	CBS 518.97, LARS 629, Cm-4	*Sida spinosa*	USA	KF178471	KF178592
C. sidae	CBS 574.97, LARS 625, ATCC 96725, 3-1-1, Cm-9	*Sida spinosa*	USA	KF178471	KF178591
C. spinosum	CBS 113171, IMI 368075, STE-U 5296	*Xanthium spinosum*	Argentina	KF178475	KF178596
C. spinosum	CBS 515.97, LARS 465, DAR 48942	*Xanthium spinosum*	Australia	KF178474	KF178595
C. tebeestii	CBS 522.97, LARS 733, 83-43	*Malva pustilla*	Canada	KF178473	KF178594
C. trifolii	CBS 128554, ICMP 12934, LARS 164, N85 ANW	*Medicago sativa*	USA	KF178476	KF178597
C. trifolii	CBS 158.83, BBA 70709	*Trifolium*	USA	KF178478	KF178599
C. trifolii	CBS 425.83	unknown	unknown	KF178477	KF178598

RESULTS AND DISCUSSION

The symptoms of watermelon fruits begin as small, sunken lesion that have a water-soaked appearance, increase in diameter and coalesce, leaving a large sunken soft area. The necrotic spots can expand and merge to cover the whole affected area. The color of the infected part darkens. Orange conidial masses may occur scattered or in concentric rings on the lesion (Figure 1).

All tested isolates caused anthracnose lesions on watermelon fruit after 10 to 14 days of incubation. No lesions developed on fruit inoculated with non colonized PDA disk. Koch's postulates were fulfilled by reisolation from inoculated watermelon fruits. Conidia shape, size, and colony morphology were identical for the original and recovered isolates.

Macroscopic and microscopic morphology characteristics of isolates LC1, LC2 and LC3 were uniform. Fungal colonies were dense aerial, initially white, becoming gray and then turning black, as the cultures aged on PDA. Colony reverse was gray to dark gray. The cultures developed black acervuli around the center of the colony. Mycelia were branched, septate, and hyaline. Conidiophores and setae formed directly from hyphae. Setae were brown, smooth-walled, 1-5 septate, 30–120 µm long. Conidia were hyaline, aseptate, straight, cylindrical to clavate, with one end round and the other truncate, 9-(10.5)-12.5 µm x 4-(4.5)-5.5 µm (Figure 2). Appressoria were single, dark brown, smooth-walled, ovate or clavate, 5.5-(6)-7.5 µm x 4.5-(5.5)-6 µm.

The morphological characteristics of our isolates are similar to those reported by Damm *et al.* (2013). However, definitive identification of *Colletotrichum* species based on morphology is difficult because isolates have overlapping ranges of conidial and colony characteristics, and because variation in morphology is accepted for isolates within a species (Sutton, 1992).

Figure 1. Anthracnose symptoms on watermelon fruit:
sunken necrotic lesion with orange conidial masses and black acervuli.

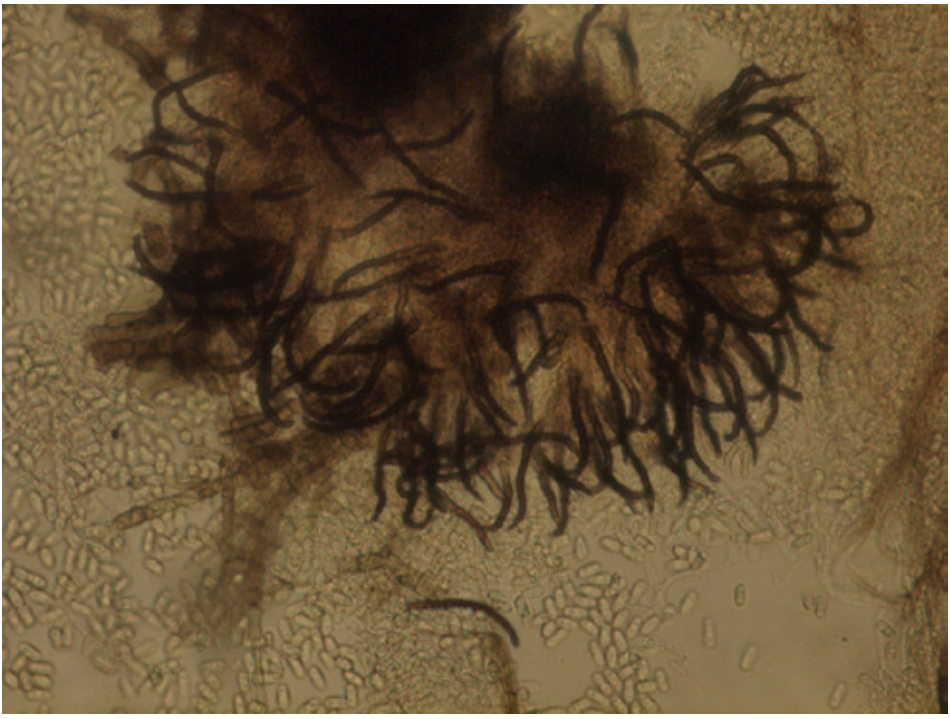

Figure 2. Morphological characteristics of isolate LC1: acervuli, setae and conidia (x400).

PCR amplifications of the ITS and TUB2 gene gave a fragment of the expected size (approximately 600 and 800 bp) and their sequences were used for classification based on a BLAST analysis.

The sequence analysis of ITS region revealed that the Serbian isolates LC1, LC2 and LC3 (GenBank Accession No. KT454386, KT454387 and KT454388) shared 100% identity with *C. orbiculare* isolate deposited in the GenBank from South Korea (JX997422). BLAST analysis of the TUB2 sequences of the three Serbian *C. orbiculare* isolates LC1, LC2 and LC3 (GenBank Accession No. KT581236, KT581237 and KT581238) shared the highest identities with 23 Indian *C. orbiculare* isolates (KP899039-61) from *Citrullus lanatus* and two Japanese *C. orbiculare* isolates (JQ005862 and KF178579).

A neighbor-joining tree (Figure 4) of 46 *Colletotrichum* species and the outgroup (*C. gloeosporioides*) was constructed based on combined alignment of ITS and TUB2 genes. Phylogenetic analysis resulted in detection of three main clades and 9 subclades within the *C. orbiculare* species complex. The first main clade is formed by *C. lindemuthianum* strains and is well supported with a bootstrap support of 99%. The second main lineage is represented by a single strain of *C. bidentis*. The third main clade consists of six subclades: the clades

representing *C. trifolii* and *C. malvarum* are well supported and grouped with each other. A sister clade is formed by *C. orbiculare* containing the largest number of strains with two smaller subclades representing *C. sidae* and *C. tebeestii* as well as a single-clade representing *C. spinosum*.

The results of our study showed that, all of the three isolates obtained from diseased tissues of watermelon in Serbia belonged to the *C. orbiculare*. Our isolates, together with isolates from Japan, the UK and the Netherlands were clustered in the branch of clade *C. orbiculare*, with high bootstrap support of 99%. The overall shape of the *Colletotrichum* reconstructed phylogenetic tree was similar to those previously reported and phylogenetic analysis resulted in the delineation of three main clades as determined by the most recent comprehensive study (Damm *et al.* 2013). Presently however, not all *Colletotrichum* species and species complexes are sufficiently known from DNA sequence data and some of them might have an intermediate position between *C. orbiculare* and other species complexes (Damm *et al.* 2013). Different gene sequences of *Colletotrichum* can be used for the detection of these taxa from *Colletotrichum* at generic level and have been successfully applied in the characterization of several *Colletotrichum* species.

Results from dual culture assay showed that *T. harzianum* had significantly greater inhibitory activity against *C. orbiculare* than the *G. roseum in vitro. T. harzianum* exhibited the strong antagonism against isolates LC1, LC2 and LC3 with a high PGI value (69%, 67%, and 70% respectively). No distinct inhibition zones were observed between antagonistic fungus and pathogens. Major mechanisms involved in the antagonistic activity of *Trichoderma* spp. were competition for space and nutrients, production of diffusible and/or volatile antibiotics, and hydrolytic enzymes like chitinase and β-1,3-glucanase (Howell, 2003). These hydrolytic enzymes partially degrade the pathogen cell wall and lead to its parasitization (Kubicek *et al.* 2001). Microscopic examination revealed that antagonist caused a wide spectrum of mycelial malformation of all tested *C. orbiculare*: abnormal stunted, highly branched hyphal tips, swollen hyphae and the vacuolar appearance of the mycelium of pathogenic fungi. Similar results were reported by Gupta *et al.* (1995), Howell (2003) and Begum *et al.* (2008).

G. roseum presented a moderate antifungal effect *in vitro* on isolates of *C. orbiculare*, LC1 (40%), LC2 (35%) and LC3 (38%). After 10 days of incubation a very weak inhibition zones were observed between *G. roseum* and all tested pathogens (2–3 mm). In these study hyphae of *G. roseum* were never observed to overlap the colony of *C. orbiculare*. In all cases isolates of *Colletotrichum* stopped growing before direct contact was made, presumable in response to diffusible inhibitors released by the antagonist. These results were similar to the results revealed by Lee and Wu (1984).

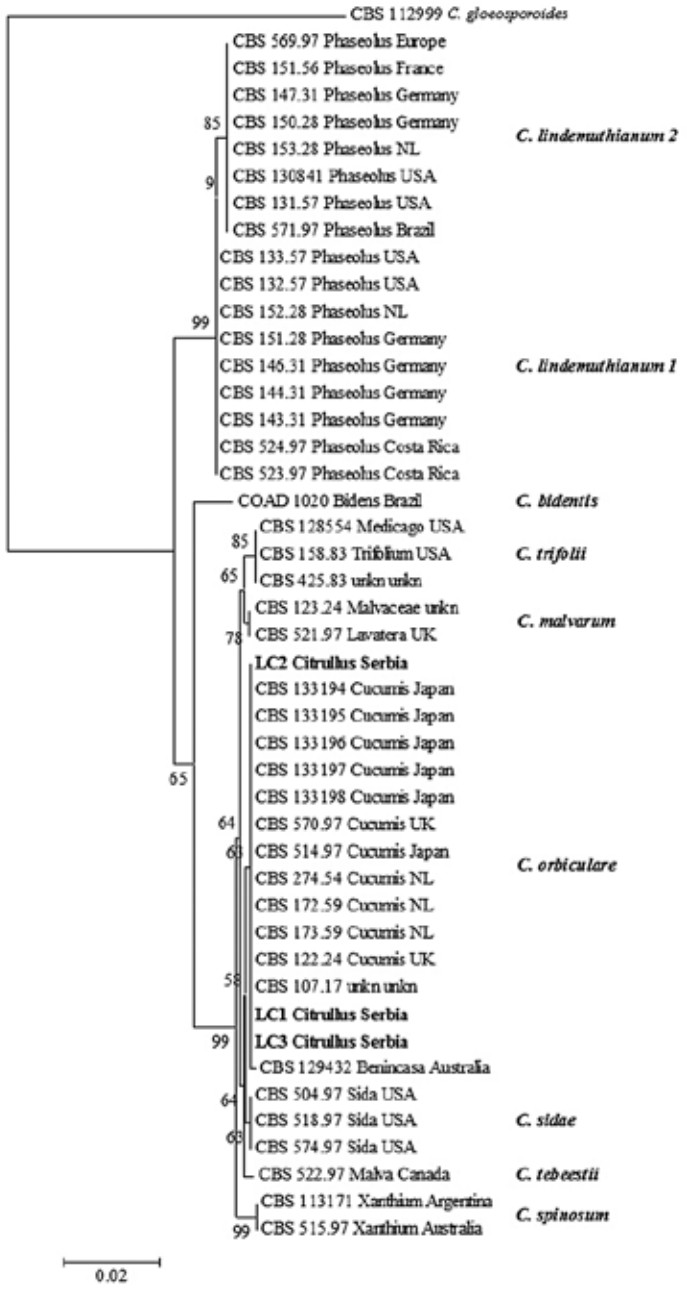

Figure 3. Neighbour-Joining tree based on analysis of combined alignment of ITS and TUB2 genes containing for 46 isolates of *Colletotrichum* species. *Colletotrichum gloeosporioides* CBS 112999 is used as outgroup. Bootstrap analysis was performed with 1,000 replicates and bootstrap values (>50%) are shown next to relevant branches. The Serbian *Colletotrichum* isolates are bolded.

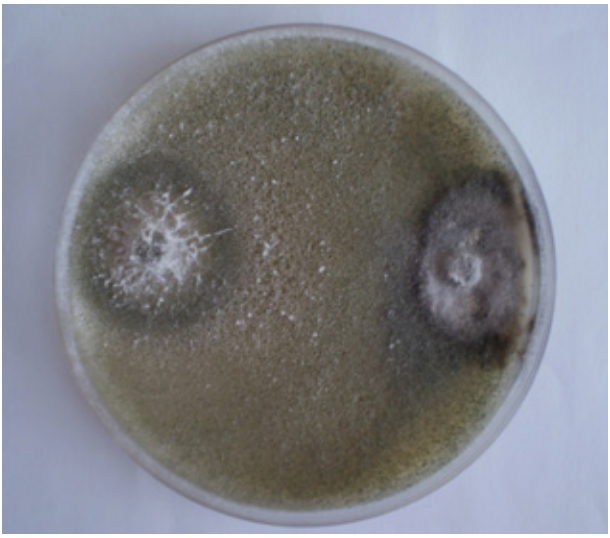

Figure 4. Antagonistic activity of *T. harzianum* against *C. orbiculare* – isolate LC1.

CONCLUSION

Identification of *Colletotrichum* spp. is a fundamental criterion in the development of more efficient control measures. In the present study all of the three isolates of *Colletottrichum* spp. from watermelon fruits were morphologically identified as *C. orbiculare* and species identification was confirmed by PCR and sequencing. To our knowledge, this is the first molecular and phylogenetic analysis of *C. orbiculare* in Serbia. The results of antagonistic activity *in vitro* identify *T. harzianum* and *G. roseum* as promising BCAs for further testing against anthracnose disease on watermelon fruits.

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Colletotrichum orbiculare СА ЛУБЕНИЦЕ: ИДЕНТИФИКАЦИЈА И *IN VITRO* ИНХИБИЦИЈА ГЉИВАМА АНТАГОНИСТИМА

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РЕЗИМЕ: Антракноза проузрокова на гљивом *Colletotrichum orbiculare* једна је од најзначајнијих болести на биљкама рода *Cucurbitaceae*. У Србији су током 2015. године прикупљени плодови лубенице с типичним антракнозним лезијама. Инфицирани плодови су са улегнутим, тамно браон до црним лезијама и масом наранџастих конидија из ацервула. У циљу идентификације проузроковача болести, с некротичног ткива узети су фрагменти, површински стерилисани и засејани на кромпир декстрозни агар (КДА). Макроскопске и микроскопске морфолошке карактеристике три изолата проучавана су након седам дана инкубације на температури од 25 ºC у условима 12^h светло/мрак. Гљиве формирају колоније беле, сиве до црне боје са густом, ваздушастом мицелијом. Конидије су хијалинске, несептиране, праве, цилиндричне до облика палице, величине 9–12,5 μm × 4–5,5 μm. Карактеризација изолата обављена је секвенцирањем ITS rDNA региона коришћењем прајмера ITS1F/ITS4 и β-tubulin 2 гена помоћу T1/Bt2b прајмера. Нуклеотидне секвенце су депоноване у NCBI банку гена (ITS Acc. No. KT454386, KT454387

и KT454388; β- тубулин 2 ген Acc. No. KT581236, KT581237 и KT581238). BLAST анализа секвенци ITS и β-tubulin 2 гена је показала да су наши изолати 100% идентични с другим *C. orbiculare* врстама из NCBI базе. Тест патогености је обављен на одабраним, здравим плодовима лубенице. Сви испитивани изолати проузрокују антракнозне лезије на плодовима лубенице, десет дана након инокулације. Антагонистички потенцијал гљива *Trichoderma harzianum* (DSM 63059) и *Gliocladium roseum* (DSM 62726) испитиван је *in vitro* према изолатима *C. orbiculare*. *T. harzianum* и *G. roseum* су резултатима ових истраживања идентификовани као биолошки агенси који се могу успешно укључити у будућа тестирања у циљу сузбијања антракнозе плодова лубенице.

КЉУЧНЕ РЕЧИ: *Colletotrichum orbiculare*, лубеница, идентификација, гљиве антагонисти