SCREENING OF ANTAGONISTIC ACTIVITY OF MICROORGANISMS AGAINST *COLLETOTRICHUM ACUTATUM* AND *COLLETOTRICHUM GLOEOSPORIOIDES*

SVETLANA ŽIVKOVIĆ[1], S. STOJANOVIĆ[1], Ž. IVANOVIĆ[1], V. GAVRILOVIĆ[1], TATJANA POPOVIĆ[2], and JELICA BALAŽ[3]

[1]*Institute for Plant Protection and Environment,* 11000 Belgrade, Serbia
[2]*Luxembourg Industries d.o.o,* 21000 Novi Sad, Serbia
[3]*Faculty of Agriculture, University of Novi Sad,* 21000 Novi Sad, Serbia

Abstract – The antagonistic activities of five biocontrol agents: *Trichoderma harzianum, Gliocladium roseum, Bacillus subtilis, Streptomyces noursei* and *Streptomyces natalensis*, were tested in vitro against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*, the causal agents of anthracnose disease in fruit crops. The microbial antagonists inhibited mycelial growth in the dual culture assay and conidial germination of *Colletotrichum isolates*. The two Streptomyces species exhibited the strongest antagonism against isolates of *C. acutatum* and *C. gloeosporioides*. Microscopic examination showed that the most common mode of action was antibiosis. The results of this study identify *T. harzianum, G. roseum, B. subtilis, S. natalensis* and *S. noursei* as promising biological control agents for further testing against anthracnose disease in fruits.

Keywords: Antagonistic activity, *Colletotrichum acutatum, Colletotrichum gloeosporioides, Trichoderma harzianum, Gliocladium roseum, Bacillus subtilis, Streptomyces noursei, Streptomyces natalensis*

INTRODUCTION

The genus *Colletotrichum* and its teleomorph *Glomerella* contain an extremely diverse number of fungi including both plant pathogens and saprophytes. Plant pathogenic species are important worldwide, causing pre- and post-harvest losses of crops. These fungi cause diseases commonly known as anthracnose of grasses, legumes, vegetables, fruits and ornamentals. The disease can occur on leaves, stems, and fruit of host plants (Sutton, 1992).

Anthracnose caused by the fungi *C. acutatum* J.H. Simmonds and *C. gloeosporioides* (Penz.) Penz. & Sacc. is an important disease in Serbia. Several host species can be affected, including the sour cherry (Arsenijević, 1984; Ivanović and Ivanović, 1992), apple (Trkulja, 2003), strawberry (Ivanović et al., 2007), pear and tomato fruits (Živković et al., 2008; 2009a). Disease outbreaks can occur rapidly and losses can be severe, especially under prolonged warm and wet weather conditions. Typical fruit symptoms include dark, sunken, and circular lesions that produce mucilaginous, pink to orange conidial masses. Under severe disease pressure, the lesions can coalesce. Affected fruits can drop prematurely from the plant and further losses occur through post-harvest infection in storage bins.

Effective control of anthracnose disease involves the use of one of, or a combination of, the following: resistant cultivars, cultural control and chemical control. The intensive use of fungicides has resulted in the accumulation of toxic compounds potentially hazardous to humans and the environment, and also in the build-up of resistance of the pathogens. In view of this, investigation and the application of biological control agents (BCAs) seems to be one of the promising approaches (Cook, 1985). Biocontrol involves the use of naturally occurring nonpathogenic microorganisms that are able to

reduce the activity of plant pathogens and thereby suppress diseases. Antagonistic microorganisms can compete with the pathogen for nutrients, inhibit pathogen multiplication by secreting antibiotics or toxins, or reduce pathogen population through hyperparasitisms.

The filamentous fungi, *Trichoderma* and *Gliocladium*, are well studied and have shown efficiency in the biocontrol of different phytopathogens such as *Alternaria, Botrytis, Colletotrichum, Diaporthe, Fusarium, Monilinia, Phytophthora, Phythium, Rhizoctonia, Sclerotinia*, and V*erticillium* (Bell et al., 1982; Yu and Sutton, 1997; Balaž et al., 2000; Begum et al., 2008; Hajieghrari et al., 2008; Imtiaj and Lee, 2008). Many strains of *Trichoderma* are strong opportunistic invaders, fast growing, prolific producers of spores and powerful antibiotic producers (Woo et al., 2006). The *Gliocladium* species are effective and versatile antagonists. These fungi have the advantage of abundant production and the long-term viability of inoculum attributes of key importance for commercial use (Sutton et al., 1997).

The strains of bacteria *Bacillus* and actinomycetes, especially those belonging to the genus *Streptomyces,* have been widely used against a number of economically important plant pathogenic fungi. *Bacillus* spp. have special characteristics that make them good candidates as BCAs. The strains of this genus are ubiquitous. They possess a resistant spore stage, produce several kinds of antifungal compounds and have showed significant inhibitory activity against *Ceratocystis ulmi* (Gregory et al., 1986), *Puccinia pelargonii-zonalis* (Rytter et al., 1989), *Euthypa lata* (Ferreira et al., 1991), *Fusarium moniliforme* (Agarry et al., 2005), *Colletotrichum, musae* (Mahadtanapuk et al., 2007; Alvindia and Natsuaki, 2009), and *C. gloeosporioides* (Havenga, 1999; Demoz and Korsten, 2006). The antagonistic activity of *Streptomyces* is usually related to the production of extracellular hydrolytic enzymes and secondary antifungal metabolites. The *Streptomyces* species have the potential for biological control of fungal diseases caused by *Phytophthora capasici* (Jo, 2005), *Phyto-*

phthora cinnamomi (Aryanta and Guest, 2006), *F. oxysporum, Botrytis cinerea,* and *Monilinia. laxa* (Lu et al., 2008), *Sclerotium rolfsii* and *C. gloeosporioides* (Prapagdee et al., 2008).

With the exception of preliminary reports (Živković et al., 2009b; 2009c), there are no publications on the biocontrol of anthracnose disease of fruits in Serbia using antagonistic microorganisms. Therefore, the objective of this study was to investigate *in vitro* the interactions of *T. harzianum, G. roseum, B. subtilis, S. noursei* and *S. natalensis* with isolates of *C. acutatum* and *C. gloeosporioides* from various fruits, to better understand their possible activity as BCAs.

MATERIALS AND METHODS

Pathogens

Five representative monoconidial isolates of *C. acutatum* and *C. gloeosporioides* were isolated from anthracnose lesions on pear, apple, sour cherry and tomato fruits (Culture Collection of Institute for Plant Protection and Environment, Belgrade). The reference isolates of *C. acutatum* (CBS 294) and *C. gloeosporioides* (CBS 516) were obtained from the Fungal Biodiversity Centre, Netherlands. The host origin and the data of the collection of isolates are indicated in Table 1. Stock cultures of each isolate were maintained on potato dextrose agar (PDA) at 4°C. Working cultures were established by transferring a stock agar plug containing the mycelium of each isolate onto PDA in Petri dishes and incubating for 7 days in darkness at 25°C.

Antagonists

The antagonistic microorganisms *T. harzianum* (DSM 6305), *G. roseum* (DSM 62726), *S. noursei* (DSM 40635), *S. natalensis* (DSM 40357) and *B. subtilis* (CFBP 4228), employed for the *in vitro* antimicrobial assay were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ), and the French Collection of Plant Pathogenic Bacteria (CFBP). *B. subtilis* was maint-

ained on nutrient agar (NA). The two *Streptomyces* species were maintained on glucose yeast extract - malts extract (GYM) agar, and PDA was used for the routine cultivation of fungal antagonists.

Antagonistic activity in vitro

The assay for antagonism was performed on PDA on Petri dishes by the dual culture method (Fokkema, 1978). The mycelial plugs (5 mm diameter) of pathogens and fungal antagonists were placed on the same dish 6 cm from each other. Isolates of C. *acutatum* and C. *gloeosporioides* were plated 3 days earlier than *T. harzianum*, reflecting the slow growth of these pathogens in culture. To test for antagonistic bacteria, a 5 mm of mycelia agar disc from pathogen cultures was placed on the one side of a Petri dish containing PDA medium. The dishes were incubated at 25°C for 24 h. A loopful of bacteria was then streaked 3 cm away from the disc of *Colletotrichum* isolates on the same dish. Paired cultures were incubated at 25°C. Dishes inoculated only with test pathogens served as controls. The experiment was repeated twice with three replications of each treatment. The percent growth inhibition (PGI) was calculated using the formula:

Table 1. Isolates of C. acutatum and C. gloeosporioides

Isolate code	Species	Host origin	Year of isolation
KC-21	C. acutatum	pear	2006
KC-23	C. acutatum	pear	2007
KC-82	C. acutatum	pear	2007
JC-4	C. acutatum	apple	2006
PC-3	C. acutatum	tomato	2007
CBS 294	C. acutatum	apple	-
KC-9	C. gloeosporioides	pear	2005
KC-11	C. gloeosporioides	pear	2005
JC-7	C. gloeosporioides	apple	2007
VC-7	C. gloeosporioides	sour cherry	2007
VC-9	C. gloeosporioides	sour cherry	2008
CBS 516	C. gloeosporioides	apple	-

$$PGI\ (\%) = KR\text{-}R1/KR \times 100,$$

where *KR* represents the distance (measured in mm) from the point of inoculation to the colony margin on the control dishes, and *R1* the distance of fungal growth from the point of inoculation to the colony margin on the treated dishes in the direction of the antagonist (Korsten et. al., 1995). The PGI was categorized on a growth inhibition category (GIC) scale from 0 to 4, where 0 = no growth inhibition; 1 = 1-25% growth inhibition; 2 = 26-50% growth inhibition; 3 = 51-75% growth inhibition; 4 = 76-100% growth inhibition.

The zone of inhibition was recorded as the distance between the fungal pathogen and the area of antagonist growth after 7 days.

T. harzianum, *G. roseum*, *S. noursei* and *S. natalensis* were tested for both antibiosis and mycoparasitic activities against isolates of C. *acutatum* and C. *gloeosporioides*. The edges of the parasitized pathogen hyphae by microbial antagonists were transferred from the dual culture dish onto clean slides after 7 days of incubation. Cover slips were mounted on the mycelia with a drop of lactophenol cotton blue (LCB). Hyphal interaction and morphology were examined under a light microscope.

Inhibition of conidial germination of C. acutatum and C. gloeosporioides

For the preparation of the bacteria suspension, a loopful of *B. subtilis* grown on an NA slant for 48 h was cultured in 250 ml Erlenmeyer flasks containing 100 ml nutrient broth. After 24 h of incubation with continuous shaking (70 rpm) at 28°C, the cells were harvested by centrifugation for 10 min at 12 000 rpm. The resulting pellet was dissolved in 10 ml of sterile distilled water. The cell concentration was adjusted to 10^8 cfu/ml with a spectrophotometer. Inoculums of the two antagonistic *Streptomyces* were produced on GYM agar for 5 days at 25°C. The mature spores were washed from the medium with 10 ml of sterile distilled water and diluted into the suspension in the concentration of 10^7 spores/ml. The cultures of

pathogens and antagonistic fungi were grown on PDA, and incubated for 7 days at 25°C. Spores were harvested by flooding the plates with 10 ml of sterile distilled water and Tween 20 (v/v 0.01%), scraping with a rubber spatula, and then filtering the suspension through double layers of cheesecloth to remove the mycelial fragments. The concentration of spores in the suspensions were determined with a hemacytometer and adjusted to 10^7 spores/ml. Fifty (50) μl of the standardized suspensions of antagonists and *Colletotrichum* isolates were mixed and transferred to sterile microscope slides. The control consisted of suspensions of *Colletotrichum* conidia in sterile distilled water. The slides were than incubated in moist chambers for 24 h using three replicate slides for each pathogen isolate. At the end of the incubation period, a drop of LCB was added to each slide to arrest germination. In this context germination was defined as a germ tube that had developed to longer than half of the cell length. The percent of germination was determined by counting 100 conidia from each pathogen isolate under a light microscope and determining the proportion that had germinated.

Statistical analysis

Basic statistical parameters were calculated and the obtained information was presented through a box-plot. Homogeneity of variances was analyzed by Levene's test. All of the comparisons of means of inhibition of mycelial growth and inhibition of conidial germination were subjected to a two factorial analysis of variance (MANOVA). The significant differences between the treatments were determined by the t-test (p=0.05). All statistical analyses were performed using STATISTIKA v.6 (StatSoft, Inc.) software.

RESULTS

Antagonistic activity in vitro

Results from the dual culture assay showed that all antagonistic microorganisms inhibited the mycelial growth of *C. acutatum* and *C. gloeosporioides*, with varying efficiencies (Figs. 1 and 2). Two fungal antagonists presented a moderate inhibition against majority of *Colletotrichum* isolates and belonged to
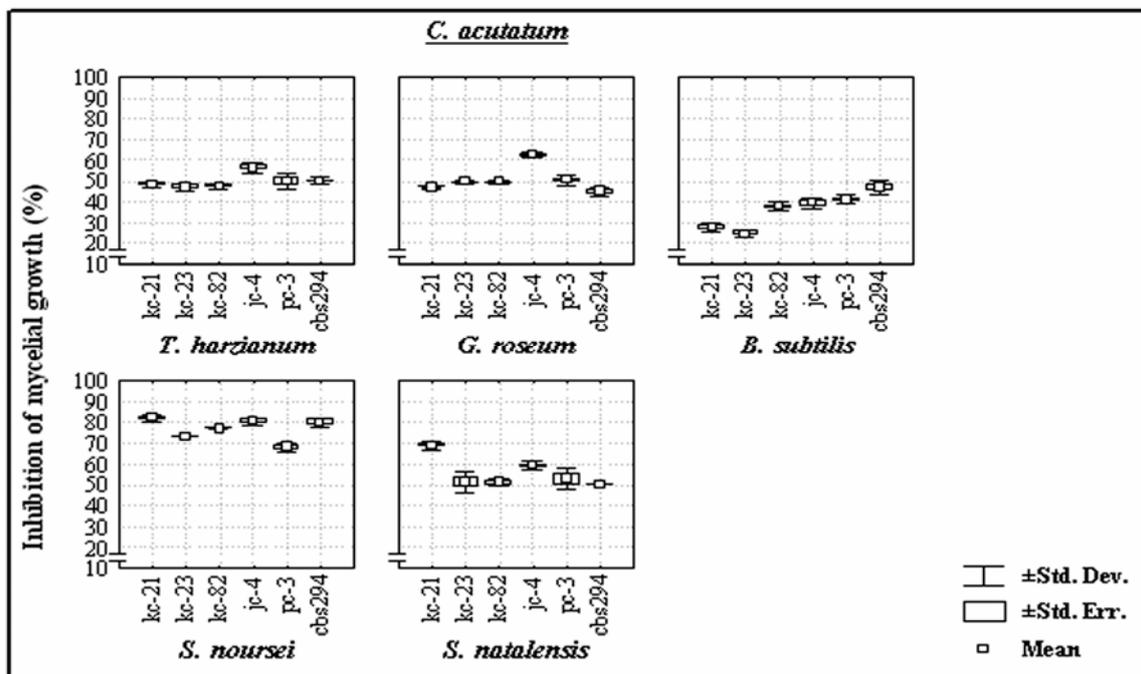

**Fig. 1.** Basic statistical parameters of inhibition of mycelial growth of *C. acutatum*.
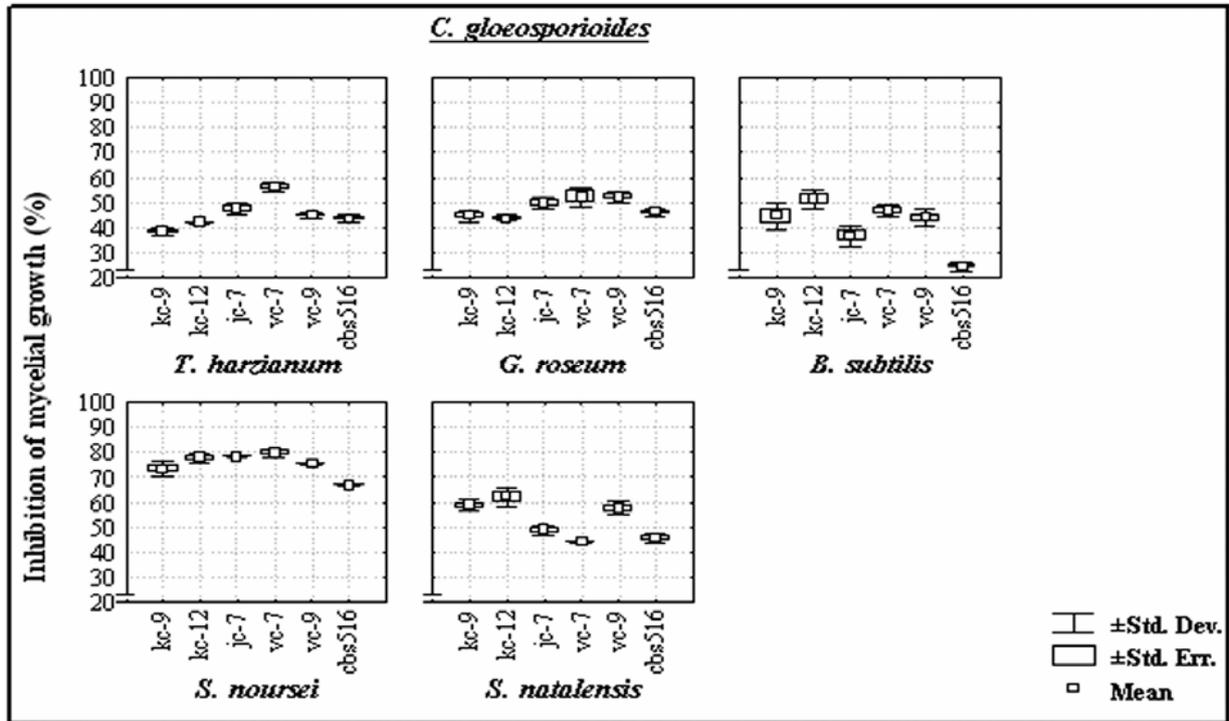
Fig. 2. Basic statistical parameters of inhibition of mycelial growth of *C. gloeosporioides*.

growth inhibition categories (GIC) 2 and 3. *T. harzianum* and *G. roseum* reduced the radial growth of the pathogens by more than 38% and 43%, respectively. Among the five microbial antagonists, *S. noursei* significantly exhibited the strongest antagonism against the isolates of *C. acutatum* and *C. gloeosporioides* with a high PGI value (67–82%), followed by *S. natalensis* (45–69%). Based on the *in vitro* dual culture experiment, *S. noursei* was classified in GIC 3 and 4, and *S. natalensis* in GIC 2 and 3. *B. subtilis* showed varying degrees of inhibitory ability against *C. acutatum* and *C. gloeosporioides*. The percentage inhibition in radial growth varied from 25% to 48%, GIC 2.

The Levene's test showed a nonhomogeneity of variances for the isolates of *C. acutatum* (F=3.130, p=0.000) and *C. gloeosporioides* (F=2.749, p=0.000).

The results of the analysis of variance (Table 2.) confirmed statistically very significantly differences (p<0.01) in the antagonistic abilities of *T. harzianum*,

G. roseum, *B. subtilis*, *S. noursei* and *S. natalensis* against fungal pathogens. The isolates of *C. acutatum* and *C. gloeosporioides* showed very significant differences in mycelial growth in the dual culture assay. Interactions between the antagonists and the isolates of *C. acutatum* and *C. gloeosporioides* were also statistically very significant.

The T- test showed that *S. noursei*, *S. natalensis* and *B. subtilis* were mutually very different and in comparison with the two fungal antagonists in the inhibition of the mycelial growth of *C. acutatum* and *C. gloeosporioides*.

Comparisons between the isolates of *C. acutatum* using the t-test revealed that isolates from the pear fruit (KC-21, KC-23 and KC-82) were statistically mutually very different and in relation with the isolates originating from the apple and tomato (JC-4 and PC-3). The reference isolate, CBS 294, was very different from KC-23 and JC-4, and only different from isolate PC-3.

Table 2. Analysis of the variance of the mycelial growth inhibition of *C. acutatum* and *C. gloeosporioides* in dual culture assay with microbial antagonists.

Species	Source of variation	*df*	*MS*	*F*	*p-level*
C. acutatum	antagonists	4	3898.454	746.932	0.000**
	isolates	5	185.194	35.483	0.000**
	antagonists x isolates	20	108.198	20.730	0.000**
	error	60	5.219	-	-
C. gloeosporioides	antagonists	4	3202.442	545.193	0.000**
	isolates	5	229.295	39.036	0.000**
	antagonists x isolates	20	111.890	19.048	0.000**
	error	60	5.874	-	-

** = very significant, p<0.01

The results of the t-test confirmed very significant differences between the isolates of *C. gloeosporioides* from the pear (KC-9 and KC-12). The isolates VC-7 and VC-9 originating from sour cherry were statistically significantly different from KC-9 and JC-7. The reference isolate CBS 516 was very different from all other isolates of *C. gloeosporioides*. This strain was the most resistant to *in vitro* inhibition by the antagonists.

Distinct inhibition zones were observed when *G. roseum*, *B. subtilis*, *S. natalensis* and *S .noursei* were used against *Colletotrichum* isolates (Fig. 3 b-e, and Table 3). However, *S. noursei* gave strong and very strong inhibition zones (13 – 22 mm), compared to *S. natalensis* (8 – 18 mm), and *B. subtilis* (5 – 12 mm). Very weak inhibition zones were revealed between *G. roseum* and the isolates of *C. acutatum* and *C. gloeosporioides* (2 -5 mm) after 7 days of incubation. No distinct inhibition zones were observed between *T. harzianum* and the isolates of fungal pathogens (Fig. 3 a).

Microscopic examination of the dual culture assay showed an alternation of the mycelium of the pathogen where it was in contact with antagonist. The most common mode of action observed was antibiosis which appeared in the co-inoculated dishes as an inhibition zone. *G. roseum*, *B. subtilis*,
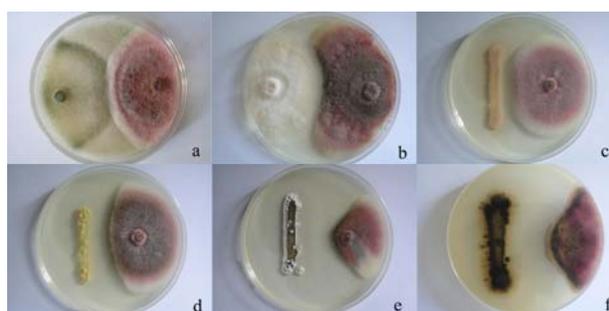

Fig. 3. Antagonistic zone between *T. harzianum* (a); *G. roseum* (b); *B. subtilis* (c); *S. natalensis* (d), *S. noursei* (e) and isolate KC-23 (*C. acutatum*); (f) Mycelial malformation of *C. acutatum* in dual culture assay with *S. noursei* (back side).

S. noursei and *S. natalensis* induced abnormally stunted, highly branched hyphal tips and swollen hyphae at the edges of the *C. acutatum* and *C. gloeosporioides* colonies (Fig. 3 f). Mycoparasitism was observed as coiling, penetration, direct contact and parallel growth alongside the host hyphae. *T. harzianum* hyphae grew initially alongside and coiled compactly around the hyphae of the isolates of *C. acutatum* and *C. gloeosporioides* (Fig. 4). The hyphae of the *Colletotrichum* isolates were observed to absorb a pigment that apparently originated from the antagonist, becoming red, before collapsing and dying (Fig. 5).
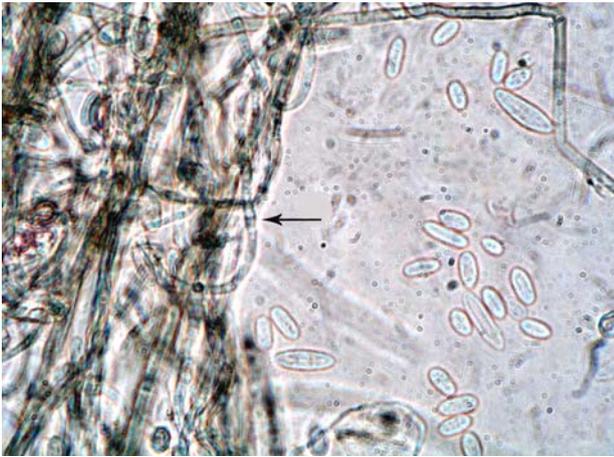
Fig. 4. Hyphal coiling by *T. harzianum.*
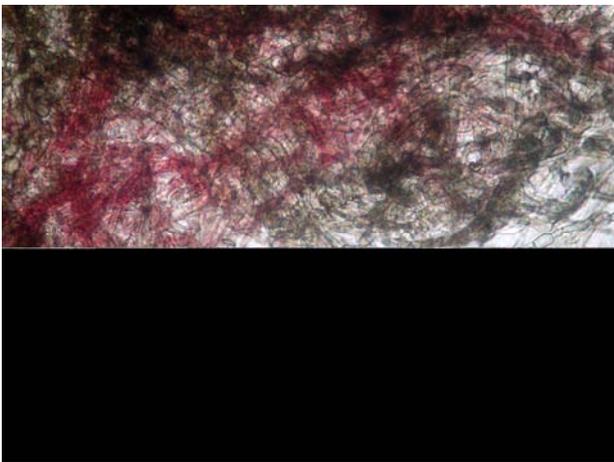
Fig. 5. Collapsing of hyphae of *C. acutatum.*

Fig. 6. Germination of conidia of C. *acutatum* in the control.
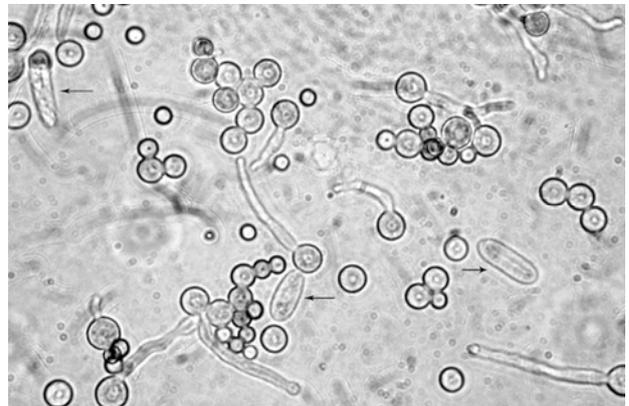
Fig. 7. Non-germinated conidia of *C. acutatum* parasitized by *T. harzianum.*

The conidia of *Colletotrichum* isolates incubated in water (control) at 25°C, swelled and after 4 h started to germinate, producing one or two germ tubes (Fig. 6). After 6 h about 50% of the conidia had germinated. However, conidia swellings were strongly limited in co-cultivation with the antagonists. They surrounded the spores of *C. acutatum* and *C. gloeosporioides* and inhibited their germination (Fig. 7). After 24 h of co-cultivation, there was significant inhibition of the germination in all mixtures with the antagonists (80– 99%), (Fig. 8 and 9). The results of inhibition on the conidial germination assay showed that the bacterial antagonist *S. noursei* presented a strong inhibitory activity against all of the *Colletotrichum* isolates (92-99%), followed by *S. natalensis* (89-96%), and *B. subtilis* (86-91%). Two fungal antagonists *T. harzianum* and *G. roseum* caused more than 86% and 89% inhibition of conidial germination, respectively. Conidia that were ungerminated after 24 h, did not germinate afterwards. The non-germinated conidia shrank and changed shape.

Inhibition of conidial germination of C. acutatum and C. gloeosporioides

The Levene's test showed a homogeneity of the variances for the isolates of *C. acutatum* ($F=1.190$, $p=0.279$), and nonhomogeneity of the variances for the isolates of *C. gloeosporioides* ($F=2.059$, $p=0.009$).

Table 3. Inhibition zone* between microbial antagonists and isolates of *C. acutatum* and *C. gloeosporioides*.

Isolate code	*T. harzianum*	*G. roseum*	*B. subtilis*	*S. noursei*	*S. natalensis*
KC-21	-	+	++	+++++	++++
KC-23	-	+	++	+++++	+++
KC-82	-	+	++	+++++	+++
JC-4	-	+	+++	+++++	++++
PC-3	-	++	++	++++	+++
CBS 294	-	+	+++	+++++	+++
KC-9	-	+	+++	+++++	++++
KC-12	-	+	++	+++++	++++
JC-7	-	+	++	+++++	+++
VC-7	-	+	+++	++++	++
VC-9	-	+	++	++++	+++
CBS 516	-	+	++	++++	+++

*Inhibition zone: - no inhibition zone; + (very weak), 0-5 mm; ++ (weak), 5-10 mm; +++ (moderate), 10-15mm; ++++ (strong), 15-20 mm; +++++ (very strong), > 2

The results of analysis of variance (Table 4.) confirmed statistically very significant differences (p<0.01) in the antagonistic activity of *T. harzianum*, *G. roseum*, *B. subtilis*, *S. noursei* and *S. natalensis*. The isolates of *C. acutatum* and *C. gloeosporioides*, and the interactions between the antagonists and isolates of *C. gloeosporioides* showed very significant differences in conidial germination of fungal pathogens. The interactions between the antagonists and isolates of *C. acutatum* were only statistically significantly different (p<0.05).

The t-test showed that the two *Streptomyces* species were statistically mutually very different and in comparison with other antagonists in all tests of inhibition of conidial germination. *B. subtilis* was significantly different from *G. roseum* only in the conidial germination assay of isolates of *C. acutatum*.

Comparisons between the isolates of *C. acutatum* using the t-test revealed that isolate KC-23 from the pear and the reference isolate CBS 295, were statistically very different. The strains from pear and tomato fruit, (KC-21 and PC-3) showed only significant differences in sensitivity by the antagonists. Also, significant differences were confirmed between the isolates of *C. gloeosporioides* from apple and sour cherry (JC-7 and VC-9). The isolate KC-9 originating from the pear, and the reference isolate CBS 516 were statistically very different from all other isolates of *C. gloeosporioides*.

DISCUSSION

In the present study, isolates of *T. harzianum* and *G. roseum* and three isolates of the bacteria *B. subtilis*, *S. noursei* and *S. natalensis* were tested *in vitro* for preliminary screening to look for potential BCAs against the pathogenic fungi *C. acutatum* and *C. gloeosporioides*. A considerable variation was observed between, as well as within the fungal and bacterial antagonists with regard to the hyphal interaction and subsequent events to the inhibition of pathogen growth and conidial germination.
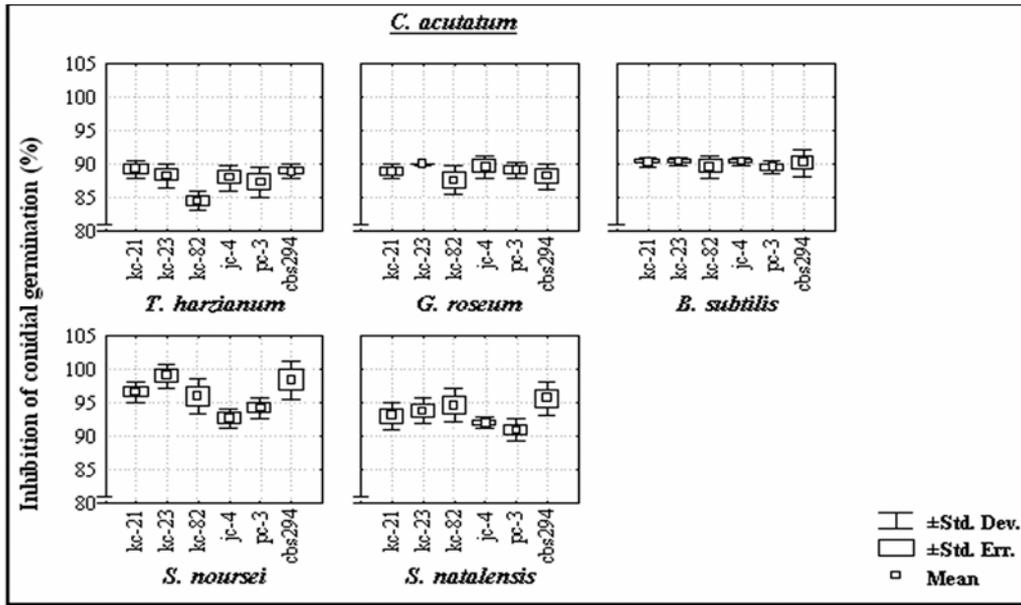
Fig. 8. Basic statistical parameters of inhibition of conidial germination of *C. acutatum*.
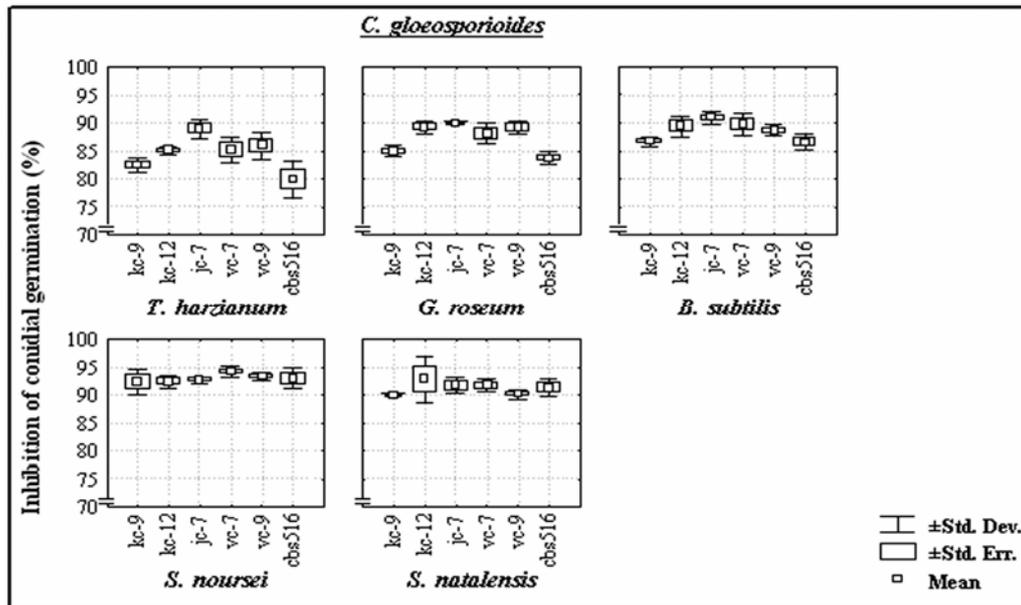

Fig. 9. Basic statistical parameters of inhibition of conidial germination of *C. gloeosporioides*.

This study has shown that colonies of *T. harzianum* grow faster than isolates of *C. acutatum* and *C. gloeosporioides*. This rapid growth gives *Trichoderma* an important advantage in the competition for space and nutrients with plant pathogenic fungi, even before it deploys its arsenal of mycotoxin (Barbosa, et al., 2001). Toxic action was evident in the various alternation of the hyphal structure of *Colletotrichum* isolates' growth together with *T. harzianum*, similar to the effects described in other systems of mixed cultures (Aryantha and Guest, 2006). The species of

Trichoderma are known to produce a number of antibiotics, such as trichodermin, trichodermol, trichotoxin, harzianum A and harzianolide (Dennis and Webster, 1971). These compounds were responsible for most of the inhibition of *Colletotrichum* isolates from the fruits in this study, and were also described previously in experiments involving the biocontrol of fungal phytopathogens (Zazzerini and Tosi, 1985; Gupta et al., 1995). The direct mycoparasitic activity of fungi of the genus *Trichoderma* has been proposed as one of the mechanisms involved in their antagonistic activity against phytopathogenic fungi. *Trichoderma* spp. attach to the host hyphae by coiling, hooks or appressorium-like structures (Elad et al., 1983). Our research showed that the major aspect of antagonism of *T. harzianum* was mycoparasitism. Most of the interaction between isolates of *C. acutatum* and *C. gloeosporiodes* and the antagonist *T. harzianum* observed in this study involved coiling and parallel growth. Begum et al. (2006) also found that the *Trichoderma* isolates coiled around the hyphae of *C. truncatum*. Competition for nutrients, mycoparasitism and antibiosis are the presumed modes of antagonism of *G. roseum* toward plant pathogens (Sutton et al., 1997). In these studies the hyphae of *G. roseum* were never observed to overlap the colony of *C. acutatum* and *C. gloeosporiodes*. In all cases, the isolates of *Colletotrichum* stopped growing before direct contact was made, presumable in response to diffusible inhibitors that were released by the antagonist. These results were similar to the results revealed by Lee and Wu (1984).

B. subtilis, *S. noursei* and *S. natalensis* inhibited radial growth by establishing a clear inhibition zone in a dual culture test. Several mechanisms are responsible for the suppression of fungal pathogens by bacteria, including competition, antibiotic and metabolite production (Compant, 2005). The inhibition of radial growth by the forming of an inhibition zone against *C. acutatum* and *C. gloeosporoides* is considered as antibiosis, whereby the antibiotic metabolites may penetrate the pathogen cell and inhibit its activity by chemical toxicity. *B. subtilis* produced several kinds of antimicrobial peptide substances such as subtilin, bacilysin, mycobacillisyn, and iturin (Yoshida et al., 2001). Light microscope investigation in this study revealed that extracellular metabolites by *B. subtilis* and *Streptomyces* strains caused cellular changes in the hyphal morphology of *C. acutatum* and *C. gloeosporioides* including hyphal swelling, distortion and cytoplasm aggregation. Similar results were observed by Sariah (1994), and Rahman et al. (2007) who reported that the fungal mycelial malformation might be due to the antibiotic metabolites produced by the bacteria, which can penetrate and cause protoplasmic dissolution and disintegration. The most efficacious antagonists in the present research was *S. noursei*. The polyene macrolide antibiotic nystatin is produced commercially by the *S. noursei*. Although it is an important antifungal agent used in human therapy (Fjaervik and Zotchev, 2005), there is no systematic report on the use of nystatin to control plant fungal diseases. Many *S. natalensis* strains have been reported to produce natamycin as the main antifungal compound. Natamycin is a macrolide polyene antifungal drug, which is widely used for the treatment of fungal keratitis and also in the food industry to prevent mold contamination of cheese and other non-sterile foods (Anton et al., 2004). The results from the study of Lu et al. (2008) suggest the potential of using natamycin as a BCA for plant fungal pathogens, such as *F. oxysporum*, *B. cinerea* and *M. laxa*.

Inhibition of spore germination was used as a bioassay to evaluate the level of antifungal activity of microbial antagonists against the isolates of *C. acutatum* and *C. gloeosporioides*. The degree of inhibition was found to be proportional to the level of chitin in the cell wall of the target fungus (Haran et al., 1996). The inhibitory effect on the conidial germination of the *Colletotrichum* strains was observed by all of the microbial antagonists. The antagonistic fungi *T. harzianum* and *G. roseum* showed moderate inhibition of conidial germination. The *Streptomyces* strains and *B. subtilis* exhibited the strongest inhibition on the

Table 4. Analysis of variance of the inhibition of conidial germination of *C. acutatum*   and *C. gloeosporioides*.

Species	Source of variation	df	MS	F	p-level
C. acutatum	antagonists	4	216.109	73.067	0.000**
	isolates	5	13.240	4.477	0.002**
	antagonists x isolates	20	6.224	2.104	0.014*
	error	60	2.958	-	-
C. gloeosporioides	antagonists	4	193.570	69.826	0.000**
	isolates	5	36.375	13.122	0.000**
	antagonists x isolates	20	6.614	2.386	0.005**
	error	60	2.772	-	-

** = very significant, p<0.01; * = significant, p<0.05

conidial germination of *C. acutatum* and *C. gloeosporioides*. Similar observations have been reported by Korsten et al. (1995), Jo (2005), Demoz and Korsten (2006), Mahadtanapuk et al. (2007), and Imtiaj and Lee (2008).

Screening is a critical step in the development of BCAs. The success of all subsequent stages depends on the ability of a screening procedure to identify an appropriate candidate. Several recent studies have shown that antagonistic microorganisms from the genus *Trichoderma*, *Gliocladium*, *Bacillus* and *Streptomyces* can help limit fungal pathogen damage in various fruits. Our results support these findings by showing that *T. harzianum*, *G. roseum*, *B. subtilis*, *S. noursei* and *S. natalensis* restrict the *in vitro* growth and conidial germination of *C. acutatum* and *C. gloeosporioides*, two of the most common and economically important pathogens of fruits. The *in vitro* results do not necessarily translate to what occurs *in planta*. Nonetheless, this study and the results are particularly useful for identifying likely candidates for biocontrol and for making educated guesses concerning the mechanisms by which they reduce pathogen damage.

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ПРОВЕРА АНТАГОНИСТИЧКОГ ДЕЛОВАЊА МИКРООРГАНИЗАМА НА *COLLETOTRICHUM ACUTATUM* И *COLLETOTRICHUM GLOEOSPORIOIDES*

СВЕТЛАНА ЖИВКОВИЋ[1], С. СТОЈАНОВИЋ[1], Ж. ИВАНОВИЋ[1], В. ГАВРИЛОВИЋ[1], ТАТЈАНА ПОПОВИЋ[2] И ЈЕЛИЦА БАЛАЖ[3]

[1]*Институт за заштиту биља и животну средину,* 11000 Београд, Србија
[2]*Luxembourg Industries d.o.o,* 21000 Нови Сад, Србија
[3]*Пољопривредни факултет, Универзитет у Новом Саду,* 21000 Нови Сад, Србија

Антагонистичко деловање пет агенаса биолошке заштите: *Trichoderma harzianum, Gliocladium roseum, Bacillus subtilis, Streptomyces noursei* и *Streptomyces natalensis*, тестирано је *in vitro* на *Colletotrichum acutatum* и *Colletotrichum gloeosporioides*, проузроковаче антракнозе плодова. Сви микробни антагонисти инхибирају пораст мицелије у тестовима двојне култивације и клијавост конидија *Colletotrichum изолата*. Две врсте рода *Streptomyces* испољавају најјачи степен антагонизма према изолатима *C. acutatum* и *C. gloeosporioides*. Микроскопско испитивање показује да је антибиоза најчешћи начин деловања антагониста. *T. harzianum, G. roseum, B. subtilis, S. natalensis* и *S. noursei* су резултатима ових истраживања идентификовани као биолошки агенси који се могу успешно укључити у будућа тестирања у циљу сузбијања антракнозе плодова.