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Antifungal properties of *Beauveria bassiana* strains against *Fusarium oxysporum* f. sp. *lycopersici* race 3 in tomato crop

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Abstract

Aim : Entomopathogenic fungus, *Beauveria bassiana* from different geographic regions, i.e. Coahuila, Nuevo Leon and Tabasco were assessed for their *in vitro* and *in vivo* antagonistic activity in tomato *Solanum lycopersicum* L., against soil borne disease, *Fusarium oxysporum* f. sp. *lycopersici* race 3.

Methodology : The strains were assessed *in vitro* (dual culture technique, lytic enzyme production and inhibition potency of culture filtrates). Strain 1215 was used to control *F. oxysporum* f. sp. *lycopersici* race 3 in tomato plants *in vivo*. The strain was applied to soil, foliar sprayed or stem injected, and plant development determined.

Results : These strains inhibited pathogen growth collapsing the mycelium, but only *B. bassiana* strain 1215 inhibited growth of *F. oxysporum* f. sp. *lycopersici* race 3 efficiently with 72% when applied in to the medium two days earlier. Lytic enzyme inhibited up to 21% of the growth of soil borne fungal pathogen *in vitro*. Application of *B. bassiana* strain 1215 to soil decreased by 1.4 the plant index disease without any adverse effect on plant height and root development.

Interpretation : Overall, the most efficient was *B. bassiana* strain 1215, which reduced the growth of *F. oxysporum* f. sp. *lycopersici* race 3 *in vitro* and decrease disease development *in vivo* without any adverse effect on plant height and root development and can be used as biological control agent against certain fungal pathogens.

In vitro
antagonism
assay

CWDEs assay

In vivo
antagonism
assay

Introduction

Tomato (*Solanum lycopersicum*) in Chiapas (Mexico) is an important vegetable crop with annual production of 2.5×10^5 tons (INEGI, 2011). However, tomato yield is low due to its susceptibility to several pathogenic fungi, bacteria, viruses and nematodes (Barone and Frusciante, 2007). The soil-borne *Fusarium oxysporum* is one of the most devastating disease ranking fifth in fungal plant pathogens, which can reduce tomato production significantly (Dean et al., 2012). Different formae speciales (f. sp.) exist for *F. oxysporum* with f. sp. *lycopersici* infecting mainly tomatoes. Tomato production is often reduced by 30 to 40%, however, sometimes it may reach up to 80% (Nirmaladevi et al., 2016). Three physiological races (1, 2 and 3) of pathogen specify are distinguished by their specific pathogenicity to tomato cultivars. They invade the root epidermis, extend into the vascular tissues and colonize the xylem vessels. Disease symptoms appear as a result of severe water stress, mainly due to vessel clogging (Nirmaladevi et al., 2016). Use of resistant cultivars is the most reliable method for disease management. However, no commercial cultivar is available with adequate levels of resistance to *F. oxysporum* f. sp. *lycopersici* and management thus, relies primarily on chemical control (Vos et al. 2014). In recent years, different studies have shown that entomopathogenic fungi, such *Beauveria bassiana*, are useful biocontrol agents against *F. oxysporum* due to their ability to infect a wide range of insect pests and the production of toxic secondary metabolites (Sung et al., 2007). *B. bassiana* has the potential to limit the growth of plant pathogens, such *Botrytis cinerea* (Bark et al. 1996), *Rhizoctonia solani* (Ownley et al., 2008a) and *Alternaria porri* (Gothandapani et al. 2014). Isolates of *B. bassiana* have been used in the greenhouse against basal rot of onion (*Allium cepa* L.) caused by *F. oxysporum* f. sp. *cepae*, and they were found to reduce the infection significantly (Flori and Roberti, 1993). This fungus also colonizes a variety of cultivated plants, such *Theobroma cacao* (Posada and Vega, 2005), *Musa spp.* (Akello et al., 2007), *Solanum lycopersicum* (Ownley et al., 2008a), *Gossypium hirsutum* (Gurulingappa et al., 2010; Lopez and Sword, 2015), *Pinus radiata* (Brownbridge et al., 2012), *Papaver somniferum* (Quesada-Moraga et al., 2009) and *Manihot esculenta* (Greenfield et al., 2016). The mechanism of antagonism of *B. bassiana* includes antibiosis (Vesely and Koubova, 1994; Bark et al., 1996), competition (Ownley et al., 2004) and induced systemic resistance (Ownley et al., 2008b). Nevertheless, studies on enzymes production, secondary metabolites or antibiotics responsible on mycelial growth inhibition of pathogenic fungi is meagre. In view of the above, the present study was carried out to control pathogens in tomato plants, *B. bassiana* was tested as a biocontrol agent of *F. oxysporum* f. sp. *lycopersici* race 3. First, the antagonistic capacity of three different strains of *B. bassiana* against *F. oxysporum* f. sp. *lycopersici* race 3 was determined *in vitro* and later, the antagonistic capacity of the most promising strain of *B. bassiana* against *F. oxysporum* f. sp. *lycopersici* race 3 was

undertaken in tomato plants *in vivo*.

Materials and Methods

Fungal microorganisms : Three strains of *B. bassiana* (19, 1205 and 1215) and one strain of *F. oxysporum* f. sp. *lycopersici* race 3 (Fo/3) were studied. The 5.8S rRNA gene sequence of strains 19, 1205, and 1215 of *B. bassiana* were deposited in the GenBank database under the accession numbers KX232464, KX232465 and KX232466, respectively, while that of strain Fo/3 of *F. oxysporum* f. sp. *lycopersici* race 3 under accession number KX232463. For all the *in vitro* experiments, disks (5 mm) from the edge of growing fungal colonies were used.

In vitro assays : Antagonistic activity: In the first experiment, each strain of *B. bassiana* was grown as dual culture with *F. oxysporum* f. sp. *lycopersici* race 3. Agar disks with a strain of *B. bassiana* were placed separately in sterile plastic round 9 cm Petri dishes containing 15 ml of PDA medium. After two days, the pathogen was placed equidistantly at the opposite side of the antagonist and incubated at 28°C for 12 days in darkness. Colony radii of the phytopathogenic fungi were measured twice a day for 12 days (Annes et al., 2010). This procedure was repeated five times for each strain.

In the second experiment, Agar disks each with a strain of *B. bassiana* were placed separately in a Petri dish equidistantly at the opposite side of the pathogen at the same time and incubated at 28°C for 12 days. Petri dishes containing only a disk with the phytopathogenic fungi served as control (Annes et al., 2010). Antagonism was classified as suggested by Mbarga et al. (2012). Grade 1 was obtained when the antagonist covered the entire surface of the medium; 1.5 grade when the antagonist covered 87.5% of the medium surface; grade 2 when the antagonist covered approximately 67% of the medium surface; 2.5 grade when the antagonist covered approximately 62.5% of the medium surface, grade 3 when the antagonist covered approximately 50% of the medium surface, grade 4 when the antagonist covered approximately 33.3% of the medium surface; grade 5 when the antagonist did not grow and the pathogen covered the entire medium surface.

Antifungal activity of extracellular metabolites : Four disks of 5 mm diameter were cut from the growing tip of 15-day old culture of each *B. bassiana* strain with the help of a cork borer, added to a flask containing 50 ml of liquid medium and incubated in a rotatory shaker at 150 rpm and 28°C for eight days. The medium was prepared as described by Rajeswari (2015). Briefly, 50 ml sterilized Czapek's liquid media ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g l⁻¹, KCl 0.5 g l⁻¹, NaNO_3 , 3 g l⁻¹; K_2HPO_4 , 1 g l⁻¹; FeSO_4 , 0.01 g l⁻¹; V8 juice, 200 ml, adjusted to 1200 ml) in a 250 ml Erlenmeyer flasks and supplemented with crab shell chitin (1% w/v) as carbon source. The mycelium was harvested by filtering the solution through a Whatman No. 1 filter paper placed on a Buchner funnel under vacuum. Cells were removed by centrifugation at 8,000 rpm at 4°C

for 20 min and the supernatant was recovered. Growth inhibitory effects of the extracellular metabolites were determined by using the radial growth inhibition assay and fungal growth inhibition was expressed as the percentage of radial growth inhibition in comparison with control (Prapagdee *et al.*, 2007). In the control treatment, only one disk of the phytopathogenic fungi was placed in the middle of the Petri dish containing PDA medium and was added with sterile distilled water. This procedure was repeated three times for each strain at different days of incubation (2, 4, 6 and 8).

Determination of enzyme activity : Activity of chitinase and β -1, 3-glucanase was quantified in the extracellular medium of *B. bassiana* as described by Reissig *et al.*, (1957) and Zheng and Wozniak (1997). Protein concentration was estimated by the protein kit (Bio-Rad), according to the method described by Bradford, using Bovine Serum Albumine as a standard. The whole procedure was performed in triplicate.

In vivo study : *B. bassiana* strain that exhibited the best antagonistic effect against *F. oxysporum* f. sp. *lycopersici* race 3 was used in the *in vivo* study with tomato plants. The *B. bassiana* strain 1215 was cultured on potato dextrose agar media at $28 \pm 2^\circ\text{C}$ for 15 days. Four disks of five mm diameter were cut and transferred into 50 ml flasks containing liquid medium (Rajeswari, 2015). The flasks were placed on a rotary shaker at 28°C and 150 rpm for eight days (Ahmed Idris *et al.*, 2007). Spores were extracted and quantified in a Neubauer chamber. The conidial suspension of *B. bassiana* was adjusted to a spore concentration of 1×10^7 conidia ml^{-1} with sterile distilled water.

Tomato plants were cultivated following the method of Montes-Molina *et al.* (2014). Commercial seeds of tomato (*Solanum lycopersicum* L.) var. Rio Fuego susceptible to *F. oxysporum* f. sp. *lycopersici* race 3 were sown in plastic containers (14.8 cm long 11.0 cm wide) filled with sterile peat moss and kept in a growth chamber. After 25 days, tomato seedlings were inoculated with a conidial suspension of *B. bassiana* and one week later the plants were inoculated, with 10 ml of *F. oxysporum* f. sp. *lycopersici* (1×10^5 conidia ml^{-1}) suspension and kept in greenhouse with a photoperiod of 12 hr and daily average temperature of $32 \pm 2^\circ\text{C}$ for 25 days. Each plant was supplied with $75 \text{ kg} \cdot \text{ha}^{-1}$ urea, per plant approximately 1.2 g, 15 and 45 days after emergence and $90 \text{ kg} \cdot \text{ha}^{-1}$ with triple 17 (17% N, 17% P as P_2O_5 and 17% K as K_2O), per plant approximately 1 g, 15 days after emergence. The plants were watered as and when needed during experimentation and were inoculated every 5th day with the antagonistic strain.

The antagonist was applied in three different ways to tomato plants, i.e., foliar spray, stem injection and soil application. Application rate and inoculation of tomato plants with *B. bassiana* strain 1215 was done as per method described by Posada *et al.* (2007). In foliar spray, 8 ml of suspension (1×10^7 conidia ml^{-1}) was

directly sprayed on leaves with the help of an atomizer. The planted soil surface was covered with plastic sheet to avoid leaching of spray solution in potted soil. In stem injection, a BD Ultra Fine™ syringe with sterile hypodermic needle 31 G was used to inject 0.2 ml suspension (1×10^7 conidia ml^{-1}) into the stem. In soil application, 8 ml suspension (1×10^7 conidia ml^{-1}) was sprayed directly on the soil surface of each pot. In control treatment, sterile water was used instead of conidial suspension. One screen house experiment was laid out in randomized block design with 140 experimental units, where factors evaluated were: *B. bassiana* method of application (foliar, stem and soil) and time (5, 10, 15, 20, 25 days). Control treatments used the same method of application of *B. bassiana* and one treatment without fungus. The whole procedure was performed in quadruplicate. All the methods were same as described in the previous experiment.

At 5-day interval for 25 days after inoculation, four containers with tomato plants of each treatment were taken to laboratory. The tomato plants were taken out from the container and the soil washed from the roots. Tomato plants were studied for plant height (cm), root length (cm), dry root weight (g), dry leaf weight (g) and disease severity. The disease development was assessed on three replicated plants per treatment using a numerical scale of 0-3; where 0 is healthy; 1 is slight infection and yellowing of leaves; 2 is severe wilting and yellowing of leaves; 3 is dead plants (Chandel *et al.*, 2010).

Statistical analysis : Data were subjected to the analysis of variance and the means were compared with a Tukey's multiple range test ($P < 0.05$).

Results and Discussion

The antagonism of the *B. bassiana* strains towards *F. oxysporum* was determined *in vitro* by the dual culture technique (Rahman *et al.*, 2009) and the bioassays showed an inefficient antagonism when both were applied to the medium at the same time (Table 1). Inhibition efficiency ranged from 22% (strain Bb-19) to 31% (strain 1215). Contact between the hyphae of strain 1215 and *F. oxysporum* occurred after 7 days. However, the radial growth of *B. bassiana* strains was slower than *Fusarium*.

The growth of phytopathogenic fungus was slower when *B. bassiana* strains were applied to the medium two days earlier to *F. oxysporum* as compared to simultaneous inoculation and both organism came in contact with each other on 4th day (Table 1). None of the antagonistic strains did overgrow the pathogen. *B. bassiana* strain 1215 was the most efficient and showed a 72% inhibition compared to strains 1205 (61%) and Bb-19 (55%). The results indicated that different *B. bassiana* strains had variable biocontrol potential. Dubey *et al.* (2007) reported that the antagonistic capacity of *Trichoderma* varied from species to species and isolate to isolate; thus growth rate is an important

Table 1 : *In vitro* bioefficacy of *B. bassiana* strains against *Fusarium oxysporum* f.sp. *lycopersici* race 3

Application of <i>B. bassiana</i> to the medium 2 days before <i>F. oxysporum</i>		
Strains	Hyphae contact (days)	Growth inhibition (%)
1215	4 ^a ± 0.4 ^b A ^c	72 ± 0.5 A
1205	4 ± 0.5 A	61 ± 0.6 B
Bb19	4 ± 0.5 A	55 ± 0.6 C
MSD ^d	0.2	0.5
Application of <i>B. bassiana</i> and <i>F. oxysporum</i> to the medium at same time		
1215	7 ± 0.7 C	31 ± 0.5 A
1205	8 ± 0.2 B	24 ± 0.6 B
Bb19	9 ± 0.4 A	22 ± 0.9 C
MSD	0.1	0.6

^a Values are means of five replicates, ^b Standard deviation of the mean, ^c Values with the same capital letter are not significantly different between strains, i.e., within the columns at P<0.05, ^d MSD: Minimum significant difference at P<0.05

Table 2 : Extracellular enzymes production potency of *B. bassiana* strains

Strains	β -1,3-Glucanase (pkat ml ⁻¹)				Chitinase (pkat ml ⁻¹)			
	Days				Days			
	2	4	6	8	2	4	6	8
1205	133 ^a ± 2 ^b B ^c	233 ± 1 A	176 ± 1 B	89 ± 1 C	28 ± 1 B	154 ± 1 A	29 ± 1 B	38 ± 1 B
1215	167 ± 2 A	126 ± 1 C	140 ± 1 B	120 ± 1 B	55 ± 1 A	68 ± 1 B	39 ± 1 A	42 ± 1 A
Bb-19	101 ± 1 C	160 ± 2 B	1128 ± 3 A	147 ± 1 A	15 ± 1 C	66 ± 1 C	17 ± 1 C	21 ± 1 C

^a Values are means of five replicates, ^b Standard deviation of the mean, ^c Values with the same capital letter are not significantly different between strains, i.e., within the columns at P<0.05

Table 3 : Biomass production of *Beauveria bassiana* strains and their inhibitory effect of extracellular enzymes against *Fusarium oxysporum* f.sp. *lycopersici*

Strains	Biomass (mg)					Inhibition (%)		
	Incubation time (day)					Incubation time (day)		
	2	4	6	8	2	4	6	8
1205	124 ^a ± 2 ^b B ^c	183 ± 3 A	190 ± 3 A	169 ± 3 AB	0	5 ± 0.3 A	15 ± 0.3 B	13 ± 0.5 B
1215	197 ± 2 A	203 ± 2 A	212 ± 2 A	199 ± 2 A	0	0 B	21 ± 0.7 A	15 ± 0.6 A
Bb-19	89 ± 1 C	29 ± 2 B	52 ± 1 B	147 ± 2 B	0	0 B	12 ± 0.2 C	11 ± 0.1 C
MSD ^d	36	35	35	39	0	0.3	0.7	0.7

^a Values are means of five replicates, ^b Standard deviation of mean values, ^c Values with the same capital letter are not significantly different between strains, i.e., within the columns at P<0.05, ^d MSD: Minimum significant difference at P<0.05

factor for colonization in to medium or a niche, which determines the efficiency of an antagonist. The growth of *F. oxysporum* was slower when *B. bassiana* strains were inoculated two days before due to the production of diffusible hydrolytic enzymes. Mycoparasites of *Trichoderma* use the same mechanism when grown together (Annes et al., 2010). Therefore, greater efficiency was achieved by inoculating the antagonist strains two days before the pathogen. Diffusible metabolites of *Trichoderma* spp., have been found to interact with hyphae of pathogens before direct contact occurred collapsing the mycelia of pathogenic fungus (Zeilinger and Omann, 2007; John et al., 2010). In the

present study, the interaction between both organisms occurred 24 hrs before direct contact and was maintained till 48 hrs, as determined through stereoscopic microscopy. However, the formation of infection structures by the antagonist, such as appressoria followed by initiation of mycoparasitism that ultimately led to the collapse of the pathogen's mycelia (Zeilinger and Omann, 2007), no formation of infection structure was observed in this study. However, inhibitory diffusible components were produced (Ownley et al., 2008a) and overgrowth of the pathogen by the antagonist is not required to inhibit the fungal pathogen (Gothandapani et al., 2014).

The production of cell wall degrading enzymes is one of the important aspect involved in the control of phytopathogenic fungi. Most of the phytopathogenic fungi possess cell walls that compromises of chitin, as a structural backbone arranged in regularly ordered layers and β -1, 3-glucan as a filling material arranged in an amorphous manner (Marcello *et al.*, 2010). This study showed that all *B. bassiana* strains could produce β -1, 3-glucanase and chitinase enzyme (Table 2) variably. The strain 1215, 1205 and Bb-19 produced the highest concentration of β -1, 3-glucanase to the extent of 167 ± 2 , 233 ± 1 and 1128 ± 3 pkat ml^{-1} , respectively on 2nd, 4th and 6th day, however, the maximum amount of chitinase was produced on 4th day by all the strains. Gomes *et al.* (2006) reported that generally the maximum amount of enzymes was being produced after 5 to 7 days, which supports the present findings.

The maximum biomass was produced by strain 1215 (212 mg), 1205 (190 mg) and Bb-19 (147 mg) on 6th and 8th day (Table 3). No correlation existed between produced amount of biomass and enzyme. The present results are contradictory with the earlier findings that showed a correlation between the amount of antifungal enzymes produced and biomass of *B. bassiana* (Bark *et al.*, 1996). All *B. bassiana* strains showed a high percentage of inhibition when their culture filtrates were applied to *F. oxysporum* at 6 days. The highest inhibition was

obtained with strain 1215 (21%) and the lowest with strains Bb.19 (12%) as presented in Table 3. Bark *et al.* (1996) reported that a culture filtrate of *B. bassiana* when added to different media resulted in 16% mycelium growth inhibition of *F. oxysporum* f. sp. *lycopersici*, while in the present study it was 21%. It might be due to enzymatic inducers as they allow the expression of genes involved in production of chitinases and glucanases production.

When *B. bassiana* strain 1215 was tested for its impact on tomato plant by different application methods (Table 4), it was noted that there was no significant difference in plant height stem and foliar application compared to water application, however soil application method showed significant increase. The roots of tomato plants were longer when *B. bassiana* strain 1205 was applied as compared to water. Dry weight of leaves and roots was similar when *B. bassiana* or water was applied as foliar or soil application, but it was lower when *B. bassiana* was injected into the stem compared to water injected plants. Application of antagonistic fungus to soil induced apical and root growth of tomato plants. Schulz and Boyle (2005) reported that this effect might be a response of the plant to endophytic infection. Chowdappa *et al.* (2013) reported that increase in the vigour of the plant is due to the ability of antagonistic fungus to enhanced production of growth hormones in tomato roots.

Table 4: Effect of *Beauveria bassiana* strain 1215 on growth parameters of tomato plants after 25 days

Treatments	Plant height (cm)	Root length (cm)	Foliar dry weight (g)	Root dry weight (g)
Water: soil	51 ^a $\pm$ 5 ^b BC ^c	30 $\pm$ 4 D	18 $\pm$ 2 ABC	12 $\pm$ 1 CD
Water: foliar	49 $\pm$ 2 C	31 $\pm$ 2 D	17 $\pm$ 3 BC	13 $\pm$ 2 BC
Water: stem	50 $\pm$ 3 C	28 $\pm$ 3 E	16 $\pm$ 3 C	12 $\pm$ 2 CD
<i>B. bassiana</i> : soil	58 $\pm$ 7 A	40 $\pm$ 5 A	18 $\pm$ 3 ABC	13 $\pm$ 2 BC
<i>B. bassiana</i> : foliar	50 $\pm$ 5 C	35 $\pm$ 3 BC	19 $\pm$ 3 AB	14 $\pm$ 3 B
<i>B. bassiana</i> : stem	51 $\pm$ 6 BC	34 $\pm$ 3 BC	13 $\pm$ 2 D	11 $\pm$ 2 D
No pathogen applied	54 $\pm$ 6 B	36 $\pm$ 4 B	20 $\pm$ 4 A	16 $\pm$ 4 A
MSD ^d	3	2	2	2

^a Values are means of five replicates, ^b Standard deviation of mean values, ^c Values with the same capital letter are not significantly different between strains, i.e. within the columns at $P < 0.05$, ^d MSD: Minimum significant difference at $P < 0.05$

Table 5: Antagonistic effect of *B. bassiana* after 25 days of biocontrol test against *F. oxysporum* f.sp. *lycopersici*

Treatments	Disease index
<i>F. oxysporum</i> + water applied to soil	2.6 $\pm$ 0.5 ^d
<i>F. oxysporum</i> + foliar applied water	2.2 $\pm$ 0.4 ^{cd}
<i>F. oxysporum</i> + stem applied water	2.6 $\pm$ 0.4 ^d
<i>F. oxysporum</i> + <i>B. bassiana</i> applied to soil	1.4 $\pm$ 0.5 ^b
<i>F. oxysporum</i> + foliar applied <i>B. bassiana</i>	1.6 $\pm$ 0.5 ^{bc}
<i>F. oxysporum</i> + stem applied <i>B. bassiana</i>	2.2 $\pm$ 0.4 ^{cd}
No fungal pathogen applied	0.4 $\pm$ 0.5 ^a

* Values are means of four replicate plants per treatment. Values in each columns followed by the same letter are not significantly different according to the Tukey's multiple range test ($P < 0.05$). Disease was scored on a categorical scale of 0-3, indicating 0 – healthy plant; 1 – slight infection with yellowing; 2 – severe infection, yellowing and wilting; 3 – death of the plant

F. oxysporum f. sp. *lycopersici* race 3 induced vascular wilting on tomato plants in all treatments (Table 5). Application of *B. bassiana* 1215 reduced the development of disease in tomato plants when applied on the soil surface compared to water application. The severity of disease in tomato plants inoculated with *B. bassiana* was lower than the plants inoculated with water after 25 days. Sangeetha *et al.* (2013) reported that enzymatic activity and release of metabolites acted directly on the pathogen and/or induced systemic resistance in plants resulting in a decrease in disease development. Sukno *et al.* (2008) reported that the amount of fungal inoculum used affected the degree of colonization in plants and infection rates. Studies in many plant species have revealed that the enzymatic activity increased as a defence mechanism against environmental stress, such as fungal infection (Jetiyanon, 2007; Shanmugam and Kanoujia, 2011). Regular application of conidial suspension of *B. bassiana* to soil might have reduced the population of *F. oxysporum* f. sp. *lycopersici* race 3, which decreased the disease symptoms in tomato plants. However, the fact that disease symptoms were not suppressed completely indicated that growth of the pathogenic fungi was not inhibited completely. Different explanations have been postulated for the continuous presence of phytopathogen. First, survival of *B. bassiana* was lower than that of *F. oxysporum* f. sp. *lycopersici*, thus antagonist had to be applied regularly to maintain the adequate antagonistic population dynamics. Secondly, application of antagonistic fungus increased the production of plant defence enzymes. *B. bassiana* produces an appressorium to penetrate the insect cuticle and the same process might be involved in the penetration of cuticle of leaf and stem cell wall or roots. This might trigger the production of plant enzymes as a defence mechanism reducing the population of *F. oxysporum* f. sp. *lycopersici*.

It was concluded that different *B. bassiana* strains in general, and *B. bassiana* strain 1215 in particular, have the ability to control growth of *F. oxysporum* f. sp. *lycopersici*, and hence they can be used to protect plants against soil borne fungal pathogens. The control of pathogens, however, not only depends on the characteristics of *B. bassiana* strains, but also on the biotic and abiotic factors, which should be considered if antagonists is being planned to use as a biological control strategy.

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