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Effect of Different Concentrations of the Suspensions of *B. amyloliquefaciens* and *T. harzianum* on the Development of *C. acutatum*

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Abstract

The effect of nine isolates of *Bacillus amyloliquefaciens* and one strain of *Trichoderma harzianum*, TR, on mycelial growth and germination of *Colletotrichum acutatum* were studied. The nine isolates were identified as *Bacillus amyloliquefaciens*. The efficacy of isolates was tested, at different concentrations. Results showed that one Bacillus isolates (Bc2) and TR were more effective at the lower concentration tested (3×10^5 CFU/ml and 10^5 conidia/ml).

Keywords

Biocontrol, Concentration, Inoculum, *Bacillus amyloliquefaciens*, *Trichoderma harzianum*, *Colletotrichum acutatum*

1. Introduction

Like all living organisms, plants have to cope with infections and diseases following the attacks of a mass of plant pathogens. These diseases may be causing only minor reduction in plant growth and capacity or they can cause much more serious damage leading to plant death in the worst case [1]. To prevent or control these diseases, growers have become increasingly dependent on agrochemicals, especially in recent decades, as agricultural production has intensified.

However, despite the high efficiency and ease of use of these products, their use or misuse has caused many problems.

The root zone is surrounded by many microorganisms producing molecules; they contain some volatile compounds that can affect the growth via different mechanisms, such as biochemical signals causing local defense reactions or systemic resistance [2] [3]. The use of these organisms for biological control against plant pathogens has received attention because of the prevalence of pathogens resistant to pesticides and their prohibitions. Benefits include a lower environmental impact, higher specificity, lower costs and identify new mechanisms for the elimination of new diseases. The genus Bacillus encompasses a wide genetic biodiversity.

Bacillus is a genus of bacteria forming endospores shaped Gram positive rod [4]. It is known to produce several antibiotics and is often found in soil and associated plants [5]. Trichoderma (teleomorph Hypocrea) is a fungal genus present in many ecosystems. Some strains have the ability to reduce the severity of plant diseases by inhibiting plant pathogens, mainly in the soil or on plant roots, thanks to their strong potential antagonist and mycoparasitic [6] [7].

The objective of this work is the evaluation of the effectiveness of suspensions of *Trichoderma harzianum* and *Bacillus amyloliquefaciens* at various concentrations to inhibit the growth of *Colletotrichum acutatum*.

2. Material and Methods

2.1. Fungal Material

2.1.1. Pathogenic

The conidial suspension of the plant pathogen *Colletotrichum acutatum* (Ca6) was obtained by adding 10 ml of sterile distilled water in Petri dishes containing cultures of *C. acutatum* of 10 to 15 days old on PDA [8], by gently rubbing the colonies using a sterile Pasteur pipette. To release the spores, the propagules suspension was filtered through sterile nylon gauze with pores of 102 µm diameter. The concentration was determined using a Malassez cell and adjusted to 10^4 spores/ml.

2.1.2. Antagonist

Each Erlenmeyer flask containing 15 ml of PDB (Potato Dextrose Broth) medium was inoculated with a disc of 5 mm diameter from the culture of *Trichoderma harzianum* and incubated for 12 days at 25˚C ± 2˚C with stirring at 200 rpm. After this period, the suspension was filtered using Whatman paper (150 mm) to remove the spores and mycelium. The suspension was adjusted to 10^8 spores/ml using a Malassez cell.

2.2. Bacterial Material

Antagonist each bacterial isolate was grown on PCA for 24 h at 37˚C. After, and by using a sterile loop, a bacterial culture was prepared by inoculating 50 ml of the LBB, by a colony of the isolate studied; in an Erlenmeyer flask of 100 ml.

Cultures were incubated at room temperature with stirring at 125 rpm for two days. After incubation, a volume of 5 ml of bacterial suspension was centrifuged at 3000 rpm for 15 min. Cells were washed twice in the same volume of sterile physiological water used, using the centrifuge under the same conditions cited in the above instructions. The bacterial cells were then suspended in 5 ml of sterile physiological water and the suspension was adjusted to 3×10^8 CFU/ml depending on the scale of Mac Farland [9].

2.3. Effect of Different Concentrations of Antagonist on

2.3.1. The Mycelial Growth

To evaluate the effect of the antagonist on the pathogenic agent in different concentrations, a disc 5 mm PDA culture medium diameter already prepared and poured into Petri dishes was cut with a prevails sterile in order to perform well in the middle of the dish [10], in the latter, a volume of 20 µl of *Colletotrichumacutatum* suspension is mixed with 20 µl of sterile distilled water (control), an antagonist bacterial suspension to 3×10^8, 3×10^7, 3×10^6, 3×10^5, 3×10^4, 3×10^3, 3×10^2, 3×10^1 CFU/mlor fungal antagonist suspension 10^8, 10^7, 10^6, 10^5, 10^4, 10^3, 10^2, 10^1 spores/ml. The plates were incubated at 25˚C for ten days after which the diameter of *Colletotrichum acutatum* was evaluated.

Percent inhibition was calculated according to following formula:

$$PI = \frac{(Dt - Di)}{Dt} \times 100$$

PI: Percentage inhibition.

Dt: diameter of the mycelial colony of *C. acutatum* in the absence of antagonist (control).

Di: diameter of the mycelial colony of C*acutatum* in the presence of the antagonist.

The number of repetitions is three dishes for each concentration combination-strain, and the test was repeated three times.

2.3.2. Spore Germination

The effect of the antagonists on spore germination of *Colletotrichum acutatum* was tested in sterile PDB (Potato Dextrose Broth). A volume of 200 µl of antagonist suspension already prepared and adjusted to various concentrations is mixed with 200 µl of Ca6 of suspension into 10 ml tubes containing 5 ml of PDB liquid medium. The tubes are incubated for 24 h at 25˚C.

To estimate whether there is a difference in the activity of the antagonists over time on the implementation of the spores germination of *C. acutatum*, this activity was evaluated either by mixing the two suspensions (antagonist and pathogen) simultaneously t_0, adding the suspension conidial suspensions after four hours of incubation of antagonists t_4 [10], or by adding the conidial suspension after eight hours of incubation of antagonists suspensions t_8. After 24 h of incubation, the number of germinated spores was recorded by calculating at least 100

spores germinated in the field optical microscope at a magnification of ×10 [10]. The test was repeated three times.

3. Results

3.1. Effect of Different Concentrations of Antagonists on Mycelial Growth

The results of the determination and the effect of different concentrations of antagonists on mycelial growth of *Colletotrichum acutatum* gave several percentage grades of development inhibiting this pathogen. The comparison of the percentages of inhibition of mycelial growth based bacterial concentration generally shows, for all isolates that inhibition of mycelial growth decreases with decreasing bacterial concentrations (Table 1). The complete inhibition of pathogen development was achieved by all bacterial isolates at 3×10^8 CFU/ml. All bacterial isolates except Bc5 isolate with inhibition of 73.83% at 3×10^7 CFU/ml for the concentration 3×10^6 CFU/ml except for the Bc5 isolate that gave an inhibition of 50.83%, All bacterial isolates inhibited growth completely Ca6. The Bc2 isolate was the only isolate that inhibition of mycelial growth of *C. acutatum* 100% at 3×10^5 CFU/ml while Bc9 isolates, Bc1, BC3 and BC8 inhibit the growth of the pathogenic agent at higher percentages 50% (68.33; 58.33; 56.67 and 53.33% respectively) (Table 1).

At 3×10^4 CFU/ml, mycelial growth was inhibited to 86.67% by Bc2 isolate; the percentages of inhibition obtained from other isolates are less than 50%.

At 3×10^3 CFU/ml, the efficiency of isolates begin to decrease at lower inhibitions to 30% apart from the Bc2 isolate that gave an inhibition of 68.33%.

At 3×10^2 CFU/ml, the percentages of inhibition obtained by Bc1 isolates, Bc3, Bc4 Bc6, Bc7, and Bc8 Bc9 is below 10%, the Bc2 isolate showed an inhibition of 29.50%, Isolate Bc5 showed no activity on the pathogen growth.

At the low concentration of 3×10^1 CFU/ml, all strains have a minimum inhibition of 0 to 0.33%, while the strain Bc2 inhibition 18.33.

Table 1. Mycelial growth inhibition percentage with different concentrations of bacterial isolates suspensions.

Bacterial strains	Concentration CFU/ml							
	3×10^8	3×10^7	3×10^6	3×10^5	3×10^4	3×10^3	3×10^2	3×10^1
Bc1	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	58.33 ± 2.01^{bc}	47.50 ± 1.44^c	15.00 ± 1.23^c	8.33 ± 0.20^{cd}	0.00 ± 0.00^b
Bc2	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	86.67 ± 1.00^a	68.33 ± 0.41^a	29.50 ± 1.61^a	8.83 ± 1.92^a
Bc3	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	56.67 ± 1.64^{bc}	46.50 ± 2.20^c	15.83 ± 1.35^c	8.00 ± 1.32^{cd}	0.00 ± 0.00^b
Bc4	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	49.17 ± 0.83^c	47.50 ± 1.63^c	19.17 ± 0.83^{bc}	11.67 ± 1.67^{bc}	0.33 ± 0.18^b
Bc5	100.00 ± 0.00^a	73.83 ± 0.73^b	50.83 ± 1.20^b	34.67 ± 0.85^d	13.50 ± 1.00^e	0.67 ± 0.44^d	0.00 ± 0.00^f	0.00 ± 0.00^b
Bc6	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	20.00 ± 0.33^e	20.00 ± 1.44^{de}	15.00 ± 0.25^c	5.00 ± 1.44^{de}	0.50 ± 0.35^b
Bc7	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	32.50 ± 1.44^{de}	23.67 ± 0.73^d	15.85 ± 0.20^c	9.42 ± 1.54^{cd}	0.17 ± 0.21^b
Bc8	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	53.33 ± 0.46^c	21.67 ± 2.2^d	12.50 ± 1.44^c	2.17 ± 0.44^{ef}	0.00 ± 0.00^b
Bc9	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	68.33 ± 0.41^b	62.83 ± 4.33^b	25.83 ± 0.83^b	15.00 ± 1.44^b	0.00 ± 0.00^b

The averages of the same column with the same letter do not significantly differ from each other at the 5% threshold.

Trichoderma harzianum has completely inhibited mycelial growth of *C. acutatum* 10^8, 10^7 and 10^6 spores/ml. Its percent inhibition at 10^5 spores/ml reached 99.33% and 95.66%; 63.66%; 30.00% and 10.27% at 10^4, 10^3, 10^2 and 10^1 spores/ml respectively (Figure 1).

The results allowed us to infer three minimum inhibitory concentrations of bacterial suspensions ie 3×10^8 CFU/ml (Bc5 isolate), 3×10^6 CFU/ml (Bc1, BC3, Bc4, Bc6, BC7, BC8 and Bc9) and weak inhibitory concentration 3×10^5 CFU/ml represented by Bc2 (Table 2) which is the single isolate was able to give an important control effectiveness in completely inhibiting the mycelial growth of Colletotrichum acutatum. And the minimal inhibitory concentration of *T. harzianum* is 10^6 spores/ml (Table 2) this antagonist could inhibit mycelial growth Ca6 even at low concentrations (Figure 1).

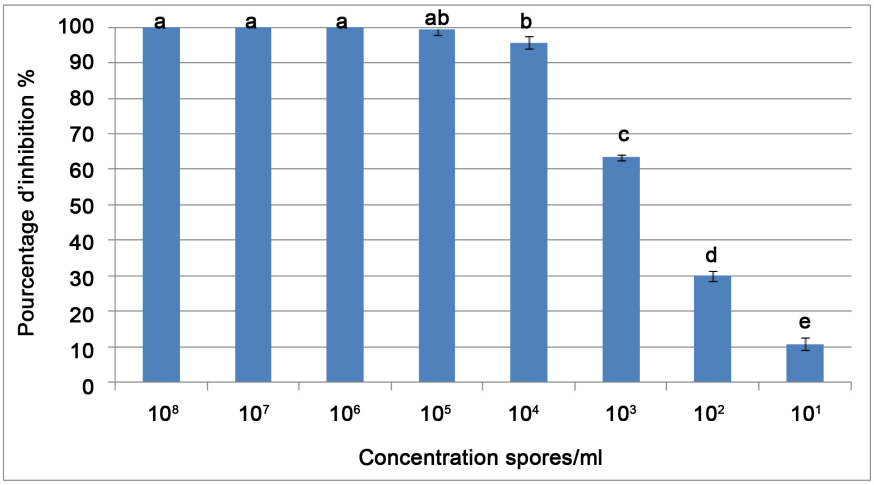

Figure 1. Mycelial growth inhibition percentage by different concentrations of suspension of *T. harzianum*.

Table 2. Determination of the minimum inhibitory concentration of antagonists' suspensions that completely inhibit mycelial growth Ca6.

Antagonist	Minimum inhibitory concentration	Unit
Bacillus bc1	3×10^6	
Bacillus Bc2	3×10^5	
Bacillus BC3	3×10^6	
Bacillus Bc4	3×10^6	**CFU/ml**
Bacillus Bc5	3×10^8	
Bacillus bc6	3×10^6	
Bacillus BC7	3×10^6	
Bacillus BC8	3×10^6	
Bacillus Bc9	3×10^6	
Trichoderma harzianum	10^6	**Spores/ml**

3.2. Effect of Different Concentrations of Antagonists on Spore Germination of Ca6

The results of this study show the percentage of inhibition of germination by *B. amyloliquefaciens* strains varies depending on the concentration of the bacterial suspension and the application time (t_0, t_4 and t_8) (Tables 3-5).

The minimum inhibitory concentrations of bacterial isolates remain the same in every application time but there are some isolates that are more effective at t_4 (Bc2, BC3, Bc4, Bc6 and BC7) other at t_0 (Bc1, Bc5, BC8 and Bc9). The application at t_8 reduces the effectiveness of the isolates to inhibit the germination of spores (Tables 3-5).

At 3×10^8 CFU/ml and 3×10^7 CFU/ml all bacterial isolates completely inhibit spore germination of Ca6 in all application time except Bc5 isolate that presented inhibition of 75.33%, 55.67% and 30.00% in t_8, t_4 and t_8 respectively 3×10^7 CFU/ml (Tables 3-5).

Table 3. Percent inhibition of the spores' germination by different concentrations of the suspensions of the bacterial strains at t_0.

Bacterial strains	Concentration CFU/ml							
	3×10^8	3×10^7	3×10^6	3×10^5	3×10^4	3×10^3	3×10^2	3×10^1
Bc1	100.00 ± 0.00^a	100.00 ± 0.00^a	96.67 ± 0.88^a	72.33 ± 0.88^d	60.00 ± 0.58^f	46.30 ± 2.02^d	29.67 ± 1.48^b	0.00 ± 0.00^b
Bc2	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	95.67 ± 2.03^a	83.33 ± 1.07^a	68.00 ± 1.15^a	54.00 ± 2.31^a	17.00 ± 1.53^a
Bc3	100.00 ± 0.00^a	100.00 ± 0.00^a	92.67 ± 1.45^b	63.00 ± 1.73^e	58.00 ± 1.15^b	16.00 ± 1.15^b	3.33 ± 0.88^e	0.33 ± 0.33^b
Bc4	100.00 ± 0.00^a	100.00 ± 0.00^a	86.33 ± 1.83^c	56.00 ± 1.53^f	52.00 ± 1.22^d	3.00 ± 0.58^b	2.00 ± 1.00^e	0.00 ± 0.00^b
Bc5	100.00 ± 0.00^a	75.33 ± 1.45^b	37.33 ± 1.20^d	27.33 ± 1.45^g	10.00 ± 1.54^f	2.67 ± 0.33^d	2.00 ± 0.58^e	0.00 ± 0.00^b
Bc6	100.00 ± 0.00^a	100.00 ± 0.00^a	60.67 ± 1.74^e	52.67 ± 1.2^f	52.33 ± 1.85^e	24.00 ± 1.00^c	19.67 ± 0.88^c	0.00 ± 0.00^b
Bc7	100.00 ± 0.00^a	100.00 ± 0.00^a	92.67 ± 1.45^b	89.33 ± 1.2^b	75.33 ± 1.45^c	66.00 ± 0.58^b	22.33 ± 1.45^c	0.00 ± 0.00^b
Bc8	100.00 ± 0.00^a	100.00 ± 0.00^a	88.67 ± 1.45^c	82.33 ± 1.45^c	39.33 ± 1.76^e	19.00 ± 0.65^d	5.33 ± 1.96^e	0.00 ± 0.00^b
Bc9	100.00 ± 0.00^a	100.00 ± 0.00^a	88.00 ± 1.53^c	72.00 ± 1.15^d	28.67 ± 0.88^d	29.33 ± 1.45^c	14.33 ± 1.2^d	0.00 ± 0.00^b

The averages of the same column with the same letter do not significantly differ from each other at the 5% threshold.

Table 4. Percent inhibition of the spores' germination by different concentrations of the suspensions of the bacterial strains at t_4.

Bacterial strains	Concentration CFU/ml							
	3×10^8	3×10^7	3×10^6	3×10^5	3×10^4	3×10^3	3×10^2	3×10^1
Bc1	100.00 ± 0.00^a	100.00 ± 0.00^a	90.00 ± 0.58^{cd}	17.33 ± 1.45^d	10.67 ± 1.20^c	5.67 ± 0.88^b	4.67 ± 1.45^c	0.00 ± 0.00^c
Bc2	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	95.33 ± 1.76^a	85.66 ± 1.20^a	35.66 ± 0.88^a	18.33 ± 0.88a
Bc3	100.00 ± 0.00^a	100.00 ± 0.00^a	92.00 ± 2.08^c	91.67 ± 1.20^e	66.00 ± 1.15^c	43.33 ± 0.88^e	29.33 ± 2.04^{ab}	4.00 ± 1.15b
Bc4	100.00 ± 0.00^a	100.00 ± 0.00^a	91.67 ± 1.20^c	60.67 ± 1.08^f	56.66 ± 1.45^d	14.00 ± 1.15^f	7.67 ± 0.88^c	0.00 ± 0.00^c
Bc5	100.00 ± 0.00^a	55.67 ± 1.45^b	25.00 ± 1.15^g	14.67 ± 1.92^g	4.33 ± 0.88^g	3.00 ± 1.15^f	0.67 ± 0.33^d	0.00 ± 0.00^c
Bc6	100.00 ± 0.00^a	100.00 ± 0.00^a	72.00 ± 2.31^e	36.67 ± 0.63^f	36.67 ± 1.45^d	24.33 ± 0.88^d	4.33 ± 1.45^c	0.00 ± 0.00^c
Bc7	100.00 ± 0.00^a	100.00 ± 0.00^a	96.00 ± 1.15^b	96.33 ± 0.77^b	92.00 ± 1.53^b	67.33 ± 1.20^b	26 ± 2.31^b	0.00 ± 0.00^c
Bc8	100.00 ± 0.00^a	100.00 ± 0.00^a	66.00 ± 1.15^f	36.67 ± 0.67^c	15.33 ± 1.76^e	5.00 ± 1.15^e	5 ± 1.15^c	0.00 ± 0.00^c
Bc9	100.00 ± 0.00^a	100.00 ± 0.00^a	86.33 ± 0.88^d	75.33 ± 0.33^d	69.33 ± 1.20^f	35.33 ± 1.20^c	2.33 ± 1.86^c	0.00 ± 0.00^c

The averages of the same column with the same letter do not significantly differ from each other at the 5% threshold.

Table 5. Percent inhibition of the spores' germination by different concentrations of the suspensions of the bacterial strains at t_8.

Bacterial strains	Concentration CFU/ml							
	3×10^8	3×10^7	3×10^6	3×10^5	3×10^4	3×10^3	3×10^2	3×10^1
Bc1	100.00 ± 0.00^a	100.00 ± 0.00^a	75.00 ± 1.53^c	13.33 ± 2.40^f	6.33 ± 1.45^f	3.67 ± 0.68^e	1.00 ± 0.57^b	0.00 ± 0.00^b
Bc2	100.00 ± 0.00^a	100.00 ± 0.00^a	100 ± 0.00^a	92.87 ± 1.45^a	81.67 ± 1.68^a	61.00 ± 1.15^a	25.00 ± 1.53^a	10.33 ± 0.88^a
Bc3	100.00 ± 0.00^a	100.00 ± 0.00^a	85.33 ± 0.88^b	70.33 ± 1.34^b	23.00 ± 1.15b^c	6.66 ± 0.66^d	1.33 ± 0.88^b	0.00 ± 0.00^b
Bc4	100.00 ± 0.00^a	100.00 ± 0.00^a	75.00 ± 1.15^c	49.33 ± 1.72^c	24.33 ± 1.45^b	2.33 ± 0.79^{ef}	0.00 ± 0.00^c	0.00 ± 0.00^b
Bc5	100.00 ± 0.00^a	30.00 ± 0.58^b	24.67 ± 1.45^f	12.00 ± 1.15^f	2.00 ± 1.15^g	1.33 ± 0.14^g	0.00 ± 0.00^c	0.00 ± 0.00^b
Bc6	100.00 ± 0.00^a	100.00 ± 0.00^a	45.33 ± 1.20^e	19.00 ± 1.73^e	17.33 ± 1.77^d	8.33 ± 0.85^c	0.00 ± 0.00^c	0.00 ± 0.00^b
Bc7	100.00 ± 0.00^a	100.00 ± 0.00^a	83.67 ± 2.33^b	76.00 ± 2.31^a	58.67 ± 2.41^{ab}	10.67 ± 0.03^b	0.00 ± 0.00^c	0.00 ± 0.00^b
Bc8	100.00 ± 0.00^a	100.00 ± 0.00^a	59.67 ± 1.20^d	20.33 ± 0.88^e	6.67 ± 1.20^e	3.33 ± 1.45^e	0.00 ± 0.00^c	0.00 ± 0.00^b
Bc9	100.00 ± 0.00^a	100.00 ± 0.00^a	71.67 ± 0.88^c	45 ± 2.64^d	12.67 ± 1.76^c	6.33 ± 1.76^d	0.00 ± 0.00^c	0.00 ± 0.00^b

The averages of the same column with the same letter do not significantly differ from each other at the 5% threshold.

Inhibition of spore germination of Ca6 decreases for other decreasing concentrations tested from 3×10^6 CFU/ml. The Bc2 isolate remains effective with inhibitory percent of 95.67%, 100% and 92.87 in t_0, t_4 and t_8 respectively at 3×10^5 CFU/ml (Tables 3-5) and Bc5 is the least effective to inhibit the growth of *C. acutatum* in all the application times.

T. harzianum completely inhibits the germination of spores of Ca6 at 10^8, 10^7, 10^6 and 10^5 spores/ml in all the application time. Its inhibition percent at 10^4 spores/ml reached 97.33; 98.68 and 99.45% in t_0, t_4 and t_8 respectively, since this concentration is decreasing, the inhibition decreases slightly in all the application time but remains important in the t_8 (Figure 2).

Inhibition of spore germination of bacterial isolates decreased from 3×10^6 CFU/ml except inhibition Bc5 isolate that starts to decrease from 3×10^7 CFU/ml and isolate Bc2 retains its power inhibitor at 3×10^6 CFU/ml in all the application times (Table 6).

And the minimal inhibitory concentration of *T. harzianum* is 10^5 spores/ml (Table 6) this antagonist was also able to inhibit the germination of spores of *C. acutatum* even at low concentrations (Figure 1).

4. Discussion and Conclusion

The microorganisms that develop in the rhizosphere are ideal for use as biological control agents, by what the rhizosphere provides as the first defense against the attack of plant pathogens [11].

In recent years, various Bacillus spp. such as *B. amyloliquefaciens*, *B. subtilis*, *B. atrophaeus*, *B. amyloliquefaciens*, *B. thuringiensis*, and *B. pumilis* were used as potential biocontrol agents against different Colletotrichum spp. T [12] [13] [14] [15] [16], studies have been made to fight against Colletotrichum spp. using Trichoderma spp. [17] [18] [19].

In this study, the effect of different concentrations of inoculums of nine isolates of *B. amyloliquefaciens* and an isolate of *T. harzianum* was tested *in vitro*

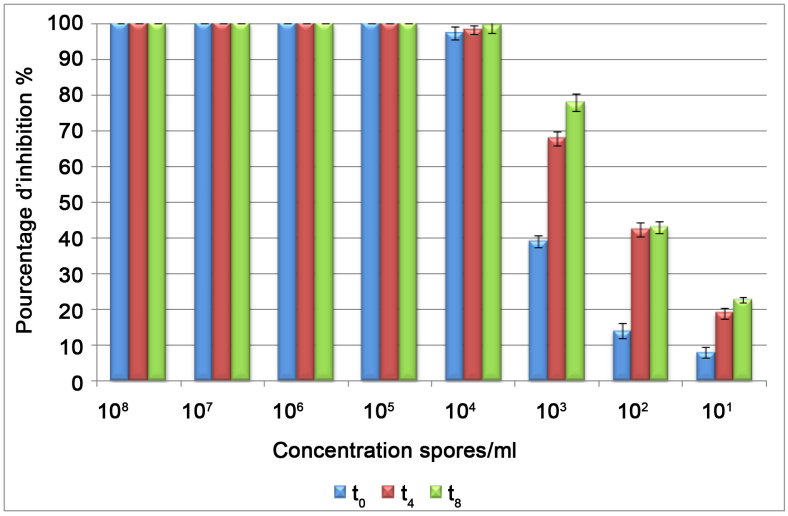

Figure 2. Percent inhibition of the spores' germination by different concentrations of the suspensions of *T. harzianum*.

Table 6. Determination of the minimum inhibitory concentration of antagonists' suspensions that completely inhibited spore germination of Ca6.

bacterial strains	Application time			Concentration
	simultaneous	preventive		
	t_0	t_4	t_8	
bc1	3×10^7	3×10^7	3×10^7	
Bc2	3×10^6	3×10^6	3×10^6	
BC3	3×10^7	3×10^7	3×10^7	
Bc4	3×10^7	3×10^7	3×10^7	**CFU/ml**
Bc5	3×10^8	3×10^8	3×10^8	
bc6	3×10^7	3×10^7	3×10^7	
BC7	3×10^7	3×10^7	3×10^7	
BC8	3×10^7	3×10^7	3×10^7	
Bc9	3×10^7	3×10^7	3×10^7	
Trichoderma harzianum	10^6	10^6	10^6	**Spores/ml**

vis-à-vis a mycelial growth and spores germination of phytopathogenic agent to find the potential of biological control at a low minimal inhibitory concentration against *C. acutatum*.

We found all the studied antagonists can inhibit or reduce mycelial growth and spore germination of *C. acutatum* Ca6. The effectiveness of *B. amyloliquefaciens* isolates to control against *C. acutatum* isolate differs from one to another, and we deduce that Bc2 submitted a total inhibition of mycelial growth of plant pathogen to MIC = 3×10^5 CFU/ml. And the minimal inhibitory concentration of *T. harzianum* obtained for inhibiting the mycelial growth of Ca6 is 10^6 spores/ml.

Comparing the minimal inhibitory concentrations of spore germination of

plant pathogen antagonistic, we observe that the majority of isolates give maximum inhibition at a concentration of 3×10^7 CFU/ml within three days of application, except for the isolate Bc2 to 3×10^6 CFU/ml and Bc5 at 3×10^8 CFU/ml. These concentrations are higher than those obtained in inhibiting mycelial growths which are 3×10^5 CFU/ml for Bc2 and 3×10^6 CFU/ml for the other isolates, except for Bc5 isolate that remains the same. Hamdache *et al*., [10] found that the values of the bacterial concentration of the same isolates used in this study are necessary for a total inhibition *Botrytis cinerea* and are low compared to those required to inhibit the germination of spores. The Bc2 isolate is the best among *B. amyloliquefaciens* strains because its percent inhibition is high relative to the others in decreasing concentration.

T. harzianum has a greater efficacy against the development of Ca6, even with the variation of its minimal inhibitory concentration of the mycelial growth and sporesgermination of the plant pathogen.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Cawoy, H., Bettiol, W., Fickers, P. and Ongena, M. (2011) Bacillus-Based Biological Control of Plant Diseases. Embrapa Meio Ambiente-Capítulo em livro científico (ALICE). https://doi.org/10.5772/17184

[2] Kai, M., Effmert, U., Berg, G. and Piechulla, B. (2007) Volatiles of Bacterial Antagonists Inhibit Mycelial Growth of the Plant Pathogen *Rhizoctonia solani*. *Archives of Microbiology*, **187**, 351-360. https://doi.org/10.1007/s00203-006-0199-0

[3] Chung, Y.A. and Rudgers, J.A. (2016) Plant-Soil Feedbacks Promote Negative Frequency Dependence in the Coexistence of Two Aridland Grasses. *Proceedings of the Royal Society B: Biological Sciences*, **283**, Article ID: 20160608. https://doi.org/10.1098/rspb.2016.0608

[4] Reva, O.N., Dixelius, C., Meijer, J. and Priest, F.G. (2004) Taxonomic Characterization and Plant Colonizing Abilities of Some Bacteria Related to *Bacillus amyloliquefaciens* and *Bacillus subtilis*. *FEMS Microbiology Ecology*, **48**, 249-259. https://doi.org/10.1016/j.femsec.2004.02.003

[5] Yu, G.Y., Sinclair, J.B., Hartman, G.L. and Bertagnolli, B.L. (2002) Production of Iturin A by *Bacillus amyloliquefaciens* Suppressing *Rhizoctonia solani*. *Soil Biology and Biochemistry*, **34**, 955-963. https://doi.org/10.1016/S0038-0717(02)00027-5

[6] Kumar, R.N. and Mukerji, K.G. (1996) Integrated Disease Management Future Perspectives, In: Mukerji, K.G., Mathur, B., Chamala, B.P. and Chitralekha, C., Eds., *Advances in Botany*, APH Publishing Corporation, New Delhi, 335-347.

[7] Viterbo, A.D.A., Wiest, A.R.I.C., Brotman, Y., Chet, I.L.A.N. and Kenerley, C. (2007) The 18mer Peptaibols from *Trichoderma virens* Elicit Plant Defense Responses. *Molecular Plant Pathology*, **8**, 737-746. https://doi.org/10.1111/j.1364-3703.2007.00430.x

[8] Es-Soufi, R., El Kbiach, M.L., Errabii, T., Saidi, R., Badoc, A., Chaveriat, L., Martin, P. and Lamarti, A. (2018) Biology and Physiology of *Colletotrichum acutatum*

Strains Causing Strawberry's Anthracnose. *Agricultural Science*, **9**, 974-990. https://doi.org/10.4236/as.2018.98068

[9] Tatagiba, J.S., Maffia, L.A., Barreto, R.W., Alfenas, A.C. and Sutton, J.C. (1998) Biological Control of *Botrytis cinerea* in Residues and Flowers of Rose (*Rosa hybrida*). *Phytoparasitica*, **26**, 8-19. https://doi.org/10.1007/BF02981261

[10] Hamdache, A., Ezziyyani, M. and Lamarti, A. (2018) Effect of Preventive and Simultaneous Inoculations of *Bacillus amyloliquefaciens* (Fukumoto) Strains on Conidial Germination of *Botrytis cinerea* Pers: Fr. *Anales de Biología*, **40**, 65-72. https://doi.org/10.6018/analesbio.40.08

[11] Suprapta, D.N. (2012) Potential of Microbial Antagonists as Biocontrol Agents against Plant Fungal Pathogens. *ISSAAS Journal*, **18**, 1-8.

[12] Guardado, V.L., Tovar, P.E., Chacón, L.A., López, G.U., Gutiérrez, M.P., Stoll, A. and Aguilera, S. (2018) Identification and Characterization of a New *Bacillus atrophaeus* Strain B5 as Biocontrol Agent of Postharvest Anthracnose Disease in Soursop (*Annona muricata*) and Avocado (*Perseaamericana*). *Microbiological Research*, **210**, 26-32. https://doi.org/10.1016/j.micres.2018.01.007

[13] Gowtham, H.G., Murali, M., Singh, S.B., Lakshmeesha, T.R., Murthy, K.N., Amruthesh, K.N. and Niranjana, S.R. (2018) Plant Growth Promoting Rhizobacteria-*Bacillus amyloliquefaciens* Improves Plant Growth and Induces Resistance in Chilli against Anthracnose Disease. *Biological Control*, **126**, 209-217. https://doi.org/10.1016/j.biocontrol.2018.05.022

[14] Zhao, P., Li, P., Wu, S., Zhou, M., Zhi, R. and Gao, H. (2019) Volatile Organic Compounds (VOCs) from *Bacillus subtilis* CF-3 Reduce Anthracnose and Elicit Active Defense Responses in Harvested Litchi Fruits. *AMB Express*, **9**, 119. https://doi.org/10.1186/s13568-019-0841-2

[15] Fuente, S.N.M., Casados, V.L.E., Garcia, P.A.P., Barboza, P.U.E., Bideshi, D.K., Salcedo, H.R., Garcia, A.B.E. and Barboza, C.J.E. (2016) The Endochitinase ChiABtt of *Bacillus thuringiensis* subsp. Tenebrionis DSM-2803 and Its Potential Use to Control the Phytopathogen *Colletotrichum gloeosporioides*. *Microbiology Open*, **5**, 819. https://doi.org/10.1002/mbo3.372

[16] Zheng, M., Shi, J., Shi, J., Wang, Q. and Li, Y. (2013) Antimicrobial Effects of Volatiles Produced by Two Antagonistic Bacillus Strains on the Anthracnose Pathogen in Postharvest Mangos. *Biological Control*, **65**, 200-206. https://doi.org/10.1016/j.biocontrol.2013.02.004

[17] Yan, Z.K. and Anh, V.T.T. (2018) Effect of Trichoderma sp. on Anthracnose Disease of Stored Chilli. *Borneo Journal of Resource Science and Technology*, **8**, 90-102. https://doi.org/10.33736/bjrst.1211.2018

[18] Alvindia, D.G. (2018) The Antagonistic Action of *Trichoderma harzianum* Strain DGA01 against Anthracnose-Causing Pathogen in Mango cv. "Carabao". *Biocontrol Science and Technology*, **28**, 591-602. https://doi.org/10.1080/09583157.2018.1468998

[19] Setiawati, W., Hasyim, A. and Hudayya, A. (2016) The Effect of Fruit Characteristics of Cayenne Pepper (*Capsicum frutescens*) and Biocontrol Agents (Trichoderma sp and Azoxystrobin) on Severity of Anthracnose (*Colletotrichum acutatum*). *Advances in Agriculture & Botanics*, **8**, 1-12.